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Carbon and Nutrient Balances in Microalgal Bioenergy System

by

Eunyoung Lee

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Environmental Engineering
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Date of Approval:
June 21, 2017

Keywords: Microalgae, Biofuel, Wastewater, Life cycle assessment, Kinetic model

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DEDICATION

This dissertation is dedicated to my parents, Gunjung Lee and Yunja Jin, and my fiancé, Youngwoon Kim, for their tremendous love and supports throughout the years of my Ph.D. study.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Qiong Zhang for her guidance, support, and mentorship throughout my time at USF. I would also like to thank my committee members, Dr. James R. Mihelcic, Dr. Sarina Ergas, Dr. Babu Joseph, and Dr. John Jermier for their advice and feedback on my research. I would like to acknowledge Dr. Jie Zhang and Dr. Meng Wang for their feedback and help. Furthermore, I would like to express gratitude to Dr. Zhang's water-energy nexus research group (Youngwoon Kim, Christine Prouty, Xiaofan Xu, Nader Rezaei, Yan Zhang, Dr. Shima Mohebbi, Dr. Nancy Diaz Elsayed, and Dr. Haiyan Duan) for their supports. Moreover, I would like to thank my REU students (Jessica Marron, Allison Wood, and Jewel Cumberbatch) for their assistance. In addition, I would also like to thank Mr. Paul Tavernier and Mr. Jeffrey Borden from the City of Northeast Clearwater Wastewater Treatment Facility for their assistance on wastewater sample provision.

This material is based upon work supported by the National Science Foundation Research Experience for Undergraduates (REU) Program grant of the United States (No. 1156905) and the National Science Foundation Partnerships for International Research and Education (PIRE) grant of the United States (No. 1243510). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the National Science Foundation.

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ABSTRACT

This research investigated life cycle environmental impacts and benefits of an integrated microalgae system with wastewater treatment system using an integrated process modeling approach combined with experimentation. The overall goal of this research is to understand energy, carbon and nutrient balances in the integrated system and to evaluate the environmental impacts and benefits of the integrated system from a carbon, nutrient, and energy perspective. In this study, four major research tasks were designed to contribute to a comprehensive understanding of the environmental and economic sustainability of the integrated system, which included development of an integrated co-limitation kinetic model for microalgae growth (Chapter 2), kinetic parameter estimation models for anaerobic co-digestion (Chapter 3), development of an integrated process model (Chapter 4), and life cycle environmental and economic assessments of the integrated system (Chapter 5).

The integrated co-limitation kinetic model was developed to understand microalgae growth in the centrate from dewatering of anaerobically digested sludge. This growth kinetic model considered four major growth factors, including Nitrogen (N), dissolved carbon dioxide (CO_2) concentrations, light intensity, and temperature. The model framework was constructed by combining threshold and multiplicative structures to explain co-limitation among these factors. The model was calibrated and validated using batch studies with anaerobically digested municipal sludge centrate as wastewater source, and the model was shown to have a reasonable growth rate predictor for *Chlorella sp*. under different nutrient levels of the centrate.

Anaerobic co-digestion was used for energy conversion process in the integrated system. To estimate methane production of anaerobic co-digestion, kinetic models commonly applied. To apply the kinetic model, determining kinetic parameters for anaerobic co-digestion of microalgae and waste activated sludge (WAS) is essential, and this research introduced two potential regression-based parameter estimation models to estimate the kinetic parameters. Using the estimation models presented, the kinetic parameters for co-digestion was able to be determined for different ratios of co-substrates with limited experiments.

In this research, the integrated process model was developed to simulate the dynamic behavior of the integrated system. The model included the microalgae cultivation, harvesting, and anaerobic co-digestion processes in the integrated system to provide a comprehensive understanding of the integrated system. For cultivation, the integrated co-limitation kinetic model was applied to estimate microalgae productivity, while the regression-based parameter estimation model was used to determine the first order kinetic parameter to estimate methane production rates for anaerobic co-digestion. The simulated microalgae productivity results were comparable to typical microalgae productivity in open pond systems. For the integrated system, removal of NH_4-N by microalgae was not efficient. In particular, the NH_4-N removal was minimal during the winter season due to low microalgae growth. As the microalgae productivity increased, the CH_4 and biosolids production increased as a result of the increased amount of the substrates from the harvested microalgae biomass. The increase of CH_4 and biosolids productions, however, was minor because of the small amount of microalgae biomass for the co-digestion.

Based on simulated data for integrated process modeling, the life cycle environmental and economic impacts of the integrated system (with different CO_2 supply areas) were evaluated and compared to the conventional wastewater treatment system. The integrated systems had a lower

carbon footprint, cumulative energy demand, and life cycle cost than the conventional system. The integrated system with 10% CO_2 sparging area was able to achieve the lowest carbon footprint. Without CO_2 addition during microalgae cultivation, the integrated system had the lowest energy balance and life cycle cost. However, there is no significant difference between the integrated and conventional systems for eutrophication potential because these systems had the same effluent quality. In terms of an energy saving with the integrated systems, the benefit of energy reduction for the wastewater treatment was greater than the energy production from the anaerobic co-digestion, compared to the conventional system. Overall, the integrated system can improve the carbon balance by reducing the life cycle energy required in the conventional system.

CHAPTER 1: INTRODUCTION

1.1 Background and Significance

Approximately 80% of the world energy demand is supplied by fossil fuels, such as petroleum, coal, and natural gas (Medeiros et al., 2015). Consumption of fossil fuels results in emissions of greenhouse gas (GHG), and GHGs are known as one or major drivers of global climate change (IPPC, 2013). To reduce GHG emissions, international commitments, such as Kyoto Protocol and Paris Agreement, promote the development of alternatives to replace fossil fuels (Kintisch, 2010). Currently, many different sources of renewable energy are being explored, such as solar, wind, geothermal, tidal, and biomass (Scott et al., 2010). Among them, fuels from biomass are one of the most feasible options because they can be stored and used directly in existing vehicle engines or commercial boiler. Depending on biomass materials, the biofuel can be classified into three generations: the first generation uses edible crops; the second generation uses agricultural residues (lignocellulosic-based biomass) or non-edible crops; and the third generation uses microalgae (Moncada et al., 2014). The first and second generation biofuels, however, have fatal drawbacks: the first generation biofuels require large amounts of arable land and compete for the land with food crops; the second generation biofuels also use large amounts of arable land and require energy-intensive processes, such as thermal pretreatment of lignocellulosic-based materials (Brennan and Owende, 2013). Due to the drawbacks of the first and second generation biofuels, third generation biofuels, which are derived from microalgae, have been considered as one of the most promising alternatives (Moncada et al., 2014).

Microalgae are photosynthetic microorganisms capable of rapid adaptation to new environments (Hsueh et al., 2009). Many microalgae species have higher biomass productivities, lipid contents, and CO_2 fixation rates than terrestrial crops (Amin, 2009; Mata et al., 2010). For this reason, microalgae are a perfect candidate for CO_2 sequestration, GHG reduction, and feedstock for biofuels. Moreover, they do not compete with terrestrial agriculture for arable land because they generally grow in water bodies such as ponds, lakes, rivers, and water reservoirs (Mata et al., 2010; Sturm and Lamer, 2011). They also have an ability to improve water quality by uptaking nutrients such as nitrogen (N) and phosphorus (P) from the poor-quality water, such as municipal, industrial, or agricultural wastewaters and by adding oxygen via their photosynthesis in the water (Becker, 1994). Because of this ability, they have been studied for wastewater treatment since the mid-1970s (Bosch et al., 1974; Judd et al., 2015). In addition, microalgae biomass can be used to produce a broad portfolio of fuels, such as biodiesel, bioethanol, and biogas (Amin, 2009; Kumar et al., 2010).

Despite their benefits, microalgae bioenergy systems must overcome a number of challenges for sustainable development of the system from a holistic perspective. Life Cycle Assessment (LCA) studies have shown that the nutrient requirements for microalgae cultivation, which is associated with fertilizer consumption, results in a high environmental impact of microalgae bioenergy even though microalgae cultures use nutrients more efficiently than biomass crops (Clarens et al., 2010; Peccia et al., 2013). This nutrient consumption may also cause another issue such as competition for fertilizer with food crops. The energy requirements for microalgae harvesting and cultivation stages results in large environmental impacts as well as operational costs (Borowizka and Moheimani, 2013; Medipally et al., 2015; Peccia et al., 2013; Rösch et al., 2012).

Due to the high-energy requirements, the microalgae-based biofuel results in a higher energy ratio (energy consumed per energy produced) than fossil-based fuel (Batan et al., 2010).

For sustainable microalgae bioenergy production, microalgae production integrated with wastewater treatment has been suggested (Kumar et al., 2011). This integration contains many beneficial synergies. In the integrated system, for example, a wastewater treatment plant can improve water quality with less energy consumption and reduce on-site carbon dioxide (CO_2) emissions. A microalgae cultivation system can receive wastewater as water and nutrient resources and CO_2 as a carbon source so that it can significantly reduce operational costs (fertilizer consumption) and environmental impacts of microalgae cultivation (Menger-Krug et al., 2012). In addition, the harvested microalgae biomass can be used as feedstock for anaerobic digestion which is an existing infrastructure in advanced wastewater treatment facilities. Since anaerobic digestion does not require a drying process for microalgae, microalgae system can reduce the costs for microalgae drying (Kumar et al., 2011). Thus, by adding harvested microalgae, the integrated system can achieve higher bioenergy production via anaerobic digestion than bioenergy produced from conventional wastewater treatment facilities.

There are many studies focused on integrated systems (Figure 1.1). The majority of prior studies investigated the effect of different nutrient loadings in wastewater on microalgae productivity, methods and efficiencies of microalgae biomass harvesting, and bio-oil and syngas production from wastewater-grown microalgae through an experimental approach (Chen et al., 2015b; Milledge and Heaven, 2013; Pittman et al., 2011; Sutherland et al., 2014). Sutherland et al. (2014) investigated the effects of nitrogen loads of wastewater on microalgae in pilot-scale high rate algal ponds. They concluded that high nitrogen loads improved microalgae productivity and nutrient removal efficiency. Such experimental approaches are important to advance our

understanding of sustainable microalgae biofuel, but are limited in terms of a comprehensive understanding of system performance under varying conditions, such as light intensity, nutrient loading, and temperature.

Several studies used a modeling approach to understand the behavior of microalgae cultivation using wastewaters or anaerobic digestion using microalgae grown in wastewaters (Bello et al., 2016; Buhr and Miller, 1983; Passos et al., 2015; Yang, 2011). Bello et al. (2016) conducted a comprehensive dynamic mathematical modelling to simulate the production of microalgae in a high rate algal pond. Through their study, they obtained a dynamic behavior of microalgae in the pond and found that the addition of CO_2 helps to regulate pH as well as to enhance biomass productivity. Passos et al. (2015) investigated methane (CH_4) production through Anaerobic Digestion Model No.1 (ADM1) using microalgae harvested from the integrated system over a year. They found that the methane yield of the microalgae averaged 0.09-0.16 L CH_4 g^{-1} COD with 15-20 day hydraulic retention time. Variability of biogas production over the year was attributed to shifting dominant microalgae species. The modeling approach is suitable to expand our understanding for the system performance, but current studies are limited to a single process, such as the cultivation process or anaerobic digestion of the integrated system. Therefore, a dynamic modeling approach, considering the entire integrated system, is required to provide a comprehensive understanding of the system performance.

Table 1.1 shows the previous studies focused on LCA for integrated systems. Some studies focused only on cultivation or harvesting processes, while others considered the whole processes. Most of these studies mainly focused on energy production with a system boundary limited to the microalgae bioenergy system without considering wastewater treatment. Only two studies considered both wastewater treatment and microalgae bioenergy systems in their system boundary

(Beal et al., 2012; Menger-Krug et al., 2012), but both failed to consider the impacts of carbon and nutrient on the performance and sustainability of the integrated system.

Carbon and nutrient balances are essential to understand and mitigate environmental impacts of the whole system. Through the carbon and nutrient balances, potential GHG and nutrient emissions can be estimated and expressed as carbon footprint and eutrophication that represent global and local environmental impacts. In particular, microalgae cultivation in the integrated system can mitigate on-site CO_2 emissions through photosynthesis. Moreover, because microalgae have an ability to remove nutrients in wastewater, it is expected to reduce indirect GHG emission through reduction of energy demand for nutrient treatment in the integrated system. Thus, a comprehensive understanding of carbon and nutrient balance is necessary to improve environmental sustainability of the integrated system.

In addition, as shown in Table 1.1, prior studies have mainly focused on bio-oil productions, which requires additional infrastructures for drying and energy conversion processes. Unlike bio-oil productions, biogas productions can be achieved through existing anaerobic digestion systems in the wastewater treatment plants. The potential biogas production via anaerobic digestion using microalgae and waste sludge has been studied (Beltrán et al., 2016; Rawat et al., 2013; Wang et al., 2013). Ajeej et al. (2015) pointed out the importance of research on biogas production from anaerobic co-digestion of microalgae and sewage sludge for the sustainability of wastewater treatment plants due to energy recovery. However, assessments on life cycle environmental impacts related to the biogas productions for the integrated systems are still lacking. Thus, a LCA study is needed to improve the understanding of the sustainability of the integrated system considering biogas production.

Based on research gaps mentioned above, important scientific questions have been raised: (1) What is an appropriate rate expression regarding the algae growth, carbon biofixation, and nutrient uptake for the integrated system?; (2) How will anaerobic co-digestion of waste-activated sludge and microalgae impact on the performance of the integrated system?; and (3) Will the integrated system be sustainable from energy, carbon and nutrient perspectives?

1.2 Scope of Research

According to the previous section, integrated systems could provide many advantages, such as nutrient and energy recoveries and CO_2 mitigation, but there are still limited studies on the life cycle benefits and impacts for the whole integrated system in terms of carbon, nutrient, and energy perspectives. Thus, the overall goal of this research is to understand energy, carbon, and nutrient balances in the integrated system based on the life cycle environmental impacts and the costs of the integrated system.

Based the goal of this study, it was hypothesized that the integrated system is a net energy producer, and carbon and nutrient neutral from a life cycle perspective (Hypothesis 1). In this study, the integrated system was based on a 5 MGD advanced wastewater treatment system. In the integrated system, a microalgae system was applied as a side-stream process in the integrated system, which obtained the centrate (dewatering of anaerobically digested sludge) as nutrient medium for microalgae cultivation. Anaerobic co-digestion of waste sludge and microalgae was used as an energy conversion process in the integrated system. The integrated system considered in this study consists of wastewater treatment pathway (including pretreatment (grit removal, bar screens), primary treatment, secondary treatment (A^2/O process: 3 stage pho-redox process), filtration, and disinfection), solid treatment pathway (including waste sludge thickening (rotary-drum thickener), anaerobic digestion, and digested sludge dewatering (centrifuge)), and

microalgae pathway (microalgae cultivation (raceway pond system) and harvesting (gravity sedimentation and centrifuge)) shown in Figure 1.2.

To identify carbon and nutrient balances for the integrated system, the integrated process model was proposed. In the integrated process model, a rate expression for microalgae cultivation is an essential element, which is explained by kinetic models for microalgae growth. However, the rate expressions for microalgae cultivation using wastewater are not well documented. Among the existing kinetic models for microalgae growth, the models considering multiple growth factors, which have been developed based on two types of co-limitation theories (multiplicative and threshold theories), were preferred over the past decade (Arrigo, 2005; Pahlow and Oschlies, 2009). The multiplicative theory assumes that all resources simultaneously affect the overall growth rate, while the threshold theory considers that the overall growth rate is affected only by the most limited resource among all resources required by cell growth (Bougaran et al., 2010). Kinetic modeling studies have mostly adopted the threshold theory to explain nutrient factors such as N and P on microalgae growth (Bougaran et al., 2010; Guest et al., 2013; Klausmeier et al., 2004). On the other hand, the multiplicative theory was often applied to describe the effect of environmental factors such as light, and CO_2 on microalgae growth (Bernard, 2011; Filali et al., 2011; Ketheesan and Nirmalakhandan, 2013; Yang, 2011). However, there is no attempt to combine the threshold and multiplicative effects on microalgae growth rate by considering all of the nutrient and environmental factors. In that sense, it was hypothesized that the combination of threshold and multiplicative relationships will be an appropriate structure of the rate expression (model predictions with $R^2>0.8$) for microalgae cultivation using wastewater as the nutrient medium (Hypothesis 2).

In the integrated system, anaerobic co-digestion of microalgae and waste activated sludge could be a feasible method for energy recovery. The co-digestion is able to improve biogas production by supplying missing nutrients from co-substrates and diluting the potential toxic substances (Mata-Alvarez et al., 2000). Based on the fact, the following hypothesis was proposed: Anaerobic co-digestion of waste-activated sludge and a certain percentage of microalgae will improve methane production rates in the anaerobic digestion step compared to conventional anaerobic digestion with the sludge only (Hypothesis 3).

To achieve the goal of this study and test the hypotheses, this research includes four tasks, shown in Figure 1.3. Through this research, two models, an integrated co-limitation kinetic model for microalgae growth and kinetic parameter estimation model for anaerobic co-digestion, were developed to test Hypotheses 1 and 2, as described in Chapter 2 and Chapter 3. The kinetic models were then used to develop an integrated process model as discussed in Chapter 4. Based on simulation data obtained from Chapter 4, life cycle environmental impacts of the integrated system were assessed in Chapter 5 to test Hypothesis 3. Chapter 6 summarizes the conclusions of this study and provides recommendations for future studies.

Table 1.1 Prior studies focused on life cycle assessment for integrated systems.

Process considered			Data source		Wastewater types		Research focused			System boundary	References
Cultivation	Harvesting	Energy conversion	Experimental data	Modeling data	Municipal wastewater	Others	Energy (or cost)	Carbon	Nutrient		
√			√			√+	√		√	M	Feng et al. (2011)
√			√		√**		√	√	√	M	Clarens et al. (2010)
√			√		√**			√	√	M	Soratana and Landis (2011)
	√		√			√+++	√			M	De Godos et al. (2011)
	√		√				√			M	Lee et al. (2009)
	√		√		√***		√	√		M	Udam et al. (2013)
√	√		√			√++	√			M	Abu-Ghosh et al. (2015)
√	√	√O, √G	√		√*		√			M, W	Beal et al. (2012)
√	√	√O	√		√**			√		M	Fortier et al. (2014)
√	√	√O	√		√**		√	√		M	Handler et al. (2014)
√	√	√O, √G	√		√*		√			M	Lundquist et al. (2010)
√	√	√G	√		√*		√		√	M. W	Menger-Krug et al. (2012)
√	√	√O, √G, √D	√		√**, √***	√+++		√	√	M	Mu et al. (2014)
√	√	√O	√		√**		√	√		M	Sander and Murthy (2010)
√	√	√O	√		√**		√			M	Sturm and Lamer (2011)
√	√	√G	√			√+++	√	√	√	M	Zhang et al. (2013)
√	√	√O	√		√**				√	M	Yang et al. (2011)

Note: D: Direct combustion; G: Microalgae based biogas; O: Microalgae bio-oil (including biodiesel and bio-jet fuel); *primary wastewater; **secondary wastewater, *** anaerobically digested wastewater; + Artificial wastewater; ++ Industrial wastewater; +++ Agricultural wastewater; M: microalgae system; W: wastewater treatment system

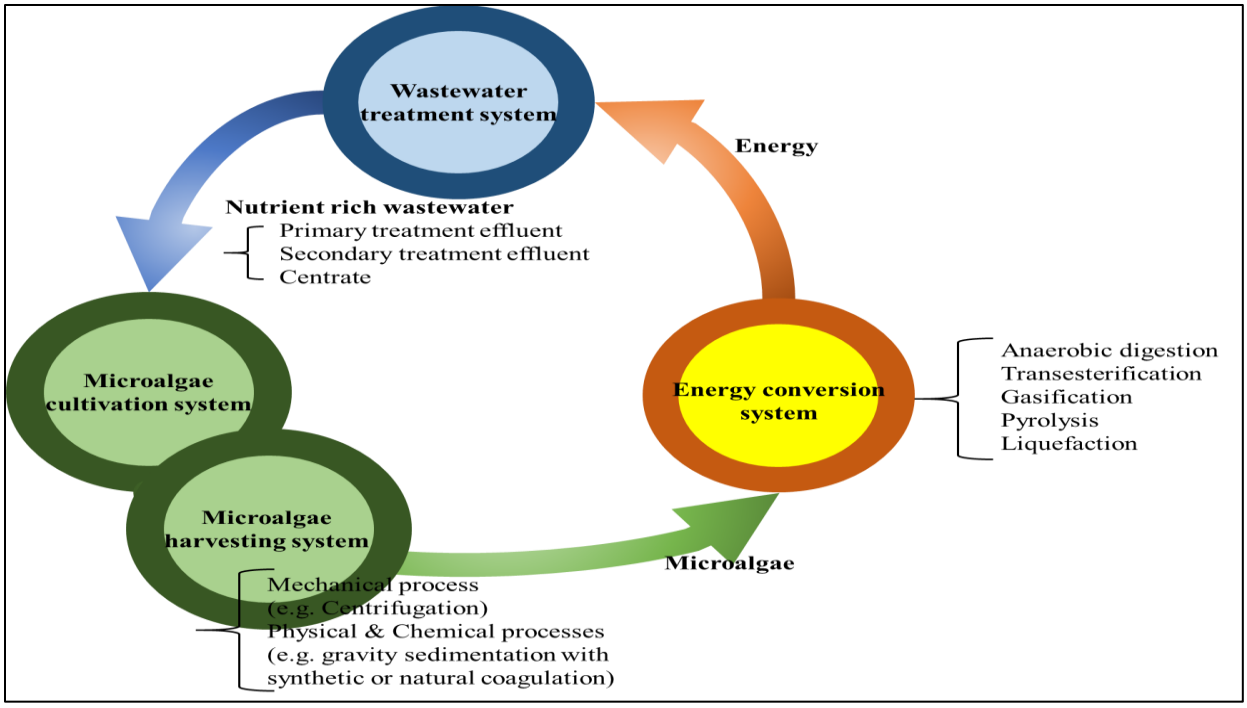


Figure 1.1 Integrated systems.

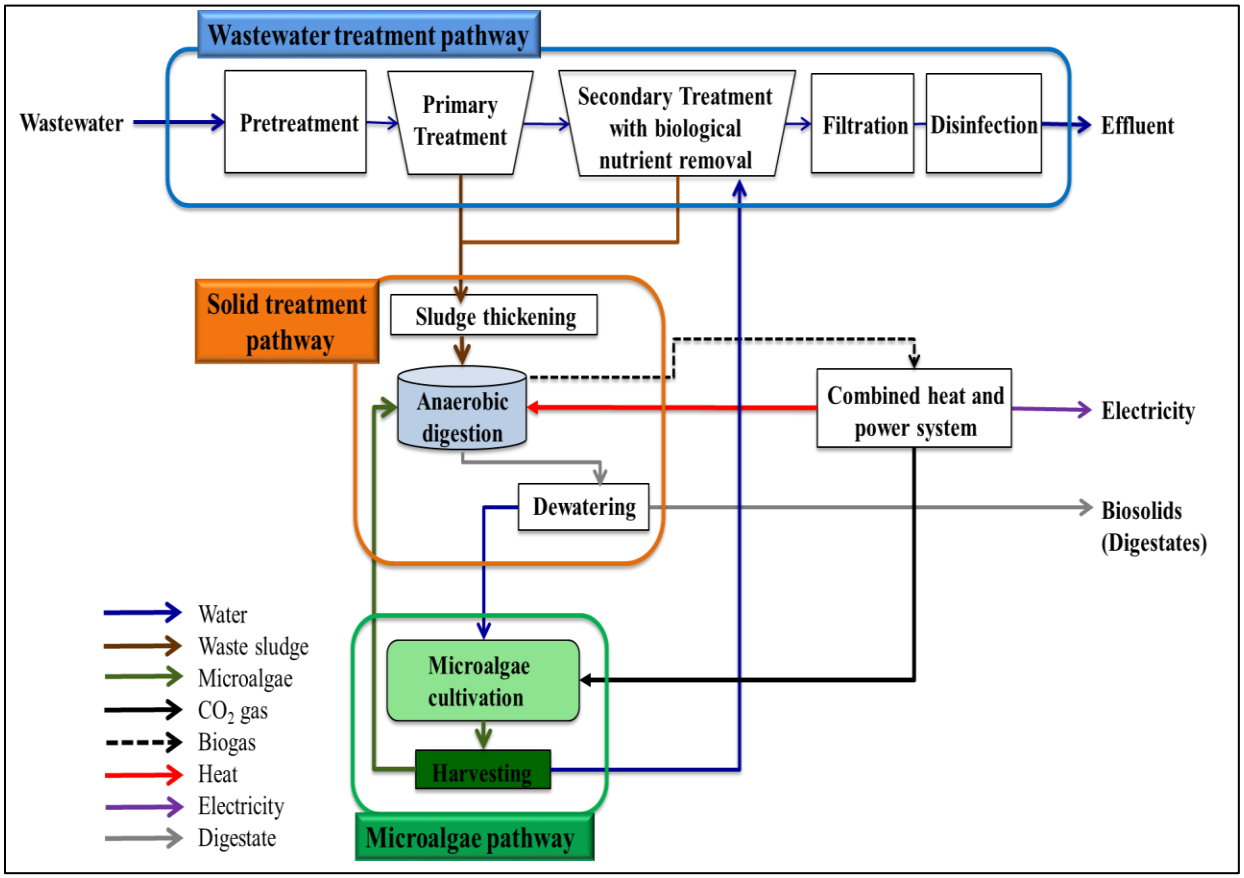


Figure 1.2 Integrated system for this research.

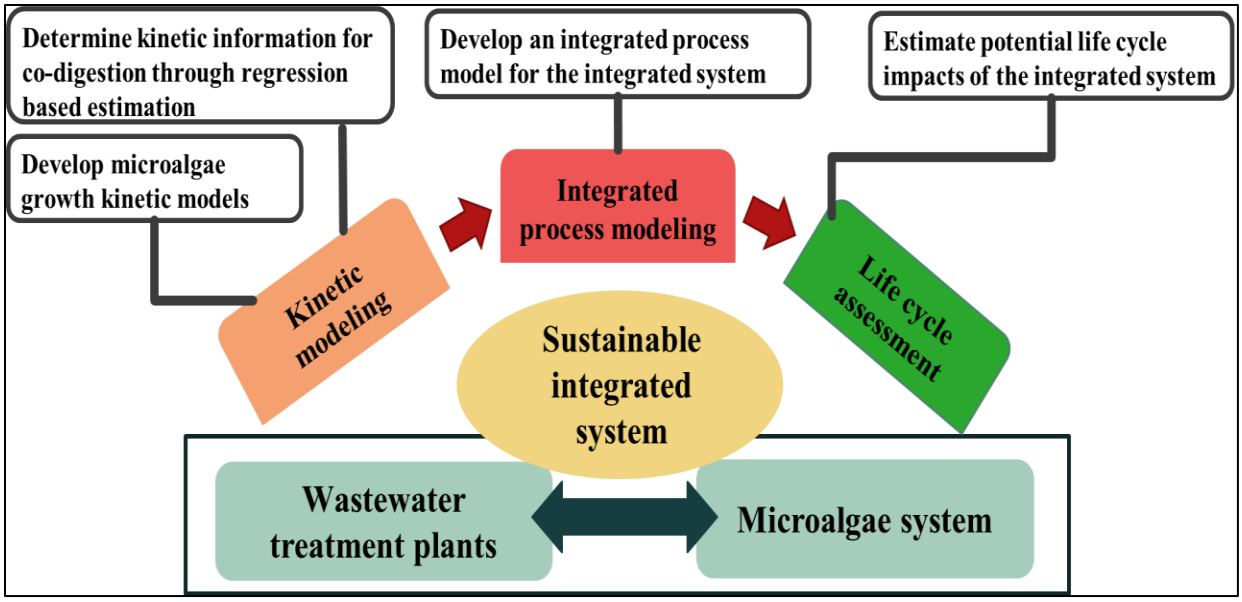


Figure 1.3 Scope of research with major research tasks.

CHAPTER 2: DEVELOPMENT OF GROWTH KINETIC MODEL FOR MICROALGAE CULTIVATION IN CENTRATE

2.1 Introduction

Microalgae-based bioenergy has received considerable interest because of distinctive advantages over other energy crops, including high solar energy yield, high biomass productivity, and low land use (Mata et al., 2010; Weyer et al., 2010). However, traditional microalgae cultivation systems face significant challenges due to the high dependency on fertilizer and freshwater as well as difficulties in full-scale bioreactor design (Singh et al., 2015; Slade and Bauen, 2013). To minimize resource consumptions for water and nutrients, microalgae cultivation integrated with wastewater has been proposed (Kumar et al., 2010; Pittman et al., 2011). Although this integrated system can significantly reduce the operational costs and environmental impacts for both microalgae cultivation and wastewater treatment, the productivity of microalgae is low due to inconsistent nutrient composition in wastewater (Lam and Lee, 2012; Menger-Krug et al., 2012). In addition, the performance of such systems varied with the types of wastewater and microalgae species (Lam and Lee, 2012; Pittman et al., 2011; Tercero et al., 2014). Thus, understanding of microalgae growth in wastewater is the key to optimize the integrated systems for successful implementation.

To date, growth kinetic models have been developed by considering the effect of a single limiting factor or multiple limiting factors (Béchet et al., 2013; Lee et al., 2015). Due to the recognition of co-limitation, the models considering multiple factors were preferred as they

[1] This chapter is based substantially on and reprinted with permission from: "Lee, E., & Zhang, Q. (2016). Integrated co-limitation kinetic model for microalgae growth in anaerobically digested municipal sludge centrate. Algal Research, 18, 15-24".

provided a better explanation of the growth which is based on either threshold or multiplicative theories (Arrigo, 2005; Kovárová-Kovar and Egli, 1998; Paerl, 1982; Pahlow and Oschlies, 2009). The multiplicative theory assumes that all resources simultaneously affect the overall growth rate, while the threshold theory considers that the overall growth rate is affected only by the most limited resource among all resources required by cell growth (Bougaran et al., 2010). Kinetic modeling studies have mostly adopted the threshold theory to explain N-P co-limitation on microalgae growth (Bougaran et al., 2010; Guest et al., 2013; Klausmeier et al., 2004). On the other hand, the multiplicative theory was often applied to describe the effect of N, light, and CO_2 on microalgae growth (Bernard, 2011; Filali et al., 2011; Ketheesan and Nirmalakhandan, 2013; Yang, 2011).

Currently, there is no growth kinetic model that considers major multiple factors including N, P, light, CO_2, and temperature, and their different co-limitation effects in the modeling framework (Lee et al., 2015). In addition, the existing modeling studies were conducted under artificial nutrient medium so that most of the studies considered only nutrient limitation conditions for microalgae growth without investigating growth inhibition caused by high nutrient concentrations. In that sense, the application of these existing kinetic models is limited for microalgae growth in wastewater because inhibition of nutrients on the growth may occur for microalgae growth in wastewater.

There have been a few attempts to develop kinetic models for microalgae growth in wastewater conditions (Coppens et al., 2014; Halfhide et al., 2015; Kasiri et al., 2015; Ruiz et al., 2013; Wu et al., 2013). Ruiz et al. (2013) proposed a kinetic model for wastewater photobiotreatment with microalgae. They applied two different kinetic models for algae growth and nutrient uptake. The growth kinetic model was based on the Verhulst model, also called as the logistic model, while the nutrient uptake model for N and P was based on the Quiroga second-

order equation (Quiroga et al., 1999). Both models were validated using experimental data for the growth of *Chlorella vulgaris* in secondary effluent of a conventional wastewater treatment plant. This growth model considered microalgae biomass concentration as the only variable in the model expression without including other growth factors particularly relevant to wastewater. Besides, to apply the growth model, the parameters (μ_{max}, $\mu_{N,max}$, $\mu_{P,max}$, X_0, X_{max}, S_{na}, Y_N, Y_P) in both growth and nutrient uptake models have to be determined. Wu et al. (2013) developed an integrated kinetic model for growth of *Scenedesmus* sp. LX1 in domestic secondary effluent in open pond systems. They applied the multiplicative theory to describe combined effects of N, P, and light intensity on the microalgae growth. However, previous studies have pointed out that N-P co-limitation on the growth follows the threshold instead of multiplicative relationship (De Groot, 1983). Furthermore, this model does not take into consideration of the effect of temperature which is one of the important factors for the growth (Davision, 1991) and cannot be easily controlled for the open pond systems. Coppens et al. (2014) developed a kinetic model based on the multiplicative theory considering inorganic carbon, N, P and light to determine the nutrient recovery potential of nitrate-storing diatoms from marine wastewater. The proposed kinetic model was able to describe the *Phaeodactylum tricornutum* growth in synthetic marine wastewater. However, similar to the limitation of Wu et al. (2013), they did not consider the different co-limitation effects of the selected factors in model framework and the impact of temperature for outdoor application. Kasiri et al. (2015) developed a non-linear dynamic model that describes the growth rate and uptake rate of *Chlorella kessleri* cultivated in oil-sands process water. The growth model in that study was based on multiplicative theory, including CO_2, light intensity, and phosphate factors, while the nutrient uptake model considered CO_2, phosphate, nitrate and ammonium. Temperature and pH were kept constant and not considered in the growth model. It was concluded that the model

adequately described the algal growth rate as well as the uptake rate of selected factors in oil sands process water. However, the criteria for selecting relevant growth factors were not clear and nitrogen as one of the important growth factors for microalgae was not considered in the growth model (Kumaer et al., 2010). Halfhide et al. (2015) investigated the performance of photobioreactor for growth of *Chlorella* sp. in anaerobically digested municipal sludge centrate. In their study, both nutrient and light were considered, and light was selected as the most limiting factor in their model based on the threshold theory. However, previous studies suggested that nutrient and light follows the multiplicative instead of threshold relationship. Also, since temperature was not included, this model is unsuitable for outdoor cultivation system.

These existing kinetic models were developed for the same domain, microalgae growth in wastewater; however, their applications are limited due to specific study conditions. Thus, a new kinetic model, which can be applied to broader conditions, is needed. Another limitation for current kinetic models for microalgae growth in wastewater is that these models fail to consider all important growth factors, including N, P, CO_2, light intensity, and temperature in the model development under wastewater conditions (Kumar et al., 2010). Furthermore, the previous studies did not consider different co-limitation effects of growth factors in the model framework. Lastly, most of the models for microalgae growth in wastewater were focused on the secondary effluent. According to Prescott (1968), green microalgae require higher N and P concentrations than other microalgal species. Besides, the use of high strength wastewaters is economically beneficial because such wastewaters are able to support higher algae biomass concentrations (Vasconcelos Fernandes et al., 2015) and consequently reduce reactor volume requirements and harvesting costs. Therefore, centrate from dewatering of anaerobic digested sludge as a side stream of municipal wastewater is a better source for microalgae cultivation because it contains very rich nutrients,

especially N and P, compared to other wastewaters. Yuan et al. (2012) and Halfhide et al. (2015) pointed out that using centrate for algae cultivation will be advantageous for wastewater treatment systems because the centrate, containing high N and P loading (up to 30% for N, 26-90% for P), flows back into the mainstream and negatively impacts the performance of biological treatment in typical advanced wastewater treatment plants (Fattah, 2012; Kotay et al., 2013).

In order to fill the gaps mentioned above, this study aims at developing an integrated co-limitation kinetic model for microalgae growth in wastewater, especially the centrate. This kinetic model considered all growth factors relevant to the centrate and different co-limitation effects (threshold and multiplicative) of selected growth factors in the model framework.

2.2 Methodology

2.2.1 Model Development

2.2.1.1 Model Factor Selection

Microalgae growth is a complex process that is affected by environmental factors and nutrition factors in aquatic systems. The environmental factors include illumination, CO_2 level, temperature, and pH, while nutrition factors consist of macronutrients (i.e., N, P, sulfur, potassium, and magnesium) and micronutrients (i.e., iron (Fe), manganese (Mn), cobalt (Co), zinc (Zn), boron (B), copper (Cu), nickel (Ni) and molybdenumand) (Juneja et al., 2013).

Considering wastewater as a culture medium for microalgae growth, not only the growth factors mentioned above but also other factors (such as predator) need to be considered. Wastewater typically contains a sufficient amount of macronutrients such as N and P, and micronutrients (Kumar et al., 2010), but the composition of nutrients and their concentrations vary with types of wastewater (Tchobanoglous et al., 2013). Thus, depending on wastewater types, the influence of the factors on microalgae growth will be different. In this study, the centrate was used

as the microalgae growth medium. Due to the characteristics of the centrate, several factors, including heterotrophic growth, heavy metals, predators, and pH, were excluded in the model. Since organic carbon in the centrate is poorly bioavailable (Yuan et al., 2012), heterotrophic growth was not considered. In terms of heavy metals, microalgae are typically tolerant to high level of heavy metals, such as Ti, Pb, Mg, Zn, Cd, Sr, Co, Hg, Ni, and Cu because polyphosphate in the algae enables storage of these metals (Narasimhan, 2010). As a result, microalgae are used for removal of heavy metals from aqueous solutions (He and Chen, 2014). Thus, heavy metals will not significantly affect microalgae growth.

Predators may play a role as a potential inhibiting factor for microalgae growth (Canovas et al., 1996; Umble and Ketchum, 1997). According to Arauzo (2003), over 2.5 mg L^{-1} un-ionized ammonia causes a significant decrease of the zooplankton (Predators) population. Since the centrate usually contains a high ammonia concentration (>300 mg L^{-1}) (Park et al., 2010; Tam and Wong, 1996; Yun et al., 1997), the inhibiting effect of predators is negligible. pH is one of the most relevant environmental factors that affect the growth of microalgae, and most microalgae prefer a neutral pH (Kumar et al., 2010). Since anaerobic digestion typically maintains neutral pH for methanogen, pH of the centrate is usually neutral which contains sufficient HCO_3^- alkalinity (Rajeshwari et al., 2000). According to Goldman et al. (1982), pH in the culture medium can be altered by a biological transformation of nitrogen species (e.g. NO_3^-, NH_4^+, and Urea). For anaerobically digested centrate, however, the pH change due to NH_4^+ uptake should be insignificant because sufficient HCO_3^- alkalinity is present in the medium (Goldman et al., 1982). Thus, pH is not considered as one of the growth factors in this study.

In terms of the environmental factors, CO_2, temperature, and light intensity are important for photosynthesis which is directly related to autotrophic microalgae growth. In terms of the

nutrition factors, N and P are the most important macronutrients for microalgae growth because they are cell elements. Therefore, by considering microalgae growth factors and constituents in centrate, CO_2, N, P, temperature, and light intensity were selected as major growth factors in the model development.

2.2.1.2 Model Framework Construction

In order to explain the effects of the selected factors on microalgae growth, this study proposed a new integrated co-limitation model framework (Eq. 2.1), which considers both multiplicative and threshold co-limitations. In terms of N and P, De Groot (1983) concluded that they follow the threshold relationship. Thus, in the model, it is assumed that the co-limitation of N and P on microalgae growth was based on the threshold theory, while the effect of other factors follows the multiplicative theory.

$$\mu = \mu_{max}\Big(\big(f(N), f(P)\big) \cdot f(CO_2) \cdot f(I) \cdot f(T)\Big) \tag{2.1}$$

where μ is the specific growth rate; μ_{max} is the overall maximum specific growth rate; *f(N)* is a function of nitrogen concentration; *f(P)* is a function of phosphorus concentration; *f(CO_2)* is a function of carbon dioxide concentration; *f(I)* is a function of light intensity; and *f(T)* is a function of temperature.

Since N is more limited than P based on the centrate characteristics, *f(P)* is eliminated from the overall rate expression, and the integrated model is reduced to Eq. 2.2.

$$\mu = \mu_{max}\big(f(N) \cdot f(CO_2) \cdot f(I) \cdot f(T)\big) \tag{2.2}$$

2.2.1.3 Rate Expression Selection for Individual Factors

Expressions of *f(N)*, *f(CO_2)*, *f(I)*, and *f(T)* were adopted from existing microalgae growth kinetic models considering only a single factor. The expressions of *f(N)* and *f(CO_2)* were selected based on the following assumptions; i) microalgae growth depends on N and aqueous CO_2

concentrations in wastewaters because intercellular N and C storages are insignificant for the growth (Andersen et al., 1991); ii) most of the bioavailable N sources in the centrate are in the form of ammonium (NH_4-N); iii) inhibition of N and CO_2 on microalgae growth may occur because of high concentrations of NH_3 in centrate (>300 mg L^{-1}) and CO_2 (>15%) (Park et al., 2010; Tam and Wong, 1996; Yun et al., 1997). Based on the assumptions above, the Andrews model was selected as the mathematical expression for *f(N)* and *f(CO_2)* to explain both limitation and inhibition effect. Although the Monod model is not able to describe the inhibition effect at high concentrations, it is widely used to describe microalgae growth under low and moderate substrate concentrations (N≤100 mg L^{-1}, CO_2 in mixture gas≤5%) with fewer kinetic parameters (Aslan and Kapdan, 2006; Goldman et al., 1974; Hsueh et al., 2009; Xin et al., 2010). Thus, the Monod model and the Andrews model were adopted for *f(N)* and *f(CO_2)* expressions under low to medium and high substrate concentrations, respectively.

In terms of selecting expressions for *f(I)*, the following assumptions were made: i) the cultivation systems contain high concentrations of microalgae because that is desirable for bioenergy feedstock production, and ii) microalgae cultivation systems are under outdoor conditions in order to reduce the energy cost from artificial illumination. Considering the first assumption, light limitation on microalgae growth may occur due to light attenuation caused by high density of the microalgae cells as well as high chromaticity of wastewaters. Among existing models considering light intensity, it was reported that the Chalker (1980) model was the best model to describe light limitations under low and medium light intensity conditions (Kurano and Miyachi, 2005). Considering the second assumption, photoinhibition for microalgae growth occurs during the central hours of the daylight period (García-Malea et al., 2006). According to Martínez et al. (2012), the Muller-Feuga (1999) model provided a good description of photoinhibition at

high light intensity. Thus, depending on the level of light intensity, the Chalker or Muller-Feuga model was adopted for *f(I)*. In terms of expressions for *f(T)*, the Arrhenius equation is most commonly used to describe the effect of temperature on microalgae growth (Béchet et al., 2013; Bissinger et al., 2008; Bordel et al., 2009; Eppley, 1972). Thus, in this study, the Arrhenius equation was used for *f(T)* and combined with expressions for light intensity since the temperature in cultivation systems will be affected by radiant heat (Morita et al., 2001).

The selected expressions were summarized in Table 2.1. In the integrated model, each factor has two possible expressions. Depending on the initial cultivation condition, one of expressions for each factor was selected for the integrated model. Since the models considering multi-factors with many parameters result in overfitting issues, therefore, applying one of the rate expressions depending on the condition could reduce the overfitting issue (Lee and Zhang, 2015).

Table 2.1 Overall expressions for the integrated kinetic model.

Factors	Model	Consideration	Rate expressions
N	Monod model	Limitation	$\mu = \boldsymbol{\mu_{max,NH4-N}} \frac{S_{NH4-N}}{\boldsymbol{K_{S,NH4-N}} + S_{NH4-N}}$
	Andrews model	Limitation and inhibition	$\mu = \boldsymbol{\mu_{max,NH4-N}} \frac{S_{NH4-N}}{\boldsymbol{K_{S,NH4-N}} + S_{NH4-N} + S_{NH4-N}^2/\boldsymbol{K_{i,NH4-N}}}$
CO_2	Monod model	Limitation	$\mu = \boldsymbol{\mu_{max,CO2}} \frac{S_{CO2}}{\boldsymbol{K_{S,CO2}} + S_{CO2}}$
	Andrews model	Limitation and inhibition	$\mu = \boldsymbol{\mu_{max,CO2}} \frac{S_{CO2}}{\boldsymbol{K_{S,CO2}} + S_{CO2} + S_{CO2}^2/\boldsymbol{K_{i,CO2}}}$
Light and Temperature	Chalker model combined with Arrhenius equation	Limitation	$\mu = \boldsymbol{\mu_{max,I}} \cdot \boldsymbol{\theta}^{T-20} \cdot \tanh(\frac{I_{av}}{\boldsymbol{I_K}})$
	Muller-Fuega model combined with Arrhenius equation	Limitation and inhibition	$\mu = \boldsymbol{\mu_{max,I}} \cdot \boldsymbol{\theta}^{T-20} \cdot \frac{2 * (1 - \boldsymbol{I_e}/\boldsymbol{I_K}) * (I_{av}/\boldsymbol{I_K} - \boldsymbol{I_e}/\boldsymbol{I_K})}{(1 - \boldsymbol{I_e}/\boldsymbol{I_K})^2 + (I_{av}/\boldsymbol{I_K} - \boldsymbol{I_e}/\boldsymbol{I_K})^2}$

2.2.2 Experimental Methods

2.2.2.1 Microalgae and Culture Medium

The Anaerobically Digested municipal sludge Centrate (ADC) was used as the culture medium in the experiments. The ADC was collected from the Northeast Water Reclamation Facility (located in Clearwater, FL). The characteristics of ADC are shown in Table 2.2. In order to prepare the ADC culture medium, glass fiber filters with pore size of 0.45 μm (Fisher Scientific; Pittsburgh, PA, G4) were used to remove particulates in the ADC. The filtered ADC was stored in a refrigerator at 4°C before the cultivation experiment.

Table 2.2 Characteristics of anaerobically digested sludge centrate.

Source	Average Concentration (mg L^{-1})
pH	7.81±0.15
Alkalinity	751±50
COD	811±74
TN	445±153
NH_4-N	397±145
NO_3-N	0.5±0.3
NO_2-N	Not detected
TP	238±59

Indigenous *Chlorella* sp. was harvested from a secondary clarifier at the Howard F. Curren Advanced Wastewater Treatment Facility in Tampa, FL. The *Chlorella* sp. was initially cultivated in the Bold 1 NV medium (Starr and Zeikus, 1993) and then cultivated in the medium containing 50% filtered ADC and 50% deionized water for adaptation before switching to 100% filtered ADC medium. The Chlorella sp. was inoculated at 22±1°C in a temperature-controlled room in 1L Erlenmeyer flasks with a working volume of 500 mL. The cultures were kept suspended by aeration (0.03% CO_2). A 24-hour continuous light (about 2000 lux) was provided by 13W fluorescent lamps.

2.2.2.2 Experimental Set-up

The experiments were conducted in 1.2 L batch-type photobioreactors containing 900 mL of the medium for 7-14 days. During the experiment, a sterile CO_2-air mixture (with a flow rate of 300ml/min) was supplied to the culture through a fine bubble diffuser. The reactors were illuminated by 13 W fluorescent lamps (24:0 h light-dark cycles) located outside of the reactor to provide the desired light intensity, and the reactors were located in a temperature-controlled room at 22±1°C. For each series of experiments, the initial microalgae concentration (expressed as Chl a) was kept constant around 0.7 ± 0.2 mg L^{-1}.

The standard cultivation conditions were: NH_4-N concentration of about 340 mg L^{-1}, light intensity of 5000 lux, and aeration with a CO_2 and air mixture gas (5% CO_2). In order to evaluate the effect of each selected factor, the conditions of the factor of interest was modified, while others were kept the same as the standard condition. The NH_4-N concentration in ADC was varied between 9-586 mg L^{-1} by dilution (The PO_4-P concentration was maintained by spiking the ADC with Monopotassium phosphate (KH_2PO_4)). The CO_2 and air mixture was varied between 0.003%-15% CO_2. Lastly, illumination was varied between 500-15000 lux. Due to radiation, culture temperature varied from 21°C to 39°C with varying illumination. All kinetic experiments were duplicated, and the samples were collected daily for 7-14 days.

2.2.2.3 Analytical Methods

Since the growth media is the real centrate, there is a possibility to contain bacteria in the centrate. According to Mara (2013), chlorophyll a concentration is a good measure of the amount of microalgae biomass and was used to represent microalgae biomass concentration in the study. Chlorophyll a was analyzed using the ethanol extraction method according to the Dutch Standard (NEN, 1981). For measurements of NH_4-N, NO_3-N, and alkalinity, samples were filtered through

0.45 μm membranes (No. 6876-2504, Whatman). NH_4-N was measured by a modified Willis et al. method (Kinyua, 2013), while NO_3-N and alkalinity were measured by Standard methods (APHA, 2012).

The pH was measured by a calibrated Orion GS9156 pH electrode meter (Thermo Fisher Scientific Inc., Waltham, MA). The temperature inside the reactor was measured using Liquid-in-Glass Partial Immersion Thermometers (Thermo Fisher Scientific Inc., Waltham, MA). Incident light intensity (I_0) from the exterior of each reactor was measured using a "30 light meter Onset® HOBO U12 data logger" (Pocasset, MA). Average irradiance (I_{av}) within each reactor was calculated using Eq. 2.3 (Grima et al., 1994; Martínez et al., 2012).

$$I_{av} = \frac{I_0}{k \cdot d \cdot X}(1 - exp(-k \cdot d \cdot X)) \tag{2.3}$$

where k is the attenuation rate (0.2 m^2 g^{-1}) (Juneja et al., 2013), d is the diameter of the reactor (m), and X is the microalgae cell concentration in the reactor (g m^{-3}).

2.2.3 Calibration and Validation

Based on the growth curve of *Chlorella* sp. (biomass concentrations vs. time), the specific growth rates were calculated using Eq. 2.4.

$$\mu_{measured} = \frac{\ln(X_2/X_1)}{t_2 - t_1} \tag{2.4}$$

where $\mu_{measured}$ is the specific growth rate calculated from experimental results (d^{-1}), X_2 and X_1 are the microalgae concentrations (mg L^{-1}) at maximum and initial microalgae concentration during exponential growth respectively, and t_2, and t_1 are the time (d) of maximum and initial microalgae concentration during exponential growth.

Parameters of the integrated model from Table 2.1 were calibrated sequentially to minimize overfitting issue. Figure 2.1 shows a flow chart of the process to determine the criteria for selecting

rate expressions and kinetic parameters for the integrated model. This process can be divided by two steps (Step1 and Step 2). Step 1 mainly focused on determination of kinetic parameters for selected rate expressions using experimental data. Step 2 is to determine limitation criteria and an overall maximum growth rate (μ_{max}).

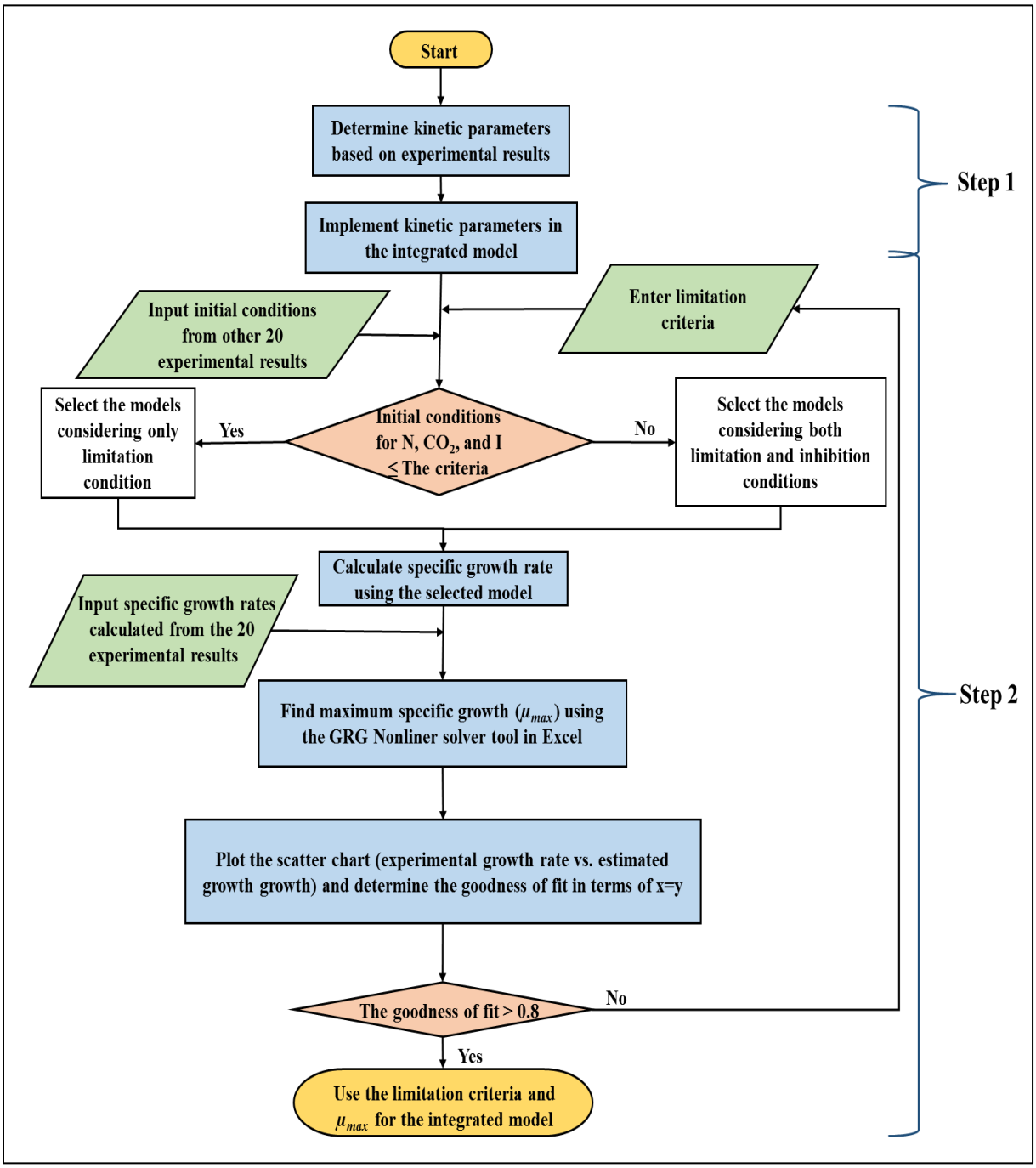


Figure 2.1 The process for determining the criteria and kinetic parameters for the integrated model.

In step 1, the kinetic parameters were determined by fitting the selected models to experimental data. These parameters were estimated by minimizing an Objective Function (*OF*, Eq. 2.5) using the Solver add-in in Microsoft Excel (Generalized Reduced Gradient Nonlinear solver tool):

$$OF = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(\mu_{measured} - \mu_{model})^2} \quad (2.5)$$

where μ_{model} is the specific growth rate estimated from the models (d^{-1}) and n is the number of the specific growth rates ($\mu_{measured}$) calculated based on the experimental data. In step 2, the criteria to select the appropriate expression between two options for each factor was selected, and then the μ_{max} in the integrated model was determined using other 20 data points. In this step, the kinetic parameters determined in step 1 were applied. The range of the criteria for different growth factors was adopted from the literature, including 150~300 mg $L^{-1}$for N, 40~220 mg L^{-1} for aqueous CO_2, and 70~200 μmol photon m^{-2} s^{-1} for light intensity. Once the model was calibrated, predicted growth rates using the integrated kinetic model were compared to calculated growth rates from another set of experimental data to evaluate whether the model can represent the real system. The goodness of fit (R^2) of more than 0.8 is generally considered as a good degree of agreement between simulation and experimental data in the case of microalgae growth simulations (Hill and Lincoln, 1981).

2.3 Results and Discussion

2.3.1 Microalgae Growth in Centrate

Figure 2.2 shows microalgae growth under different culture conditions with varying initial NH_4-N concentrations, aqueous CO_2 concentrations and light intensities. In this study, *Chlorella* sp. showed effective growth in the real centrate. Li et al. (2011) reported that microalgae (*Chlorella*

vulgaris) growth rates in centrate are comparable with those using different types of municipal wastewater (e.g., activated sludge extract, primary settled sewage, and primary clarifier effluent). In addition, they observed the highest final biomass of the microalgae grown in the centrate among different wastewaters.

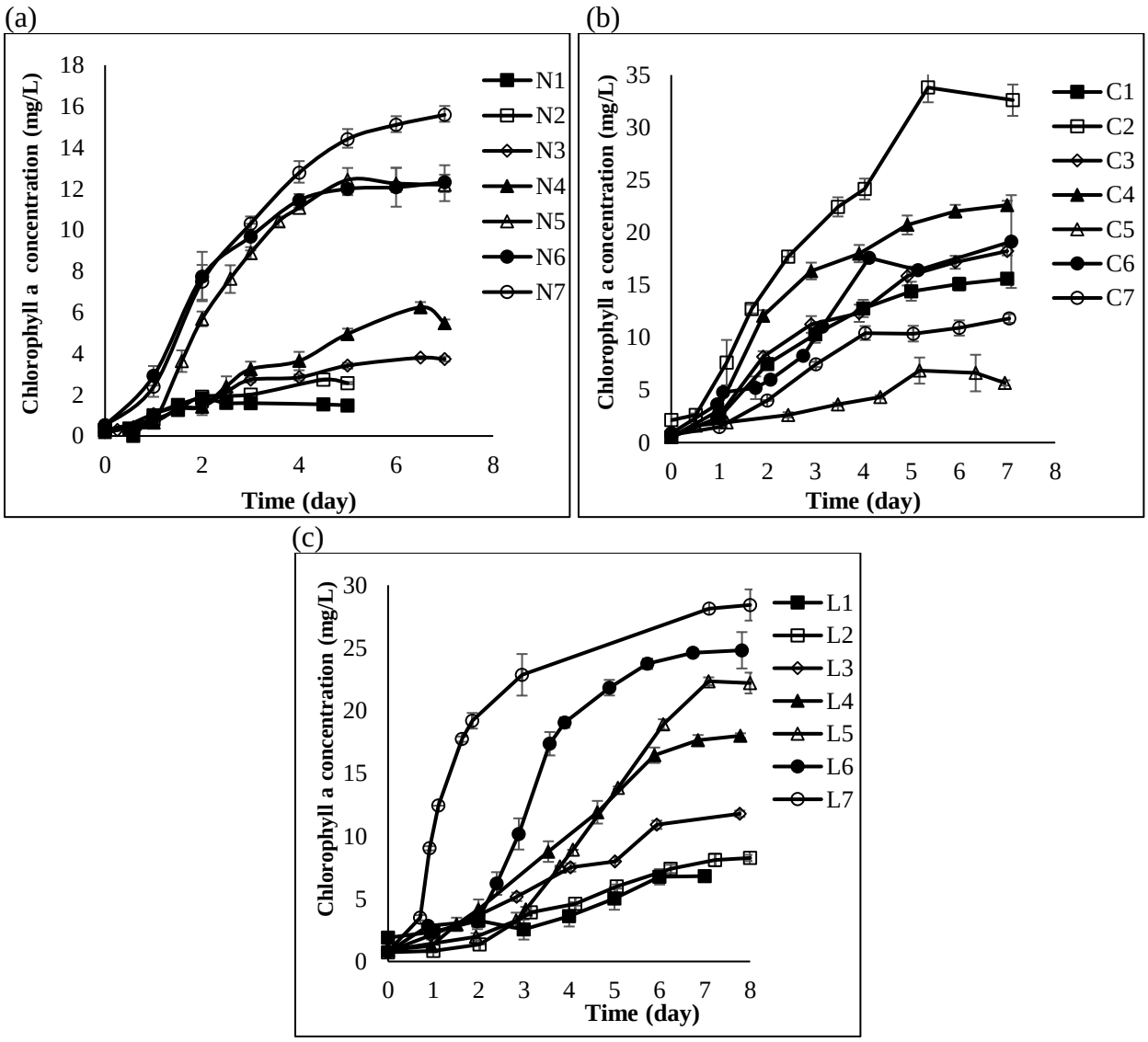


Figure 2.2 Total microalgae chlorophyll a concentrations under different growth conditions: (a) Varying initial NH_4-N concentrations, (b) Varying initial aqueous CO_2 concentrations, and (c) Varying initial light intensity with temperature. Note: N1=9.9 mg L^{-1}, N2=27.1 mg L^{-1}, N3=47.2 mg L^{-1}, N4=88.8 mg L^{-1}, N5=139.2 mg L^{-1}, N6=226.1 mg L^{-1}, N7=586.1 mg L^{-1}, C1=8.5 mg L^{-1}, C2=12.9 mg L^{-1}, C3=19 mg L^{-1}, C4=26 mg L^{-1}, C5= 92 mg L^{-1}, C6=122 mg L^{-1}, C7=200 mg L^{-1}, L1=6.8 μmol photon m^{-2} s^{-1}, L2=12.2 μmol photon m^{-2} s^{-1}, L3=17.6 μmol photon m^{-2} s^{-1}, L4=27 μmol photon m^{-2} s^{-1}, L5=77 μmol photon m^{-2} s^{-1}, L6=86.4 μmol photon m^{-2} s^{-1}, and L7=459 μmol photon m^{-2} s^{-1}.

Figure 2.2 (a) shows the changes in chlorophyll a concentration at various initial NH_4-N concentrations ranging from 9.9-586 mg L^{-1}. It was observed that the final chlorophyll a concentration increased from 1.8 mg L^{-1} to 15.8 mg L^{-1} with the increase in initial NH_4-N concentration. Previous study of Aslan and Kapdan (2006) observed similar results of the increase of final chlorophyll a concentrations as a result of the increase of initial NH_4-N concentrations using *Chlorella vulgaris*.

Figure 2.2 (b) shows the variation of chlorophyll a concentration at different aqueous CO_2 concentrations. The culture with 12.9 mg L^{-1} aqueous CO_2 resulted in the highest final chlorophyll a concentration. In the study of Mortensen and Gislerød (2015), 140 mg L^{-1} dissolved CO_2 was an optimal condition for growth of *Chlorella sorokiniana* in artificial growth medium at 28°C. Our optimum aqueous CO_2 concentration for microalgae growth is much lower than that of Mortensen and Gislerød (2015) because cultivation conditions such as temperature and growth medium are different. According to Beardall and Raven (2004), increases in cultivation temperature result in increased metabolic activity and growth of microalgae. Our cultivation temperature was 22±1°C, which is 6°C lower than the study of Mortensen and Gislerød (2015) so that lower temperature leads to lower metabolic activity which requires less CO_2. In addition, Mortensen and Gislerød (2015) cultivated the microalgae in artificial growth medium containing the essential nutrients under the balanced condition for optimum growth. However, the centrate contains imbalanced nutrients for microalgae growth which may cause nutrient limitation or inhibition.

Figure 2.2(c) shows the result for variation of chlorophyll a with time at different initial light intensities. Due to radiation from light, the temperature of culture increased from 21°C to 40°C with the increase in initial incident light intensity. In Figure 2.2 (c), L5 (under light intensity of 77 μmol photon m^{-2} s^{-1}) showed the longer lag phase than other conditions, which is mainly due

to unhealthy inoculum. The final chlorophyll a significantly increased from 6.8 mg L^{-1} to 28 mg L^{-1} with the increase in the light intensity as well as temperature, and the culture with 459 μmol photon m^{-2} s^{-1} (40°C) resulted in the highest final chlorophyll a concentration. As shown in Figure 2.2, the initial NH_4-N and aqueous CO_2 concentrations as well as initial light intensity significantly affect total chlorophyll a concentration of microalgae, which is in agreement with the results of previous studies (Cheirsilp and Torpee, 2012; Choi and Lee, 2013; Yun et al., 1997).

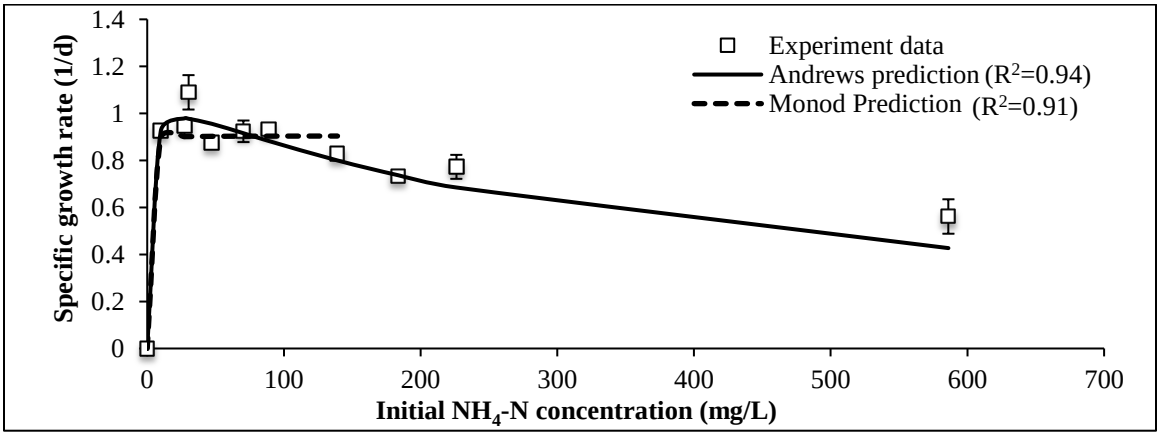
Based on the experimental data during the exponential growth stage in the growth curves of *Chlorella* sp. (chlorophyll a concentrations vs. time), the specific growth rates were calculated using Eq. 2.4. Under the different initial NH_4-N concentrations, the specific growth rates were varied from 0.56 to 1.1 d^{-1}. When initial CO_2 concentrations varied, the specific growth rates changed from 0.76 to 1.18 d^{-1}. Under light intensity conditions of 6.8-459 μmol photon m^{-2} s^{-1}, the specific growth rates varied from 0.22 to 1.73 d^{-1}. Compared to studies of Lam and Lee (2012) and Li et al. (2011), the growth rates in this study are within the ranges of the previous studies.

2.3.2 Parameter Determination

Figure 2.3 shows the observed $\mu_{measured}$ and fitted μ as a function of initial NH_4–N concentrations, initial aqueous CO_2 concentrations, and initial I_{av}. It was observed that the Monod and Chalker expressions produced a good agreement with experimental data at low and moderate substrate and light conditions, respectively (NH_4–N ≤ 150 mg L^{-1}, CO_2 ≤ 50 mg L^{-1}, and light intensity ≤ 90 μmol photon m^{-2} s^{-1}). The Andrews and Muller-Feuga expressions fit better to the growth rate data at all ranges of NH_4–N and CO_2 concentrations and light intensity; however, both require fitting one more parameter. The Monod and Chalker expressions are kept in the integrated model because of their simple mathematical formula and fewer parameters to be determined. The difference should be made, however, in applying these expressions because they were developed

based on different assumptions. The Monod and Chalker expressions were developed considering non-inhibitory conditions, while the Andrews and Muller-Fuega expressions were developed considering both limitation and inhibitory conditions.

(a)



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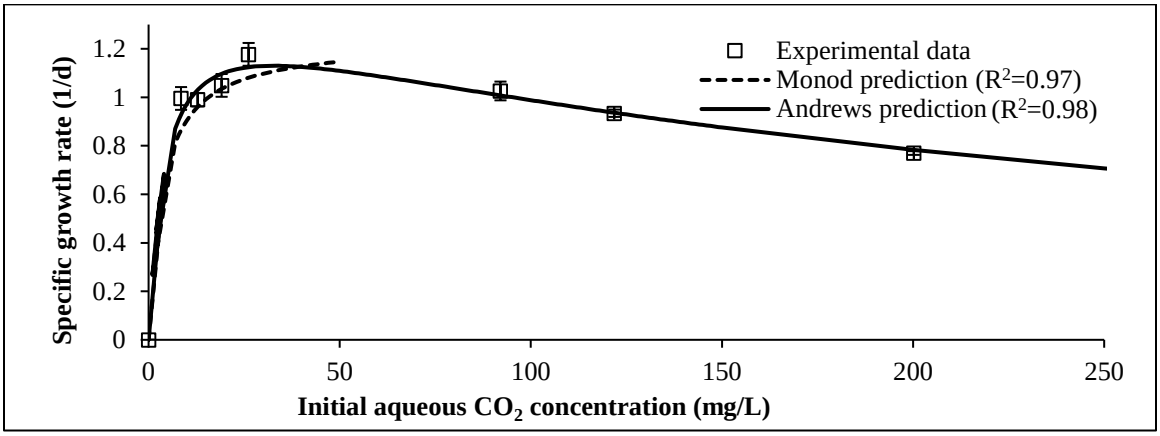

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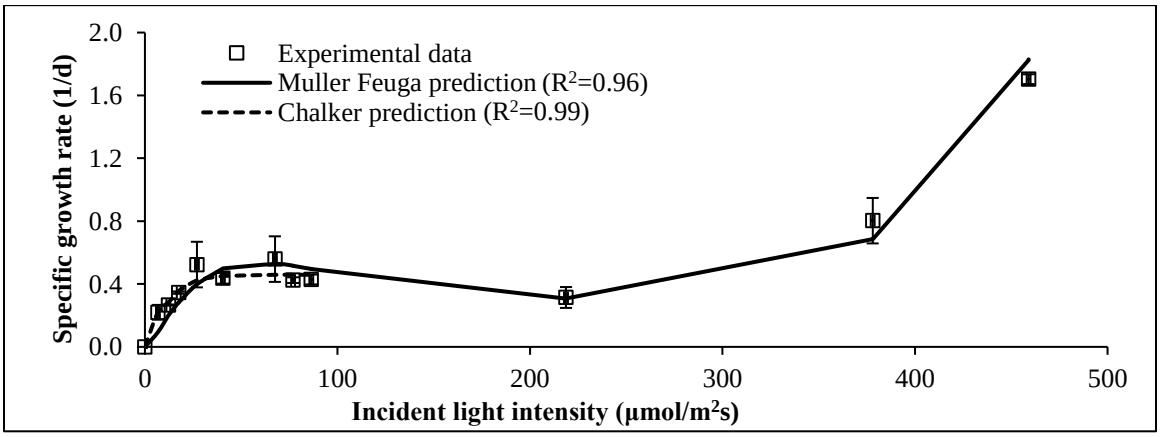


Figure 2.3 Specific growth rate as a function of growth factors: (a) Growth rate vs. NH_4-N concentration, (b) Growth rate vs. CO_2 concentration, and (c) Growth rate vs. light intensity.

Estimated kinetic parameter values are shown in Table 2.3 and compared to values obtained from literature. The half saturation constant in the Monod expression for N was comparable with the values from Kim et al. (2013). They studied N and P removal from municipal secondary wastewater effluent by *Chlorella vulgaris* and determined the maximum specific growth rate (μ_{max}, h^{-1}) and the half saturation coefficient ($K_{S,N}$, mg L^{-1}) in the Monod equation to be 0.01245 h^{-1} (0.299 d^{-1}) and 0.0696 mg L^{-1}, respectively.

Table 2.3 Calibrated model parameters. Note: [1] Kim et al. (2013), [2] Novak and Brune (1985), [3] Ketheesan and Nirmalakhandan (2013), [4] Kurano and Miyachi (2005), and [5] Li et al., (2011).

Factors	Applicable ranges	Models	Parameters	Comparison
N	NH_4-N ≤150 mg L^{-1}	Monod model	$K_{s,N}$=0.1 mg L^{-1}	μ_{max}=0.01245 h^{-1} $K_{S,N}$=0.0696 mg L^{-1} [1]
	NH_4-N >150 mg L^{-1}	Andrews model	$K_{s,N}$ =1.78 mg L^{-1} $K_{i,N}$=364 mg L^{-1}	Best fit value
CO_2	Aqueous CO_2≤50 mg L^{-1}	Monod model	$Ks,_{CO2}$=3.60 mg L^{-1}	μ_{max}=0.014-0.07 h^{-1} (0.336-1.68 d^{-1}) $Ks,_{CO2}$=0.03-0.36 mM (1.32-15.8 mg L^{-1}) [2]
	Aqueous CO_2>50 mg L^{-1}	Andrews model	$Ks,_{CO2}$=4.26 mg L^{-1} Ki=250 mg L^{-1}	μ_{max}=2.0 d^{-1} $Ks,_{CO2}$=0.0009 mol/m^3 (0.04 mg L^{-1}) Ki=180 mol/m^3 (7922 mg L^{-1}) [3]
Light and Temperature	Light intensity≤90 μmol photon m^{-2} s^{-1}	Chalker model combined with Arrhenius equation	I_K=16.98 μmol photon m^{-2} s^{-1} Θ=1.35	μ_{max}=0.115 h^{-1} (μ_{max}=2.76 d^{-1}) I_K=150 μmol photon m^{-2} s^{-1} [4]
	Light intensity>90 μmol photon m^{-2} s^{-1}	Muller-Feuga model combined with Arrhenius equation	I_e=1 μmol photon m^{-2} s^{-1} I_K=54.7 μmol photon m^{-2} s^{-1} Θ= 1.16	Best fit value
Maximum growth rate (d^{-1})			μ_{max}=0.7 d^{-1}	μ_{max}=0.677 d^{-1} [5]

The I_K of the Chalker model in our study was lower than the value of Kurano and Miyachi (2005). They examined the effect of light intensity on microalgae growth in the artificial growth medium, and reported that the growth rate of *Chloroccum littorale* is in accordance with Chalker model (μ_{max}=0.115 h^{-1} and I_k =150 μmol photon m^{-2} s^{-1}). Lower I_K value in our study is attributed to the lower light intensity range used in the experiment (0-90 μmol photon m^{-2} s^{-1}) than that of the Kurano and Miyachi's study (2.3-1060 μmol photon m^{-2} s^{-1}). In addition, the differences for cultivation temperature, microalgal species, and nutrient conditions may also cause the difference.

As shown in Table 2.3, the value of $K_{S,CO2}$ for the Monod model was in agreement with Novak and Brune (1985), who reported that $K_{S,CO2}$ for *Chlorella* sp. varies in a range of 0.03–0.36 mmol L^{-1} (1.32–15.8 mg L^{-1}). In terms of the Andrews model for CO_2, the value of $K_{S,CO2}$ is higher while the value of K_I is lower than those in the previous study of Ketheesan and Nirmalakhandan (2013). They adopted the Andrews model to explain the effect of CO_2 on growth of *Scenedesmus* sp. and *Nannochloropsis salina* in the airlift-raceway reactor using the artificial growth medium. The difference is probably caused by different microalgal species (*Scenedesmus* sp. and *N. salina* in Ketheesan and Nirmalakhandan (2013) vs *Chlorella* sp. in our study), different growth medium (artificial growth medium in Ketheesan and Nirmalakhandan (2013) vs real centrate in our study). Growth condition as well as microalgal species affect the growth kinetics (Panikov, 1995).

The estimated kinetic parameters for the selected expressions were applied, and then the overall maximum growth rate for the integrated model as shown in Eq. 2.2 was determined using other experimental data. The overall maximum growth rate was 0.7 d^{-1} (R^2=0.82) which is comparable to that (0.677 d^{-1}) from Li et al. (2011) (growth of *Chlorella* sp. in centrate). The R^2s between the experimental and the predicted growth rates at different culture conditions are all above 0.8 (Figure 2.3).

2.3.3 Model Performance

Figure 2.4 presents the comparison of the growth rates predicted by the integrated model (μ_{model}) versus the calculated growth rates ($\mu_{measured}$) based on another 20 sets of experimental data that were not used for model calibration. The result in Figure 2.4 apparently shows that most of data points are closer to the line of y=x and the R^2 of the linear trend line of y=x was 0.91. This result indicates that the predicted growth rates are close to measured growth rate and the integrated model developed is able to predict the microalgae growth rate in centrate.

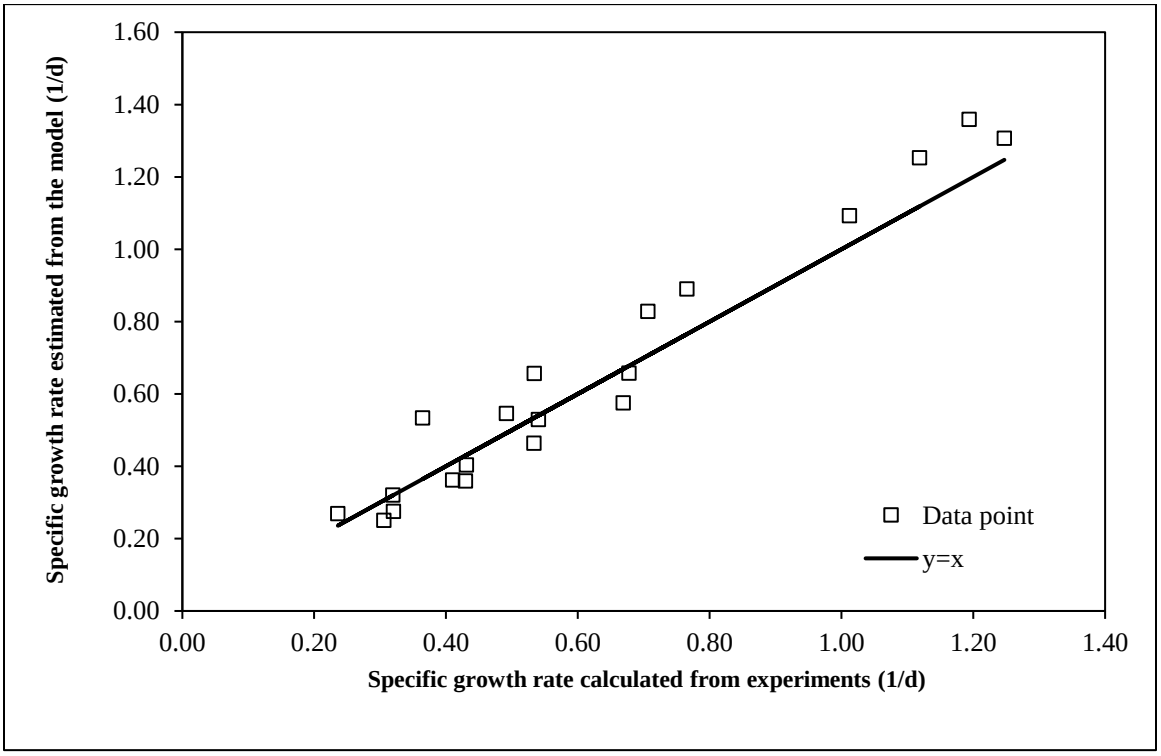


Figure 2.4 Plot of the predicted specific growth rates versus calculated growth rates based on experimental data.

The integrated model was also tested using published data from the studies that applied wastewaters as a growth medium. The N:P ratios of these wastewaters were below 16, which indicate N is more limited than P for microalgae growth and meets the requirement for Eq. 2.2. Figure 2.5 shows the microalgae biomass concentration predicted using our model and the microalgae biomass concentration obtained from literature. In the study of Yuan et al. (2012),

Chlorella sp. was cultivated in the centrate obtained from a lab-scale anaerobic digestion reactor with the initial cultivation conditions of 208.2 mg N L^{-1}, 0.02 pCO_2 (29.6 mg CO_2 L^{-1}), 9000 lux (121.5 µmol m^{-2} s^{-1}), and 20 °C. The model used the Andrews expression for N, Monod expression for CO_2, and Muller-Feuga expression for light intensity combined with Arrhenius equation for temperature to predict the microalgae growth under given conditions.

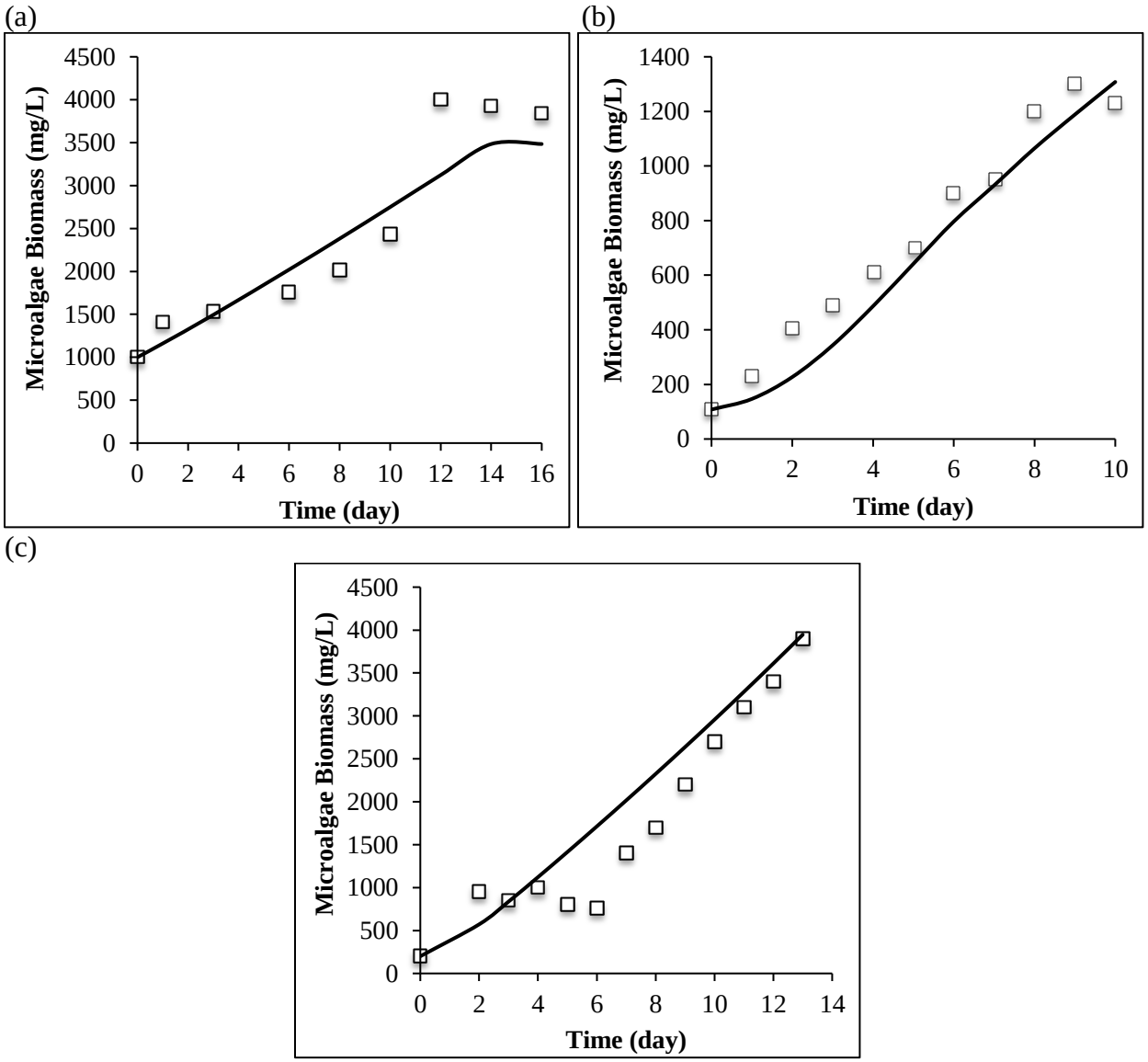


Figure 2.5 Published experimental data and modeling results for validation: (a) Data obtained from Yuan et al. (2012) (R^2=0.87), (b) Data obtained from Cabanelas et al. (2013) (R^2=0.93), and (c) Data obtained from Cheng et al. (2015) (R^2=0.86). Note: symbol (□): experimental data, line (-): Model simulation.

In Cabanelas et al. (2013)'s study, *Chlorella vulgaris* was grown in anaerobically digested municipal sludge centrate obtained from their local wastewater treatment plant with the initial cultivation conditions of 130 mg N L^{-1}, 0.01 pCO_2 (14.8 mg CO_2 L^{-1}), 150 µmol m^{-2} s^{-1}, and 20 °C. In this case, the integrated model combining the Monod expression for N, Monod expression for CO_2, Muller-Feuga model for light intensity, and Arrhenius equation for temperature was applied. As shown in Figure 2.5 (a) and (b), the model was able to describe the microalgae growth trend during the experiment period (R^2=0.85 in figure 2.5 (a), R^2=0.92 in figure 2.5 (b)).

Figure 2.5 (c) shows the simulation result compared with the experimental data obtained from Cheng et al. (2015). Cheng et al. (2015) used *Chlorella pyrenoidosa* to remove the nutrients from undiluted anaerobically digested effluent of swine manure. Their initial conditions were 1093 mg N L^{-1}, 0.15 pCO_2 (222 mg CO_2 L^{-1}), 6000 lux (81 µmol $m^{-2}s^{-1}$), and 27 °C. Thus, the integrated model was constructed by integrating the Andrews expressions for N and CO_2, Chalker expression for light intensity, and Arrhenius equation for temperature. The model was not able to capture the growth trend between day 2 and day 6; however, the result shows that the overall model simulation agrees with the experimental data to a good degree (R^2=0.88). Although the wastewater used in Cheng et al. (2015) is not the same as anaerobically digested municipal sludge centrate, the model can still describe the overall microalgae growth. Thus, the integrated model framework developed in this study is applicable to *Chlorella* species grown in wastewaters that have N:P ratios below 16.

2.3.4 Model Limitations

Although the integrated model developed is useful to estimate microalgae growth in wastewaters, the model contained several limitations for application. First, the parameters provided in this study are obtained from the experiments using the centrate and indigenous

Chlorella sp. According to Liu and Zachara (2001), kinetic parameters estimated from batch experimental data result in major uncertainties of the model predictions. Careful manipulation of experimental conditions can improve accuracy of kinetic parameters and therefore reduce uncertainties of model predictions. Since the experimental design of 24:0 h light-dark cycle was used in this study, the kinetic parameters determined for light intensity may not be applicable for cultivation conditions with different light-dark cycles. In addition, the estimated kinetic parameters for microalgae growth may be different, depending on the sources of the wastewater or microalgae species. Thus, in order to increase the usability of the model as well as to improve the model prediction, the model should be tested for other types of wastewaters and the calibration may be required for different types of wastewaters. Second, appropriate rate expressions for P in the integrated model need to be investigated. In this study, microalgae growth in the centrate was not limited by P so that P was not considered as a major factor in the final growth kinetic model. Thus, developing the robust integrated model would require suitable rate expressions of P for future applications. In addition, organic carbon needs to be considered as growth factors in order to explain microalgae growth in wastewater containing organic carbon (such as effluent of primary wastewater treatment), since microalgae (e.g., *Chlorella* sp.) have an ability to use organic carbon as a carbon source for their growth. Future research may focus on these limitations to improve the applicability of the model to various types of wastewaters.

2.4 Conclusions

To describe the microalgae growth in anaerobically digested municipal sludge centrate, an integrated co-limitation kinetic model was constructed by incorporating N, CO_2, light, and temperature factors. The model framework combining threshold and multiplicative theories was able to explain the relationship of the selected factors on microalgae growth. The model was

calibrated using experimental data from lab scale batch reactors cultivating *Chlorella* sp. in the centrate and validated through the experimental data in this study as well as data obtained from literature. The model developed was able to predict the microalgae growth rate well for all the growth conditions investigated. This model can be used in bioreactor design as well as process control and optimization of microalgae cultivation systems integrated with wastewater.

CHAPTER 3: KINETIC PARAMETER ESTIMATION MODEL FOR ANAEROBIC CO-DIGESTION OF WASTE ACTIVATED SLUDGE AND MICROALGAE

3.1 Introduction

Anaerobic digestion technology has been used in waste management for several purposes such as waste stabilization, solids reduction, and energy production (Angelidaki et al., 2003; Kythreotou et al., 2014). With the increasing interest in protecting environments and producing renewable energy, this technology becomes more popular due to its ability to produce biogas from waste (Kythreotou et al., 2014). However, anaerobic digestion of some substrates such as waste activated sludge, agricultural waste, and microalgae results in low biogas yield, because the substrate has low organic loadings (low carbon content) and high ammonia concentrations that negatively impact on the activity of methanogens during anaerobic digestion (Mata-Alvarez et al., 2014). Anaerobic co-digestion, which is the simultaneous digestion of two or more substrates, could be a feasible option not only to overcome this drawback by supplying missing nutrients from co-substrates and diluting the potential toxic substances, but also to stimulate synergistic effects on microorganisms (Mata-Alvarez et al., 2000). Many substrates, including animal waste, sewage sludge, municipal organic solid waste, agricultural waste, fats, oil, grease, and microalgae have been used for co-digestion (Mata-Alvarez et al., 2014). In particular, studies on anaerobic co-digestion using microalgae have been increased for the last decade because microalgae have an ability to treat wastewater with high biomass productivity (Pittman et al., 2011). Due to this ability,

[2] This chapter is based substantially on and reprinted with permission from: "Lee, E., Cumberbatch, J., Wang, M. & Zhang, Q. (2017). Kinetic parameter estimation model for anaerobic co-digestion of waste activated sludge and microalgae *Bioresource Technology*, 228, 9-17".

microalgae have been used for nutrient recovery in nutrient rich wastewater such as rejecting water integrated with microalgae cultivation and subsequent production of biogas from the co-digestion using Waste Activated Sludge (WAS) and microalgae can be one of the most promising options for renewable energy production at wastewater treatment plants (Ajeej et al., 2015; Wang et al., 2016).

Anaerobic co-digestion has the same mechanism as anaerobic digestion that consists of a series of biological conversion processes in which multiple microorganisms break down biodegradable organic substances, and these processes are described by four major steps, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Batstone et al., 2002; Gavala et al., 2003; Vavilin et al., 2008). It is generally accepted that hydrolysis and methanogenesis are rate limiting steps in the anaerobic digestion process (Gavala et al., 2003; Ariunbaatar et al., 2014). Due to enzymatic activity by hydrolytic bacteria to break down the large organic matters, hydrolysis is considered to be a slow reaction. On the other hand, methanogenesis is considered as another rate limiting step, because methanogenic bacteria require complex environmental conditions that are hard to maintain in digesters. For example, nitrogen contents between 3.5 and 8.7% in the substrates may result in methanogenesis inhibition (Cotsta et al., 2012). When the pH drops below 7.0 as a result of fast acidogenesis and acetogenesis steps, the activity of the methanogens is inhibited (Schwede et al., 2013). For the co-digestion of microalgae and WAS, hydrolysis and methanogenesis can be also considered as the rate-limiting steps because microalgae affect these steps (Costal et al., 2012). For instance, a hemicellulose composition of the microalgae cell wall impacts on the hydrolysis of the co-digestion (Northcote et al., 1958; Wang et al., 2013). Also, a high ammonia concentration resulting from degradation of protein content in microalgae negatively affects the methanogenic bacteria activity (Mairet et al., 2011).

The rates of these two steps have been described by different kinetic models, such as the first-order kinetic model, Monod model, and Andrews model. (Kythreotou et al., 2014). Among these models, the first-order kinetic model was mostly used to explain the rate of hydrolysis, whereas the Monod model was commonly applied in kinetic modeling of methanogenesis. Vavilin et al. (2008) reviewed existing kinetic models for the hydrolysis of particulate organic materials in anaerobic digestion. For anaerobic digestion of complex organic substrate, they suggested a modified first-order kinetic model taking into consideration of non-biodegradable fraction of the substrate. In addition to improving the rate expression of the kinetic models, the determination of the kinetic parameters is critical for the overall model prediction.

The kinetic parameters are usually obtained from kinetic studies using an experimental approach (Lübken et al., 2015). This approach provides accurate kinetic information under specific conditions, but it requires time, energy, labor, and cost to obtain the results. There are many kinetic studies for anaerobic digestion, especially anaerobic digestion of sludge from wastewater treatment plant which has been well documented by Gavala et al. (2003). Based on the previous kinetic studies, it is found that majority of the studies focused on single substrates and limited studies dealt with determining the kinetic parameters for co-digestion. Costal et al. (2012) investigated methane production potential of anaerobic co-digestion of *Ulva* sp. and WAS in batch mode at mesophilic conditions. The parameters of the first-order kinetic model for different ratios of co-substrates were determined in the study (Costal et al., 2012). Neumann et al. (2015) studied anaerobic co-digestion of lipid-spent *Botryococcus braunii* with WAS and glycerol. They also determined the kinetic parameters for the first-order kinetic model under different ratios of the co-substrates. Zen et al. (2015) evaluated the technical feasibility of anaerobic co-digestion of mixed microalgae and food waste in batch tests and explained the kinetics of methane production using

the first order kinetics. The results from these prior studies showed that kinetic parameter values were different between single and multiple substrates. Depending on a ratio of co-substrates on a volatile solid basis (or percentage), the kinetic parameters for the co-digestion can be quite different. In addition, the kinetic information for co-digestion of WAS and microalgae was very limited. Extensive experiments therefore need to be conducted in order to obtain kinetic parameters under different ratios of co-substrates.

This study aims at providing an alternative approach for estimating the kinetic parameters for co-digestion of microalgae and WAS under different ratios of co-substrates with limited kinetic experiments. The proposed kinetic parameter estimation models considered key factors which are ratios of co-substrates and the kinetic parameters for the single substrate. Among the existing kinetic models, the most applicable ones were selected - the modified first-order kinetic model for hydrolysis and the Monod model for methanogenesis (McCarty and Mosey, 1991; Vavilin et al., 2008). To demonstrate the applicability of the parameter estimation models, the models were applied to the published data from literature.

3.2 Methodology

3.2.1 Experimental Method

3.2.1.1 Microalgae Cultivation

Indigenous *Chlorella sp.* was cultivated in 2L batch glass photo-bioreactors in two times diluted real centrate. The enrichment and identification of the algal species was done as described in Halfhide et al. (2015). The centrate was collected from the Northeast Water Reclamation Facility, NWRF (located in Clearwater, FL), which contains 397±145 mg NH_4^+-N/L and 238±59 mg TP/L. In order to remove particles, the centrate was filtered through glass fiber filters (Fisher Scientific, USA) with pore size of 0.45 μm. The detailed characteristics and preparation of the

centrate were described in Lee and Zhang (2016). The reactors were maintained at 22±1°C in a temperature-controlled room. The cultures were kept suspended by aeration (0.03% CO_2). A 24 h continuous light (about 9000 lux) was provided by 13W fluorescent lamps.

3.2.1.2 Anaerobic Digestion Reactor Set-up

Batch-type anaerobic digestion experiments were performed in duplicates of 100 mL glass serum bottles with a working volume of 40 mL for 20 days. The reactors were maintained at 35°C and manually mixed twice each day. Anaerobic digested sludge and WAS were collected from NWRF. The anaerobic digested sludge was used as inoculum for the tests. The waste activated sludge was prepared by gravity setting or centrifugation, while the microalgae were harvested by centrifugation (3000 rpm, 15 minutes), in order to reach targeted Volatile Solids (VS) concentrations (5%). The characteristics of WAS, microalgae, and inoculum are shown in Table 3.1. To evaluate the effect of varying microalgae and WAS ratios on digestion performance, microalgae and WAS were added to the reactors to achieve the following mass (VS) composition: 100% WAS, 5% microalgae with 95% WAS, 10% microalgae with 90% WAS, 25% microalgae with 75% WAS, 40% microalgae with 60% WAS, 50% microalgae with 50% WAS, 75% microalgae with 25% WAS and 100% microalgae. A Substrate to Inoculum ratio (S/I) of 1 g VS/g VS was used for all experiments. Each bottle was purged with N_2 gas before sealing to remove oxygen.

Table 3.1 Characteristics of waste activated sludge, microalgae, and inoculum.

Parameters	Microalgae	Waste activated sludge	Anaerobic inoculum
TS (g/L)	76.5±3	21.1±1.2	26.7±4.5
VS (g/L)	48.7±1.8	15.2±0.8	18.8±3
COD (g/L)	73.8±0.2	20.9±0.6	11.4±0.9
TN (mg/L)	1120±57	1590±74	739±20
TP (mg/L)	136±13	272±19	562±18

3.2.1.3 Analytical Methods

Total Chemical Oxygen Demand (COD), soluble COD, ammonium (NH_4^+-N), Total Solids (TS), VS, pH, biogas volume, methane content of the biogas were measured in this study. Total and soluble CODs were measured according to Standard Methods (5200B) using Orbeco-Hellige MR COD kits (Kit number 2420711, testing Range 0-1500mg/L). Standard Methods were used for TS and VS (Method 2540), pH (Method 2320B) measurements (APHA, 2012). Measurement of nitrogen in NH_4^+-N was adapted from a modified Willis et al. (1996) method by Kinyua (2013). Samples filtered through 0.45µm membrane filters (Fisherbrand™ General Filtration Membrane Filters, USA) were collected for soluble COD and NH_4^+-N analysis. Biogas volume was manually measured from the headspace of each digester by injecting a 50 mL glass syringe (Poulten and Graf Ltd., Germany) (Ashekuzzaman, and Poulsen, 2011; Wang et al., 2016). At each sample event, methane content in the biogas were measured through liquid displacement of CO_2 dissolved in alkaline solution (Ergüder et al., 2001).

3.2.2 Kinetic Models Applied

The concept of hydrolysis generally includes disintegration, solubilization and enzymatic hydrolysis as described in most of the literature (Vavilin et al., 2008; Batstone et al., 2002). The modified first-order kinetic model includes non-biodegradable fraction of the substrate, which are able to account for slow or non-degradable materials in the substrate (Vavilin et al., 2008). The adopted modified first-order kinetic model is described;

$$r_{hyd} = -k_{hyd} \cdot (S_P - \beta S_{P0}) \tag{3.1}$$

where r_{hyd} is the rate of hydrolysis, kg m^{-3} d^{-1}, S_p is the particle substrate concentration, kg m^{-3}, S_{p0} is the initial particle substrate concentration, kg m^{-3}, k_{hyd} is the first-order rate coefficient, d^{-1}, and

β is the non-biodegradable fraction of the substrate. In batch mode, the differential equation was written as follows:

$$\frac{dS_P}{dt} = r_{hyd} \tag{3.2}$$

Methanogenesis is the most sensitive step for anaerobic digestion process and the rate is described by the Monod type model (Lawrence and McCarty, 1970; Pavlostathis et al., 1986);

$$\frac{dM}{dt} = r_m = \frac{k_m \cdot V}{K + V} \tag{3.3}$$

where M is the methane production, mL g^{-1} VS, r_m is the rate of methane production, mL g^{-1}VS d^{-1}, K is the half saturation coefficient, mg L^{-1}, V is the Volatile Fatty Acid (VFA) concentration, mg L^{-1}, k_m is the maximum substrate utilization rate, mL g^{-1} d^{-1}.

In batch system such as Bio-Methane potential (BMP) assay, the ratio of S/I is an essential parameter that is able to affect the accumulation of VFA as well as the production of methane (Alzate et al., 2012). It is often observed that the digestion was inhibited by accumulation of VFA at a high S/I ratio (Alzate et al., 2012; Zhao et al., 2014). Zhao et al. (2014) reported that there was no sign of VFA inhibition for anaerobic digestion of microalgae at the S/I ratio $\leq$ 1.0. According to Costal et al. (2012) who studied the co-digestion of WAS and microalgae with S/I ratio of 2.8, it was reported that there was no inhibition of methanogenesis from accumulation of VFA, because its concentrations were below 50 mg L^{-1}. Since the S/I ratio of this study was below 2.8, inhibition of methanogenesis was therefore not considered. In anaerobic digestion, methane production is proportional to produced VFA (Rahman et al., 2013; Kamalak et al, 2002) (Eq. 3.4).

$$M_t - M = \alpha \cdot V \tag{3.4}$$

where α is the conversion coefficient ($\alpha = M_t/V_t$), V_t is the total VFA concentration, mg L^{-1}, V is the VFA concentration, mg L^{-1}, M is the accumulated methane production (CH_4) at 35°C at the time

t, mL g^{-1}VS, and M_t is the total methane production for 20 days at 35°C, mL g^{-1}VS. Substituting Eq. 3.4 to Eq. 3.3 resulted in the final kinetic expression as listed in Table 3.2. The parameters, including k_{hyd}, β, k_m, and K', were determined by fitting the integrated forms (Table 3.2) to experimental data using minimization of an Objective Function (OF, Eq. 3.5);

$$OF = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(C_M - C_P)^2} \tag{3.5}$$

where n is the number of data points, C_M is the particulate COD concentrations or the methane productions from experiments, and C_P is the predicted particulate COD concentrations or the methane productions from the model. This determination was achieved by using a Generalized Reduced Gradient (GRG) nonlinear solver tool in Microsoft Excel. R^2, which is a common method to evaluate the model fit, was calculated and provided.

Table 3.2 Kinetic models and integrated equations for anaerobic digestion.

Steps	Models	Kinetic models	Integrated forms
Hydrolysis	Modified 1st order model	$r_{hyd} = -k_{hyd} \cdot (S_P - \beta S_{P0})$	$S_P = (1-\beta)S_{P0}e^{-k_{hyd}t} + \beta S_{P0}$
Methanogenesis	Monod type model	$r_m = \frac{k_m \cdot \frac{M_t - M}{M_t}}{K' + \frac{M_t - M}{M_t}}$ *K'=K/V_t	$K' \ln\left(1 - \frac{M}{M_t}\right) - \frac{M}{M_t} + \frac{k_m}{M_t}t = 0$

3.2.3 Development of Kinetic Parameter Estimation Models

Anaerobic digestion kinetics are generally affected by several factors such as temperature, pH, types of substrates, mixing, and S/I ratio (Manea et al., 2012). The factors of temperature, pH, mixing, and S/I ratio are relatively constant for anaerobic digestion at mesophilic domain: temperature is generally kept in 35-37°C; mixing is usually applied to provide homogenized conditions for the digestion; pH is kept at a neutral condition; and S/I ratio is usually applied in

the range from 0.5 to 3. Since anaerobic co-digestion keeps these factors constant, co-substrate types and ratios are considered as major factors in the co-digestion kinetic modeling. Previous studies showed that the kinetic parameter values varied according to the type of substrates (Gavala et al., 2003; Vavilin et al., 2008) and the ratios of co-substrate (Costal et al., 2012; Neumann et al., 2015; Zen et al., 2015). In prior studies, most of the kinetic parameter values for the co-digestion were higher than the parameters for one of the single substrates. In addition, the maximum kinetic parameter value was found at the certain ratio of co-substrates. For example, the k_{hyd} values increased for the co-digestion of microalgae and WAS when the ratios of microalgae increased to the threshold point (this point refers to the best combination for anaerobic co-digestion of microalgae and WAS), and then gradually decreased to the value for the single substrate. Thus, types and ratios of co-substrates were selected as indicators in the parameter estimation model. In the model, kinetic parameters for a single substrate were used as a substitute for substrate types because they are directly related to the types of substrate.

For model framework, hyperbolic and inverse tangent relationships were adopted in order to explain the trend of kinetic parameter for co-digestion, which are shown in Eq. 3.6 and 3.7. In general, the hyperbolic and inverse tangent-based equations are able to provide the S-shaped curve. These functions are often used in modeling of biological systems. For example, the hyperbolic function was applied to describe growth kinetics and enzyme kinetics, while the inverse tangent function was used in soil respiration (Adair et al., 2008; Del Grosso et al., 2005; Panikov, 1995). At a transition point of the curve, the inverse tangent function has slightly steeper slope representing the greater rate of change compared with hyperbolic function.

In the models, the constant *a* is introduced to avoid infinite value in denominator as well as to explain the synergetic effect of the co-substrates. For example, when constant *a* decreases,

the K_E value increases. In other words, the lower a value indicates that substrates have the higher synergetic effect in co-digestion.

$$K_E = \frac{K_A * P_A}{P_A + a} + (K_{WAS} \cdot P_{WAS}) \tag{3.6}$$

$$K_E = K_A \cdot \mathrm{ATAN}\left(\frac{P_A}{a}\right) + (K_{WAS} \cdot P_{WAS}) \tag{3.7}$$

where K_E is the estimated kinetic parameters, which could be the first-order rate coefficient or the maximum substrate utilization coefficient for co-digestion of WAS and microalgae. K_{WAS} and K_A are the first-order rate coefficients or the maximum substrate utilization coefficient for anaerobic digestion of waste activated sludge only and anaerobic digestion of microalgae only, respectively, P_A and P_{WAS} are the percentage of microalgae and waste activated sludge by mass of volatile solid, respectively.

Constant a was calibrated by minimizing the total relative error between the parameters determined from experiments and the parameters estimated from the models. In order to evaluate the performance of the estimation models, Akaike Information Criterion (AIC) was used as a statistical index (Motulsky and Christopoulos, 2004).

$$AIC = nln\left(\frac{RSS}{n}\right) + 2(N+1) + \frac{2(N+1)(N+2)}{(n-N-2)} \tag{3.8}$$

where RSS is the residual sum of squares, n is the number of data points, and N is the number of model parameters. The highest quality of the model will result in the smallest AIC. Also, R^2 and normalized root mean squared error (NRMSE) were calculated to provide additional information about the goodness of fit for the models. The NRMSE was calculated based on following relationship;

$$NRMSE = \frac{\sqrt{\sum_{n=1}^{n} \frac{(P-C)^2}{n}}}{h} \times 100 \quad (3.9)$$

where *P* is the predicted kinetic parameters using the models, *C* is the kinetic parameters determined from the experimental data in this study or the published studies, and *h* is the mean of the kinetic parameters C. If the model accurately predicts the kinetic parameter (e.g., k_{hyd} or k_m), R^2 should be close to 1, and AIC tends to be low. According to Jamieson et al. (1991), a model simulation was considered to be acceptable when the NRMSE is less than 30%. Based on the statistical indices, better models for hydrolysis and methanogenesis were selected using the experimental results from this study. The models were then tested using the data from published studies and the NRMSE criteria (NRMSE<30%) was used in this case to determine the acceptability of the model.

3.3 Results and Discussion

3.3.1 Kinetic Parameters for Hydrolysis and Methanogenesis

The modified first-order kinetic model and Monod type kinetic model were applied for hydrolysis and methanogensis in the co-digestion of microalgae and WAS, respectively. Figure 3.1 shows simulated and experimental results for hydrolysis. The simulated data showed a relatively good fit to experimental data for Particulate Chemical Oxygen Demand (PCOD) with $R^2 > 0.8$. The R^2 of the simulated result for 100% waste activated sludge was 0.68, which was lower than the values for other co-substrate ratios. Due to the uneven particle size as well as amount of particles in the samples, the change of PCOD value for 100% WAS were not noticeable during days 0-3. Thus, this affected the goodness of fit of the model. Based on the results, the modified model was able to explain the hydrolysis of microalgae and WAS during the co-digestion. Shimizu et al. (1993) and Morand and Briand (1999) also applied the first-order kinetic model considering

non-degradable portions for anaerobic digestion of WAS and microalgae (*Ulva sp.*), respectively. They also concluded that the modified first-order kinetic model was able to explain the hydrolysis of these substrates in their studies.

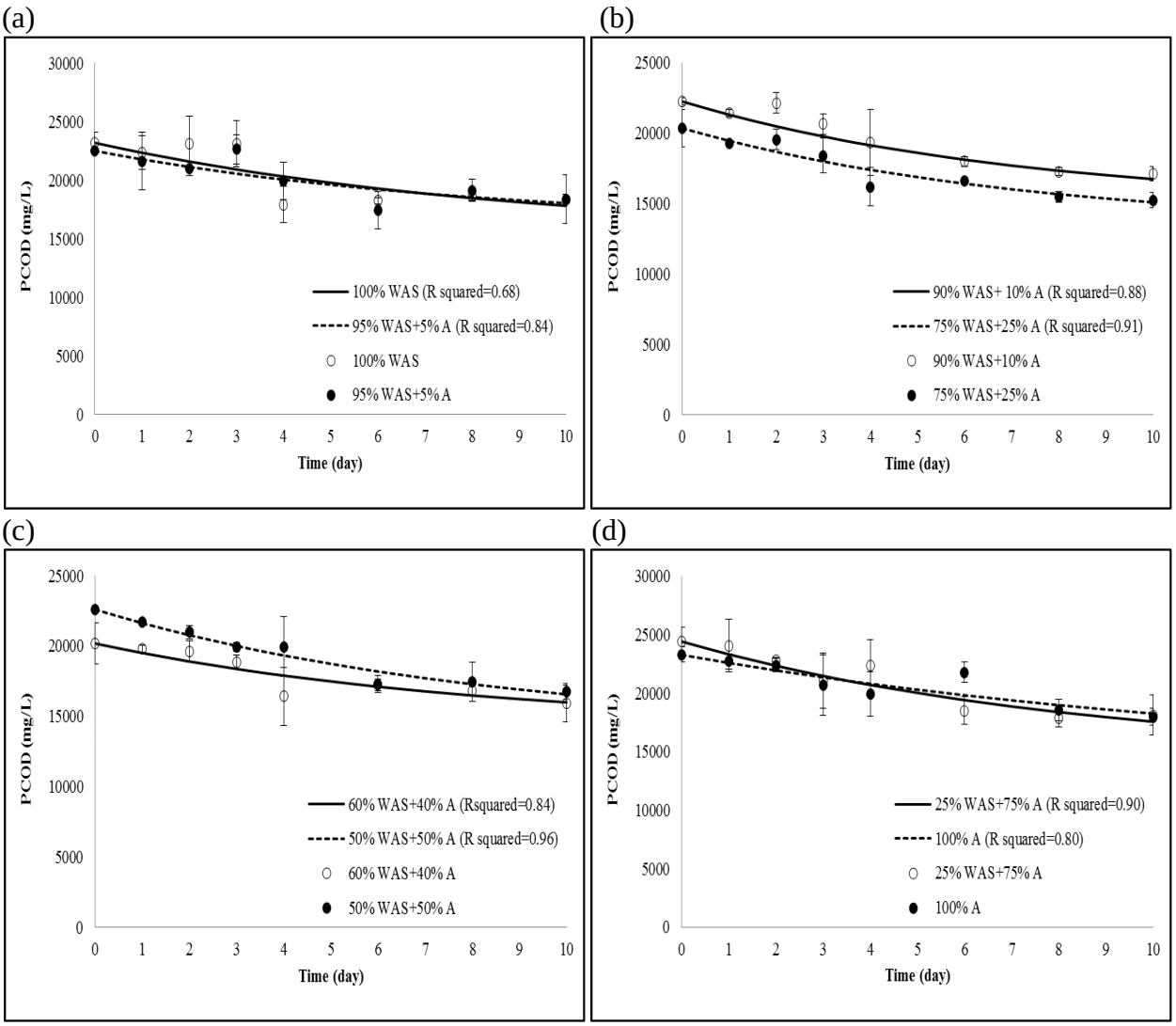


Figure 3.1 Simulated and experimental data for particulate chemical oxygen demand (PCOD) with different compositions of volatile solids (VS): (a) 100% wasted activated sludge (WAS) and 5% microalgae (A) with 95% WAS, (b) 10% A with 90% WAS and 25% A with 75% WAS, (c) 40% A with 60% WAS and 50% A with 50% WAS, and (d) 75 % A with 25% WAS and 100% A. Note: Symbols (○ and ●): Experimental data; solid and dashed lines: simulated results.

Simulated results using the Monod model and experimental results of methane accumulation for different ratios of microalgae and WAS are shown in Figure 3.2. The Monod

model slightly overestimated accumulated methane during days 10-18, but the production trend was well described with $R^2 > 0.9$ for all compositions. Siegrist et al (2002) also reported that the Monod type kinetic model was able to describe the conversion of VFA from waste sewage sludge to methane in anaerobic digestion.

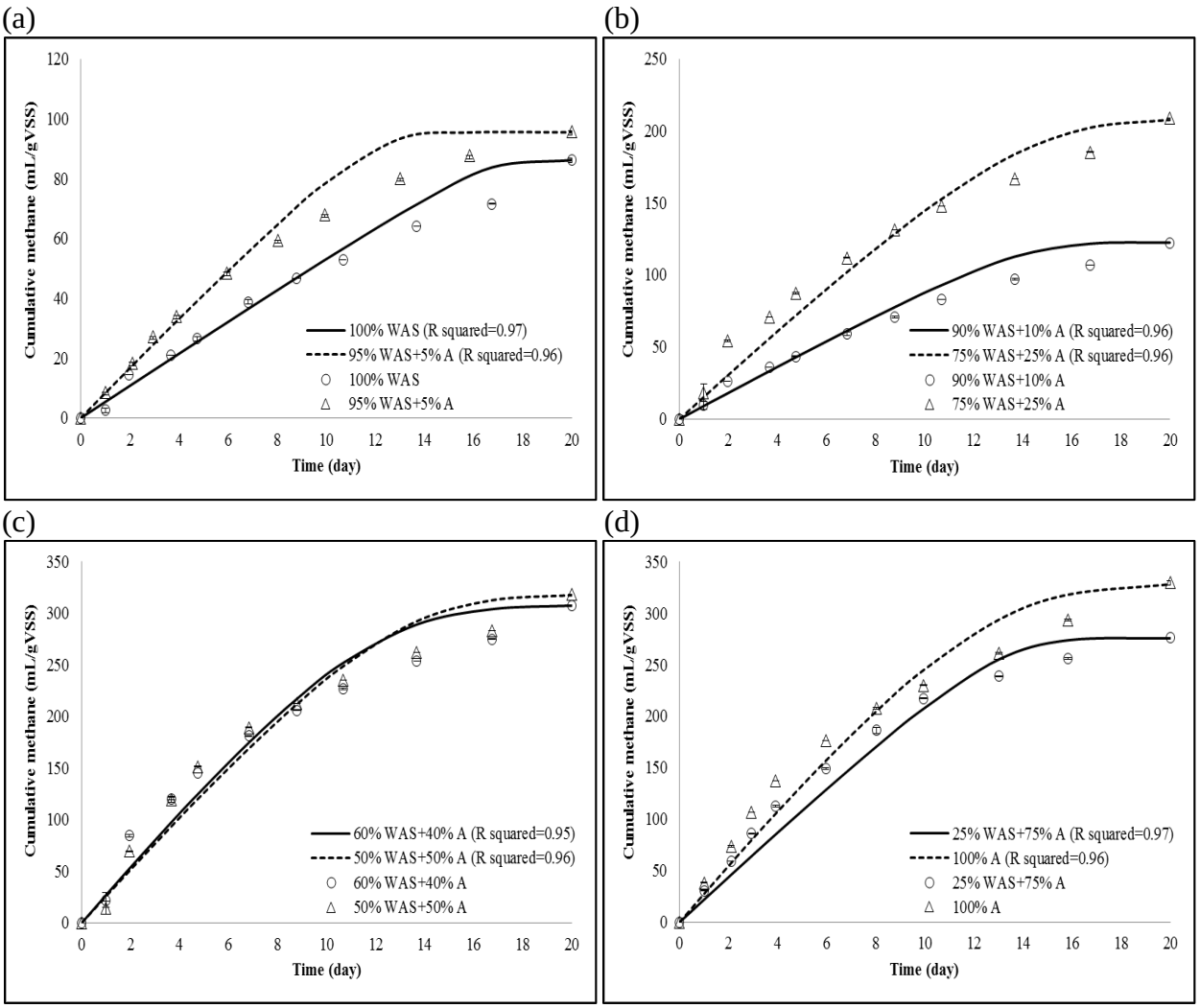


Figure 3.2 Simulated and experimental data for cumulative methane with different compositions of volatile solids (VS): (a) 100% waste activated sludge (WAS) and 5% microalgae (A) with 95% WAS, (b) 10% A with 90% WAS and 25% A with 75% WAS, (c) 40% A with 60% WAS and 50% A with 50% WAS, and (d) 75 % A with 25% WAS and 100% A. Note: Symbols (○ and Δ): Experimental data; solid and dashed lines: simulated results.

The hydrolysis and methanogensis kinetic parameters obtained from the models are presented in Table 3.3. Based on the kinetic parameter values for hydrolysis obtained from

experimental data, the values of the kinetic parameters were increased, as the microalgae proportion increased from 0 to 10%. This result indicates that the co-digestion can improve the reaction rate of the hydrolysis. It was observed that the composition of 10% microalgae with 90% WAS has the highest k_{hyd} value (0.14) and the k_{hyd} for the composition of 75% WAS with 25% microalgae was the second highest (0.13). These results indicate that the co-digestions of microalgae and WAS at these ratios were able to achieve the synergetic effect on the substrate biodegradability. However, the values were gradually decreased, as the microalgae increased from 25 to 100% in the substrates. It is because increasing microalgae that have complex cell walls slows down hydrolysis (Frigon et al., 2013; Wang et al., 2013). Costal et al. (2012) reported that the first-order kinetic model successfully described the hydrolysis for the co-digestion of microalgae and WAS. They observed that the highest value of k_{hyd} was obtained in the co-digestion of 15% VS microalgae with 85% VS mixed waste sludge (primary and secondary waste sludge), and concluded that this composition would have the best synergetic effect for the co-digestion. Their study also observed the similar trend that the k_{hyd} value reached to the maximum value and then decreased as percentage of VS microalgae increased.

Table 3.3 Kinetic parameters for hydrolysis and methanogenesis. Note: S/I ratio: substrate to inoculum ratio; VS: volatile solids, WAS: waste activated sludge, and A: microalgae.

S/I ratio (gVS/gVS)	Substrate (% by VS)		Modified 1st order equation			Monod type model		
	WAS	A	k_{hyd} (1/d)	β	R^2	k_m (mL/gVSS-d)	K'	R^2
1	100	0	0.11	0.65	0.68	5.56	0.03	0.97
1	95	5	0.12	0.72	0.84	8.89	0.06	0.96
1	90	10	0.14	0.67	0.88	9.81	0.07	0.96
1	75	25	0.13	0.65	0.91	17.4	0.12	0.96
1	60	40	0.12	0.70	0.84	32.3	0.18	0.95
1	50	50	0.12	0.61	0.96	30.0	0.15	0.96
1	25	75	0.12	0.60	0.90	23.7	0.07	0.97
1	0	100	0.07	0.59	0.80	32.8	0.18	0.96

On the other hand, it was observed that the kinetic parameter values for methanogenesis obtained from experimental data increased with increasing microalgae ratio in the substrates. This is because the cell contents of microalgae (high lipid contents) may improve methanogen activity and increase the rate of methanogenesis. Based on the Table 3.3, 100% microalgae resulted in the highest k_m value (32.8), and the k_m for the composition of 60% WAS with 40% microalgae was the second highest (32.3), but these two values were comparable.

3.3.2 Estimation of Kinetic Parameters by the Proposed Models

Based on the kinetic parameters for the single substrates and ratio of microalgae in co-substrate, the k_{hyd} and k_m for different compositions of WAS and microalgae were estimated using the kinetic parameter estimation models (Eq. 3.6 and 3.7). Figure 3.3 shows the results for estimated parameters from the models and kinetic parameters obtained from experiments as listed in Table 3.3. The constants in Eq. 3.6 and 3.7 for hydrolysis were 0.10 and 0.38 respectively, while the constants in Eq. 3.6 and 3.7 for methanogenesis were 0.21 and 0.55, respectively. Based on the results, both models were able to estimate the kinetic parameters for the first-order kinetic model and Monod model. In order to find the better model to estimate the kinetic parameters for the co-digestion, R^2, AIC, and NRMSE for hydrolysis and methanogenation were established as listed in Table 3.4.

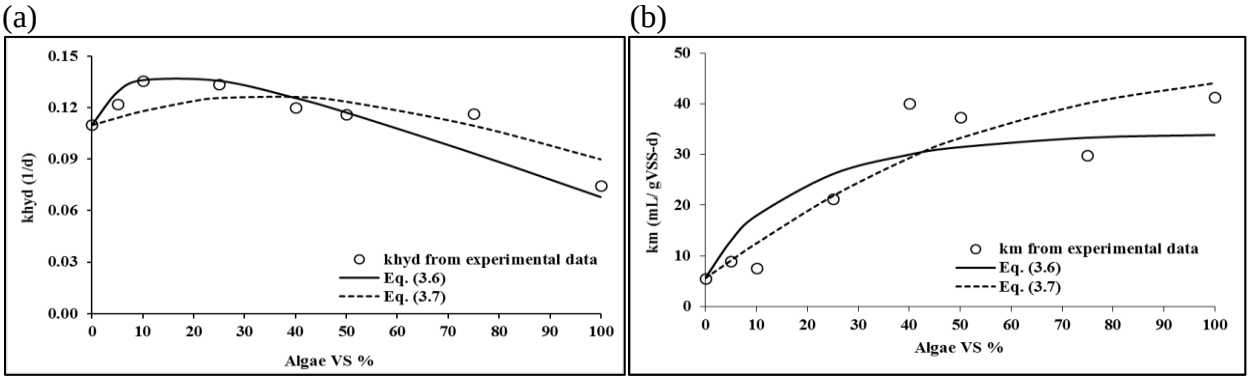


Figure 3.3 Comparisons for simulated data from two models: (a) Hydrolysis and (b) Methanogenesis.

Table 3.4 Goodness of fit for two kinetic parameter estimation models.

Items	Hydrolysis		Methanogenesis	
	Eq. (3.6)	Eq. (3.7)	Eq. (3.6)	Eq. (3.7)
R^2	0.73	0.67	0.80	0.82
AIC	-70.4	-68.9	29.4	28.5
NRMSE	2.8%	3.1%	9.8%	8.6%

In Figure 3.3(a), it was shown that the two proposed models were both able to capture the trend of hydrolysis that was discussed in the section 3.3.1. However, Eq. 3.6 with hyperbolic relationship more closely estimated the kinetic parameters of hydrolysis between 0-50% microalgae in the substrates. When comparing the two models, Eq. 3.6 was the better estimation model for hydrolysis, which has the higher R^2, lower AIC, and lower NRMSE than Eq. 3.7. On the other hand, Figure 3.3(b) showed that the inverse tangent relationship more closely estimated k_m values for lower microalgae ratio in the substrates (below 30%). Thus, Eq. 3.7 was better for estimating k_m values for methanogenesis which has the higher R^2, lower AIC, and lower NRMSE than Eq. 3.6.

The kinetic parameter estimation models were also tested using published data for the co-digestion. Eq. 3.6 was applied for estimating k_{hyd}, while Eq. 3.7 was used for estimating k_m. The results for estimating k_{hyd} are shown in Figure 3.4. Figure 3.4(a) presents the results for the kinetic parameters obtained from Costal et al. (2012) and the parameters estimated from the model. The model was able to estimate k_{hyd} values with a NRMSE of 29% with the several values underestimated. This might be due to properties of the sludge used in Costal et al. (2012). In their study, the mixture of primary sludge and waste activated sludge was used, and this mixture might affect the hydrolysis kinetics.

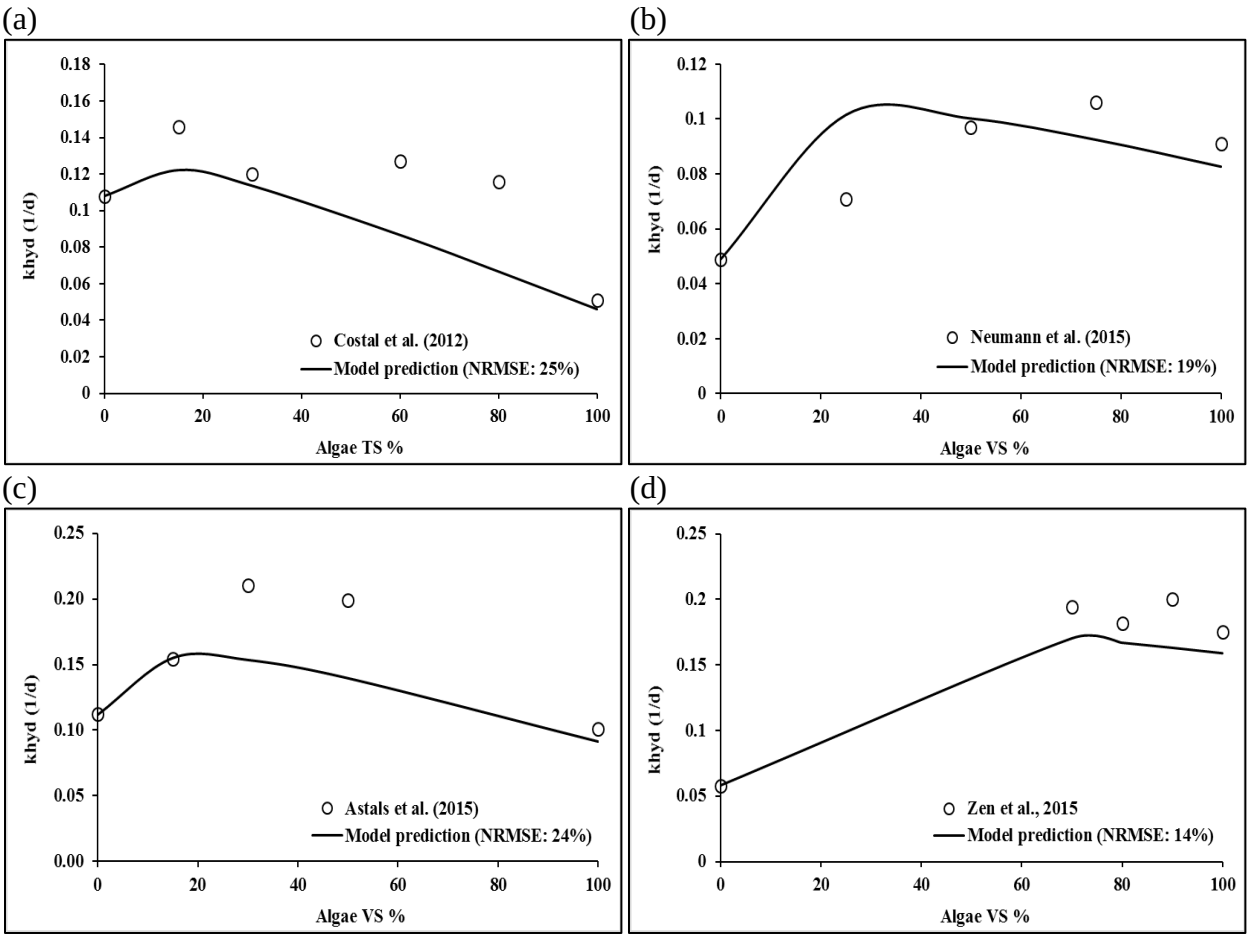


Figure 3.4 Comparisons between k_{hyd} predicted from the proposed model and k_{hyd} estimated from literature data: (a) Costal et al. (2012), (b) Neumann et al. (2015), (c) Astals et al. (2015), and (d) Zen et al. (2015).

Figure 3.4(b) shows kinetic parameters from Neumann et al. (2015) and estimated kinetic parameters using the proposed model. The data for the kinetic parameters from their study have a distinctive shape, and this is different to the results shown in Figure 3.3(a). In their results, the values of the kinetic parameters were increased, as the microalgae increased from 0 to 75%. This is because they used lipid-spent microalgae that have already broken down microalgae cell walls from lipid extraction, and the cell disruption resulted in the increase in the hydrolysis rate (Ramos-Suárez and Carreras, 2014). In this condition, the kinetic parameter value for microalgae was higher than that of the WAS (Typically, the kinetic parameter value for microalgae is lower than that of WAS), and this affected the trend of the kinetic parameter for co-digestion as a function of

substrate ratio. For this case, the proposed model slightly overestimated the k_{hyd} values at 25% lipid-spent *Botryococcus braunii* with 75% WAS and slightly underestimated the k_{hyd} values at 75% lipid-spent *Botryococcus braunii* with 25% WAS. Although the model did not provide the good curve trend, the model's NRMSE was 27%, which is considered to be within the acceptable range for model prediction.

Astals et al. (2015) investigated anaerobic co-digestion of pig manure and algae (*Scenedesmus sp.*) under mesophilic condition. They assessed kinetics and substrate biodegradability for hydrolysis using the first-order kinetic model. Figure 3.4(c) shows the results for the kinetic parameters obtained from experiments and estimated from the proposed model. The model underestimated the kinetic parameters at compositions of 30% and 50% microalgae, due to the constant *a* in the model. The proposed model was developed to estimate the kinetic parameter values for microalgae and WAS, and the constant *a* reflected the synergetic effect of the microalgae and WAS. However, in Astals et al. study the pig manure was used as one of co-substrates, which has different characteristic to WAS. Although the different co-substrates were used, the model was still able to determine the kinetic parameters with the NRMSE of 24%.

Zen et al. (2015) applied *Egeria densa* which is an aquatic plant, and they evaluated the feasibility of anaerobic co-digestion of *Egeria densa* and WAS with different VS ratios. It was observed that the model slightly underestimated k_{hyd} values due to the same reason as discussed above (Figure 3.4 (d)). Although the estimated kinetic values were lower than the values obtained from experiment results, the model was able to estimate the kinetic parameters with a low NRMSE (14%). In general, Eq. 3.6 was able to estimate the k_{hyd} value within acceptable range.

Figure 3.5 shows the comparison of k_m values predicted using Eq. 3.7 and obtained from published data by Wang et al. (2013), Gordon (2015), Kim and Kang (2015), and Lu and Zhang

(2016). Gordon (2015) investigated the co-digestion of microalgae and WAS as a method to recover and recycle nutrients in algal biofuel systems. They conducted the co-digestion of microalgae (*Chlorella sp.*) and WAS by varying the ratio of microalgae to WAS in the substrates. The kinetic parameters for the Monod model were obtained from their experimental results for average cumulative methane production, which is shown in Table A.1(Appendix A). It was found that the Monod model fit their experimental data well with R^2>0.84. As shown in Figure 3.5 (a), estimated k_m from the model was close to k_m values obtained from experimental results (NRMSE=17%). For 25% microalgae in the substrates, the kinetic parameter value obtained from experimental data was higher than the predicted kinetic parameter value.

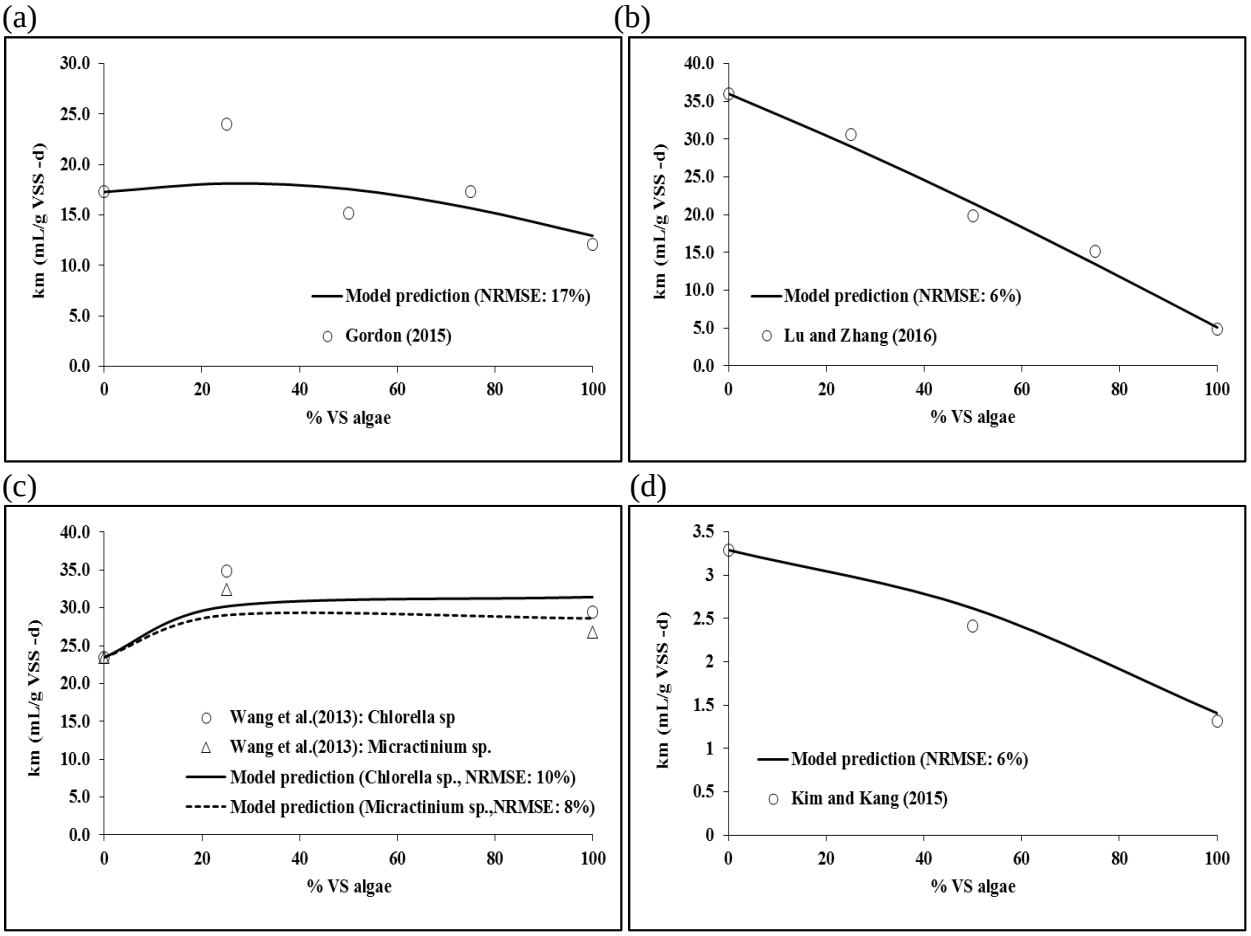


Figure 3.5 Comparisons between k_m predicted from the proposed model and k_m estimated from literature data: (a) Gordon (2015), (b) Lu and Zhang (2016), (c) Wang et al. (2013), and (d) Kim and Kang (2015).

Lu and Zhang (2016) evaluated the feasibility of using septic sludge and microalgae (*Chlorella* sp.) for anaerobic co-digestion. Based on their biogas data, the kinetic parameters were determined, which is shown in Table A.2 (Appendix A). Figure 3.5 (b) shows the k_m values with different microalgae and septic sludge compositions. The model estimations were very close to k_m values obtained from experimental data (NRMSE<7%) and the model was able to capture the distinctive curve trend that was different to the results shown in Figure 3.3 (b); the values were decreasing with an increase of microalgae.

Wang et al. (2013) investigated anaerobic co-digestion of microalgae (*Chlorella sp.* and *Micractinium sp*) and WAS. The Monod model well described the production of biogas for the co-digestion, and the kinetic parameters are presented in Table A.3 (Appendix A). In Figure 3.5(c), the estimated k_m from the model and the k_m calculated from experimental data were compared. It was found that the k values were slightly underestimated, but the model was able to estimate k_m with a NRMSE of less than 10%.

Kim and Kang (2015) evaluated methane production by the anaerobic co-digestion of different mixtures of food waste leachate, microalgal biomass (*Chlorella sp.*), and raw sewage sludge. Methane production data for the co-digestion of microalgae and raw sewage sludge were taken from their study, and the methane production kinetics were described via fitting their experimental data to the Monod model. The kinetic parameters were shown in Table A.4 (Appendix A). The k_m values of their study were lower than other studies because of low initial volatile solid concentrations for the co-substrate. Figure 3.5(d) compared the k_m values predicted from the proposed model and estimated from experimental data. The model closely estimated the k_m values for the co-digestion with a NRMSE of 6%.

Based on the results presented above, the proposed models can generally predict the kinetic parameters in the first-order kinetic for hydrolysis and the Monod model for methanogenesis in co-digestion of microalgae and WAS as well as other combinations with microalgae within the acceptable range. The observed fluctuations in the estimates might be related to two factors: original kinetic parameter values for single substrates and the constant *a* in the model.

The kinetic parameter values for single substrate were one of the important factors in the kinetic parameter estimation model. Thus, it is important to obtain accurate kinetic parameter values for single substrates from the experiments in order to improve the model prediction. Another important factor that affected the model estimation was the constant *a*. In this study, the model was developed to estimate the kinetic parameters for the co-digestion of microalgae and WAS. Thus, the model component, particularly the constant *a*, was used to explain the synergetic effect of these two substrates. When the model applied for the other substrates, it was observed that the model predictions were underestimated or overestimated, because the synergetic effect will be different for different substrates. In order to apply for other co-digestion cases, the constant *a* also needs to be determined based on substrates characteristics, such as particle size and C/N ratios. Therefore, future research may focus on these limitations to improve the applicability of the model to various substrates for the co-digestion.

3.4 Conclusions

In order to estimate the kinetic parameters for anaerobic co-digestion of microalgae and WAS, two estimation models were proposed based on the kinetic values for single substrates and the ratios of the co-substrates. It was observed that the model using a hyperbola function was better for the estimation of the first-order kinetic coefficient, whereas the model using inverse tangent function closely estimated the Monod kinetic parameters. When the models were applied to other

cases in the published studies, they were able to estimate kinetic parameters in those studies within an acceptable range even under different conditions from this study.

CHAPTER 4: AN INTEGRATED PROCESS MODEL FOR MICROALGAE BIOENERGY PRODUCTION COUPLED WITH WASTEWATER TREATMENT

4.1 Introduction

Microalgae have been investigated for nutrient removal from wastewater since the mid-1970s and have shown a potential for nutrient recovery/removal in wastewaters including domestic, industrial, and agricultural (Bosch et al., 1974; Cai et al., 2013; Hernández et al., 2013; Judd et al., 2015). With increasing recognition of the environmental impacts from fossil-based fuels, microalgae-based bioenergy became attractive due to the distinctive characteristics of microalgae (Yuan et al., 2012). Autotrophic microalgae have a capability to mitigate carbon dioxide (CO_2) emissions by converting energy-poor CO_2 to energy-rich organic carbon through photosynthesis. During photosynthesis, they improve dissolved oxygen level in water. In addition, they have high growth rates and an ability to produce a high amount of lipids per cell, compared to other energy crops (Amin, 2009; Mata et al., 2010).

Because of these characteristics, integration of microalgae cultivation into wastewater treatment has been introduced as a sustainable option (low energy consumption, high energy production, low greenhouse gas emissions, and high nutrient recovery) for wastewater treatment as well as microalgae biomass production (Kumar et al., 2010). For example, if the integrated system is applied, microalgae can recover/remove nutrients, such as nitrogen (N) and phosphorus (P) in wastewater. Furthermore, through photosynthesis, they are able to reduce on-site CO_2 emissions and improve the dissolved oxygen level. In addition, harvested microalgae biomass can be used as feedstock to produce biodiesel or biogas. Thus, this system has a high potential to reduce

the operational costs and environmental impacts for microalgae cultivation and wastewater treatment (Beal et al., 2012; Clarens et al., 2010; Menger-Krug et al., 2012).

There are many studies that considered such integrated systems, but most of these studies focused on only one aspect of the system, either microalgae cultivation using wastewater or energy conversion using microalgae biomass via lab-scale or pilot-scale experiments (Chen et al., 2015b; de Alva et al., 2013; Fathi et al., 2013; Milledge and Heaven, 2013; Pittman et al., 2011; Sutherland et al., 2014) or via modeling approach (Bello et al., 2016; Broekhuizen et al., 2012; Buhr and Miller, 1983; Mairet et al., 2011; Yang, 2011). Although these studies were able to provide some information for the integrated systems, they are limited in terms of a holistic understanding of the overall system. In addition, since most of the existing studies were based on experimental research, extensive resources and time were required.

To successfully implement the integrated system, the understanding of the performance of the overall system is important. A few studies have attempted to investigate the overall integrated system, instead of the single component of the system (Drexler et al., 2014; Menger-Krug et al., 2012; Sturm and Lamer, 2011). For example, Sturm and Lamer (2011) investigated an energy balance of microalgae production in open ponds using secondary effluents followed by biodiesel production. Based on their experimental results, they reported that microalgae bioenergy production was energetically favorable by utilizing wastewater. Menger-Krug et al. (2012) evaluated energy and nutrient (N, P) balances of an integrated microalgae system with a wastewater treatment plant. By using stoichiometric relationships as well as published experimental results, they concluded that the suggested system was able to improve energy balances without adding external resources, but the system lowered effluent water quality in terms of chemical oxygen demand, total nitrogen, and total phosphorus compared to a conventional

system, due to lower nutrient removal efficiencies of the microalgae system. Drexler et al. (2014) assessed the potential of microalgae production from municipal secondary effluents with biodiesel and biogas production by using a dynamic mass balance model for microalgae production and energy conversion based on previous experimental results. The results showed that the proposed system was able to generate US $1 M profit under high energy price-resource scarce conditions (Drexler et al., 2014).

Although these studies have shown that the integrated systems were beneficial under the specific conditions considered, it may be difficult to evaluate the benefits under different conditions because these studies were based on experimental results or theoretical quantitative relationships. Microalgae productivity varies with changing environmental conditions, such as temperature, light intensity, CO_2 supply (Drexler et al., 2014; Park and Craggs, 2010). This variation has impacts on treated wastewater quality, microalgae productivity, and bioenergy production. In addition, the previous studies were focused on energy balances with little information on nutrient and carbon balances. In order to predict the performance of the integrated system in terms of carbon uptake, nutrient removal, and energy production under varying environmental conditions, such as temperature and light intensity, an integrated process modeling is an alternative approach to provide a better understanding of the overall system as well as saving resources and time for additional experimentation (Galí et al., 2003). Therefore, the goal of this study was to develop an integrated process model, considering microalgae cultivation, harvesting and energy conversion (biogas production) processes, for evaluating the performance of the integrate system in terms of carbon, nutrient and energy. The integrated system of this research includes microalgae cultivation using anaerobically digested sludge centrate, harvesting process

with gravity sedimentation and centrifugation, and bioenergy production from anaerobic co-digestion of microalgae and waste sludge.

4.2 Materials and Methods

4.2.1 Overview of Integrated System

Figure 4.1 presents the layout of a hypothetical integrated microalgae system with wastewater treatment. In this study, the wastewater treatment plant was modeled as an advanced wastewater treatment facility with additional biological nutrient removal (A^2/O process) (Electric Power Research Institute, 2013; Tchobanoglous, 2003). It was assumed that the average influent flow of the plant is approximately 5 MGD (18,927 m^3 d^{-1}) and the effluent meets requirements of typical Florida permit (i.e., < 5 mg L^{-1} biochemical oxygen demand (BOD_5), 5 mg L^{-1} total suspended solids (TSS), 3 mg L^{-1} total nitrogen (TN) and 1 mg L^{-1} total phosphorus (TP)) (The Florida Senate, n.d.). The wastewater influent was based on typical composition of wastewater obtained from Seiple et al. (2017) and Spellman (2013): 230 mg L^{-1} BOD_5, 260 mg L^{-1} TSS, 53 mg L^{-1} TN, and 13 mg L^{-1} TP. In the integrated system, microalgae cultivation process was applied to treat the centrate from a dewatering process for anaerobically digested solids. It was assumed that the cultivation took place in an open raceway pond with a 7-day hydraulic retention time. After cultivation, microalgae were harvested by gravity sedimentation and centrifugation. The harvested microalgae biomass was used for anaerobic co-digestion with wasted sludge produced from primary and secondary treatment. The waste sludge was theoretically estimated based on Tchobanoglous et al. (2003), which was approximately 4,437 kg total suspended solid per day (primary sludge: 2,911 kg d^{-1}; secondary sludge: 1,526 kg d^{-1}). Anaerobic co-digestion was assumed to be operated under mesophilic conditions with a 20-day solid retention time. Produced

biogas was assumed to be used to generate electricity and heat via an on-site combined heat and power (CHP) system.

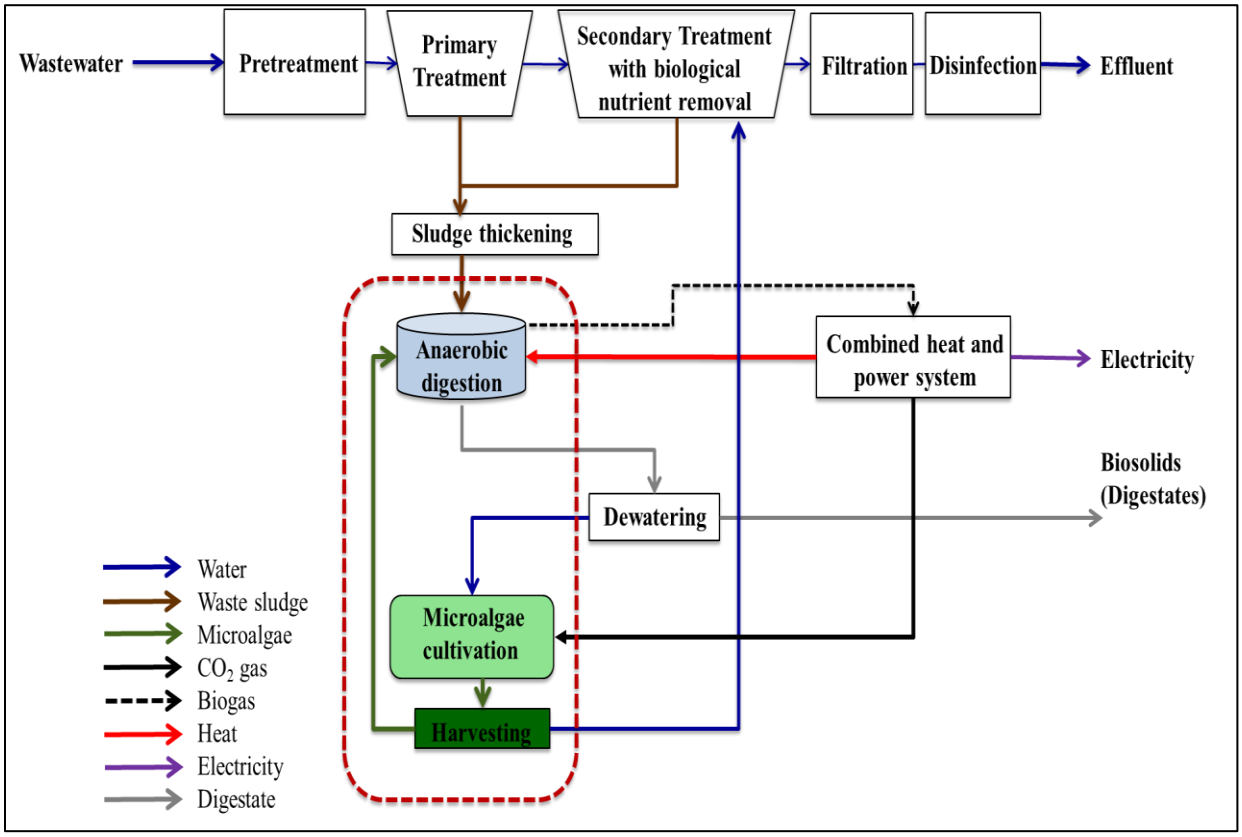


Figure 4.1 Process flow diagram of the integrated system. Note: Red dashed box is the system boundary of the integrated system.

4.2.2 Description of Integrated Process Model

The integrated process model consists of cultivation, harvesting, and anaerobic co-digestion. This section describes the assumptions and operation conditions for the integrated system.

4.2.2.1 Model Description for Cultivation

Cultivation was assumed to take place in the open raceway pond which has 329 m^3 of working volume (0.3 m depth) and 7-day hydraulic retention time in Tampa, FL. It was assumed that the centrate contained about 445 mg L^{-1} TN (NH_4-N: 397 mg N L^{-1}) and 238 mg L^{-1} TP, which is based the actual centrate characteristics from anaerobic digestion (Lee and Zhang, 2016). Also,

it was assumed that the bioavailable organic carbon is negligible and there is no microalgae in the centrate. In this system, a major nitrogen source is ammonium (NH_4-N) in the centrate.

Light intensity and temperature data were obtained from typical meteorological database for Tampa (TMY3, n.d.). In this study, the water loss due to evaporation was calculated based on Lam et al. (2001). The evaporation rates were shown in Appendix B. The combustion gas from the CHP system (assumed 5% CO_2 in combustion gas) was used to provide CO_2 through fine bubble diffusers. It was assumed that the combustion gas was applied only during the daytime (light cycle). The fine bubble diffusers were placed at intervals along the flow path at the bottom of the pond. It was assumed that the pond maintains a constant neutral pH (about pH 7.1-7.8) due to the CO_2 sparging. Since pH was kept near neutral, the dominant N species is NH_4^+ and NH_3 stripping was not considered.

Microalgae cultivated in this system are an indigenous *Chlorella* sp. The initial microalgae concentration was 100 g m^{-3}. The microalgae growth rate was described by an integrated co-limitation kinetic model (Eq. 4.1) that was introduced in Chapter 2. The model considers nitrogen as the limiting factor compared with phosphorus and CO_2 concentrations, temperature and light intensity as additional growth limiting factors. The expressions of each function in Eq. 4.1 are shown in Table 4.1. The decay rate of microalgae was modeled by Eq. 4.2 (Yang, 2011).

$$r_g = \mu_{max} f(N) \cdot f(CO_2) \cdot f(I_{av}, T) \cdot X \tag{4.1}$$

$$r_d = k_d \cdot X \tag{4.2}$$

where r_g is the microalgae growth rate (g m^{-3} d^{-1}); r_d is the microalgae decay rate (g m^{-3} d^{-1}); μ_{max} is the maximum specific growth rate (d^{-1}); *f(i)* is the function of *i*; *N* is the nitrogen concentrations (g m^{-3}); CO_2 is the aqueous CO_2 concentrations (g m^{-3}); I_{av} is the average irradiance in the culture

(μmol photon m^{-2} s^{-1}); T is the temperature (°C); X is the microalgae biomass concentrations (g m^{-3}); and k_d is the decay constant for microalgae (d^{-1}), which was obtained from Yang (2011).

Table 4.1 Overall expressions for the growth kinetic model (Lee and Zhang, 2016).

Factors	Applicable ranges	Rate expressions
N	$S_N \leq 150$ mg L^{-1}	$f(N) = \frac{S_N}{K_{S,N} + S_N}$
	$S_N > 150$ mg L^{-1}	$f(N) = \frac{S_N}{K_{S,N} + S_N + S_N^2/K_{i,N}}$
CO_2	$S_C \leq 50$ mg L^{-1}	$f(CO_2) = \frac{S_C}{K_{S,C} + S_C}$
	$S_C > 50$ mg L^{-1}	$f(CO_2) = \frac{S_C}{K_{S,C} + S_C + S_C^2/K_{i,C}}$
Light and Temperature	$I_{av} \leq 90$ μmol photon m^{-2} s^{-1}	$f(I_{av}, T) = \theta^{T-20} \cdot \tanh(\frac{I_{av}}{I_K})$
	$I_{av} > 90$ μmol photon m^{-2} s^{-1}	$f(I_{av}, T) = \theta^{T-20} \cdot \frac{2 * (1 - I_e/I_K) * (I_{av}/I_K - I_e/I_K)}{(1 - I_e/I_K)^2 + (I_{av}/I_K - I_e/I_K)^2}$
	Average light intensity $(I_{av}) = \frac{I_0}{k \cdot z \cdot X}(1 - \exp(-k \cdot z \cdot X))$	

**Nomenclature:* S_N: NH_4-N concentrations (g m^{-3}); $K_{S,N}$: Half-saturation constant of NH_4-N concentrations (g m^{-3}); $K_{i,N}$: Inhibition constant of NH_4-N concentrations (g m^{-3}); S_C: aqueous CO_2 concentrations (g m^{-3}); $K_{S,C}$: Half-saturation constant of CO_2 concentrations (g m^{-3}); $K_{i,C}$: Inhibition constant of high CO_2 concentrations (g m^{-3}); θ: Arrhenius temperature coefficient; I_e: Light energy compensation point for high light intensity (μmol photon m^{-2} s^{-1}); I_k: Light saturation point for high light intensity (μmol photon m^{-2} s^{-1}); I_0: incident light intensity (μmol photon m^{-2} s^{-1}); k:Light attenuation rate (m^2 g^{-1}); z: depth of the reactor (m); X: microalgae concentrations (g m^{-3})

As mentioned above, microalgae were assumed to be cultivated in the raceway pond. In general, flow in raceway ponds is characterized as plug flow. However, since the proposed cultivation system supplied CO_2 gas during the daytime, the cultivation system was modelled as a combination of Plug Flow Reactor (PFR) and a Completely Mixed Flow Reactor (CMFR), as shown in Figure 4.2 (a).

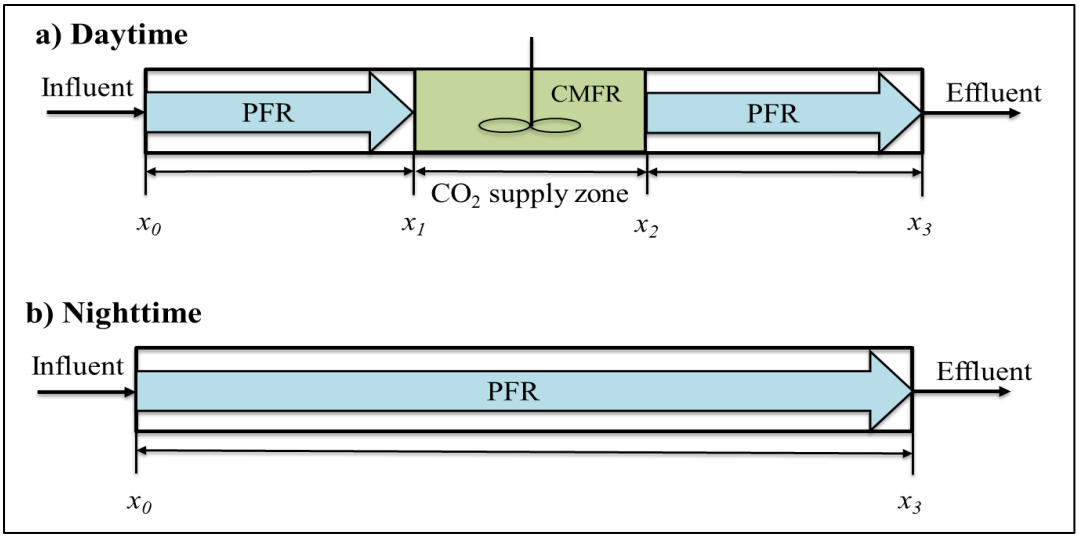


Figure 4.2 The reactor configuration. Note: x_0~x_3 is the overall distance of water flow in the raceway pond, x_1~x_2 is the CO_2 supply zone, PFR is the plug flow reactor, and CMFR is the completely mixed flow reactor.

The CO_2 supply zone (x_1~x_2) in Figure 4.2 (a) that accounts for 25% of the pond area was characterized as being completely mixed and modelled as a CMFR, while the other zones (x_0-x_1 and x_2-x_3) were modelled as PFRs. During the nighttime, the cultivation system was modelled as a PFR, shown in Figure 4.2 (b). For PFR, the mass balance equations of microalgae, NH_4-N, and dissolved CO_2 were expressed in Eq. 4.3, 4.4, and 4.5.

$$-\frac{Q_t}{A}\frac{\partial X}{\partial x} + r_g - r_d = \frac{\partial X}{\partial t} \tag{4.3}$$

$$-\frac{Q_t}{A}\frac{\partial S_N}{\partial x} - \frac{r_g}{Y_N} = \frac{\partial S_N}{\partial t} \tag{4.4}$$

$$-\frac{Q_t}{A}\frac{\partial S_C}{\partial x} - \frac{r_g}{Y_C} + K_L a_S (C_S - S_C) = \frac{\partial S_C}{\partial t} \tag{4.5}$$

For CMFR, the balances of microalgae, NH_4-N, were modeled as follows.

$$\frac{Q_t}{V}(X_{in} - X) + r_g - r_d = \frac{\partial X}{\partial t} \tag{4.6}$$

$$\frac{Q_t}{V}\left(S_{N\_in} - S_N\right) - \frac{r_g}{Y_N} = \frac{\partial S_N}{\partial t} \tag{4.7}$$

The mass balance of dissolved carbon CO_2 for CMFR was expressed in Eq. 4.8. This equation included mass transfer between the atmosphere and the surface of the pond and the mass transfer between fine gas bubble and the culture solution. The CO_2 mass transfer for gas bubbles (*f*) was described by Eq. 4.9, which considers the depth of the reactor and the volume fraction of gas holdup.

$$\frac{Q_t}{V}\left(S_{C\_in} - S_C\right) - \frac{r_g}{Y_C} + f + K_L a_S (C_S - S_C) = \frac{\partial S_C}{\partial t} \tag{4.8}$$

$$f = \frac{1}{z}\varepsilon \int_0^z K_L a (Y_S - S_C) dz \tag{4.9}$$

where r_g and r_d are microalgae growth and decay rates, respectively (g m^{-3} d^{-1}); Q_t is the flow rate of the pond (m^3 d^{-1}); S_N is the nitrogen concentrations (g m^{-3}); x is the distance from the entrance along the flow path; X is the microalgae biomass concentration (g m^{-3}); S_C is the aqueous CO_2 concentration (g m^{-3}); S_{N_in} is the influent nitrogen concentrations of the CMFR (g m^{-3}); X_{in} is the influent microalgae concentrations of the CMFR (g m^{-3}); S_{C_in} is the influent aqueous CO_2 concentrations of CMFR (g m^{-3}); Y_N and Y_C are the yield coefficients with respect to nitrogen and carbon (g g^{-1}); K_La is the overall mass transfer rate constant for CO_2 from fine bubble, (d^{-1}); and ε is the volume faction of gas holdup. The specific equations of K_La and ε are provided in Appendix B. K_La_S is the overall mass transfer rate constant for the pond surface (d^{-1}); A is a cross sectional area of the pond (m^2); C_S and Y_S is the liquid-phase concentrations of CO_2 in equilibrium with air and fine bubbles, respectively (g m^{-3}) which is shown in Appendix B; f is the rate of CO_2 mass transfer from the fine bubbles suggested by Yang (2011) (g $m^{-3}d^{-1}$); z is the depth of the reactor (m); and T is a cultivation temperature (°C). The kinetic parameters for microalgae growth were obtained from Chapter 2. Table 4.2 lists the numerical values of the model parameters used in this study.

Table 4.2 Summary of model parameters for cultivation.

Parameters		Values
Centrate flow rate from anaerobic digestion (m^3 d^{-1})	Q_t	47
Cross sectional area (m^2)	A	2.1
Depth of microalgae cultivation reactor (m)	z	0.3
Yield coefficient of nitrogen (g Biomass g^{-1}Nitrogen)	Y_N	8.8
Yield coefficient of carbon (g Biomass g^{-1} CO_2)	Y_C	0.52
Gas flow rate (vvm)	Q_{gas}	0.02
Number of diffuser per unit area (ea m^{-3})	n	250
Diameter of diffuser (m)	d_0	0.05
Overall CO_2 mass transfer coefficient for surface of the pond (d^{-1})	$K_L a_S$	18.3
Overall CO_2 mass transfer coefficient for fine bubbles (d^{-1})	$K_L a$	823.2
volume faction of gas holdup	ε	0.001
Maximum specific growth rate (d^{-1})	μ_{max}	0.7
Arrhenius temperature coefficient for growth with low light intensity	θ	1.35
Arrhenius temperature coefficient for growth with high light intensity	θ	1.16
Half-saturation constant of low NH_4-N concentrations (g m^{-3})	$K_{S,N}$	0.1
Half-saturation constant of high NH_4-N concentrations (g m^{-3})	$K_{S,N}$	1.78
Inhibition constant of high NH_4-N concentrations (g m^{-3})	$K_{i,N}$	364
Half-saturation constant of low CO_2 concentrations (g m^{-3})	$K_{S,C}$	3.6
Half-saturation constant of high CO_2 concentrations (g m^{-3})	$K_{S,C}$	4.26
Inhibition constant of high CO_2 concentrations (g m^{-3})	$K_{i,C}$	250
Light energy compensation point for high light intensity (μmol photon m^{-2} s^{-1})	I_e	1
Light saturation point for high light intensity (μmol photon m^{-2} s^{-1})	I_k	54.7
Light saturation point for low light intensity (μmol photon m^{-2} s^{-1})	I_k	16.98
Light attenuation rate (m^2 g^{-1})	k	0.2
Decay rate (d^{-1})	k_d	0.05

4.2.2.2 Mass Balance for Harvesting

In the harvesting stage, it was assumed that there is no microalgae growth, and the microalgae biomass is harvested through gravity sedimentation and centrifugation. Microalgae sedimentation velocity depends on microalgae species, ranging from 0.04-14.1 m d^{-1} (Chen et al., 2015a; Park et al., 2011). Ras et al. (2011) reported the settling velocity for *Chlorella* sp. is 3.575 m d^{-1} in their experimental results, achieving a concentration 20 times higher than the concentration in the cultivation after an hour. In this study, cone-shaped sedimentation tanks were

applied for gravity sedimentation. Also, it was assumed that the concentration of microalgae after the sedimentation process was 20 times higher than the concentration in the culture stream.

For centrifugation, Milledge et al. (2013) reviewed the harvesting technologies for microalgae, and they reported that microalgae with cell sizes in the range of 3-30 μm are suitable materials for disc tack centrfuges, which is able to obtain a microalgal solid content ranging from 2-25%. Based on this fact, it was assumed that about 10% microalgae biomass (as total solid) was achieved (100 kg m^{-3}) through centrifugation in this study. In this system, this harvesting process was modeled with 90% harvesting efficiency (Chen et al., 2011). After harvesting stage, the microalgae biomass was used for the anaerobic co-digestion, while the liquid was delivered to the final filtration system in the main stream to achieve additional solid removal. A simple mass balance for biomass in the harvesting system at steady-state can be written as below:

$$\alpha \cdot Q_{Out} \cdot X = Q_{out,centrifuge} \cdot X_c \quad (4.10)$$

where Q_{out} is the effluent flow rate of the microalgae slurry in sedimentation (m^3 d^{-1}); $Q_{out,centrifuge}$ is the concentrated microalgae slurry flow rate from centrifugation (m^3 d^{-1}), X is the concentrations of microalgae biomass in sedimentation (g m^{-3}); Xc is the concentrations of microalgae biomass after centrifugation (g m^{-3}); and α is the harvesting efficiency, which is shown in Table 4.3.

Table 4.3 Summary of model parameters for the harvesting.

Parameters		Values
Effluent flow rate of the microalgae slurry in sedimentation (m^3 d^{-1})	Q_{out}	3.75
Concentrated microalgae slurry flow rate (m^3 d^{-1})	$Q_{out,centrifuge}$	0.24-3
Harvesting efficiency	α	0.9

4.2.2.3 Model Description for Anaerobic Co-Digestion

It is generally accepted that anaerobic co-digestion has the same bio-conversion processes as anaerobic digestion, which typically includes hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Batstone et al., 2002; Gavala et al., 2003; Vavilin et al., 2008). To reduce the

complexity of anaerobic co-digestion process modeling, this study simulated the process as two steps, which is shown in Figure 4.3. It was assumed that there was no accumulation of volatile fatty acids (VFA) and methane in the liquid phase. In the co-digestion, hydrolysis was considered as step 1 (Figure 4.3) that the complex organic matter (co-substrates: mixture of microalgae and wasted sludge) was converted to soluble organics. This step was modeled using the modified first order kinetics, which considered non-biodegradable fraction of the substrates. Acidogenesis, acetogenesis, and methanogenesis were combined and considered as step 2. Step 2 was described by first order kinetics.

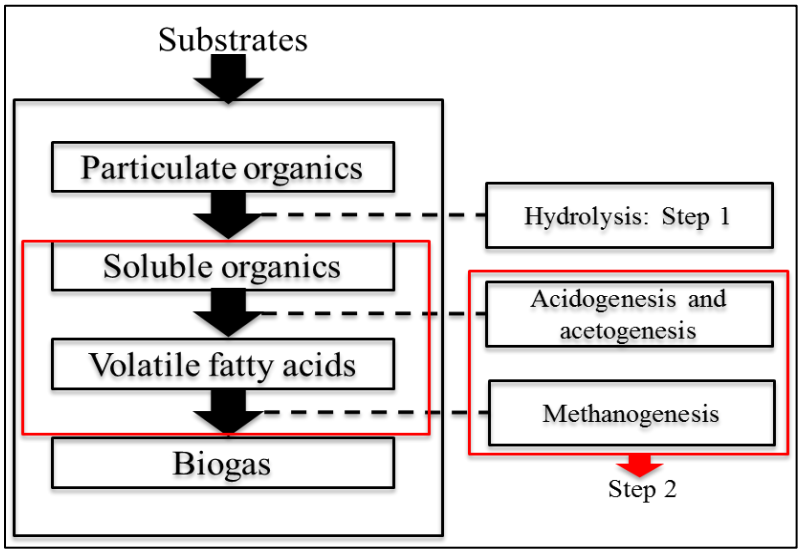


Figure 4.3 Diagram of the simplified anaerobic co-digestion model.

It was assumed that the co-digestion was operated in a cylindrical completely mixed flow rector (liquid working volume: about 1,750 m^3) under mesophilic conditions (35°C) with a 20-day retention time. The influent and effluent flow rates of the digester were assumed as constant (Q_{in}=Q_{out}=Q_{ad}). The mass balance equations of the simplified processes are shown as follows.

$$\frac{dS_{PCOD}}{dt} = \frac{Q_{ad}}{V_{ad}}\left(S_{PCOD,in} - S_{PCOD}\right) - k_{hyd}\left(S_{PCOD} - \beta S_{PCOD,0}\right) \quad (4.11)$$

$$\frac{dS_{SCOD}}{dt} = \frac{Q_{ad}}{V_{ad}}\left(S_{SCOD,in} - S_{SCOD}\right) + k_{hyd}\left(S_{PCOD} - \beta S_{PCOD,0}\right) - k_m \cdot S_{SCOD} \quad (4.12)$$

$$\frac{dG_{CH4}}{dt} = -\frac{Q_{ad\_gas} \cdot G_{CH4}}{V_{ad\_gas}} + \alpha_{ad\_m} \cdot k_m \cdot S_{SCOD} \cdot V_{ad} \quad (4.13)$$

$$\frac{dG_{CO2}}{dt} = -\frac{Q_{ad\_gas} \cdot G_{CO2}}{V_{ad\_gas}} + \alpha_{ad\_C} \cdot k_m \cdot S_{SCOD} \cdot V_{ad} \quad (4.14)$$

where $S_{PCOD,in}$ and $S_{SCOD,in}$ are influent concentrations of particulate chemical oxygen demand (PCOD) and soluble chemical oxygen demand (SCOD), respectively (g m^{-3}); S_{PCOD} and S_{SCOD} are effluent concentrations of PCOD and SCOD, respectively (g m^{-3}); Q_{ad} and ; Q_{ad_gas} are the liquid and gas flow rate, respectively (m^3 d^{-1}); V_{ad} and V_{ad_gas} are the liquid and gas volume of the digester, respectively(m^3); G_{CH4} and G_{CO2} are methane and CO_2 productions, respectively (m^3); α_{ad_m} and α_{ad_C} are the conversion factors for methane gas and CO_2, respectively, (m^3 g^{-1}); and k_{hyd} and k_m are kinetic coefficients of step 1 and step 2, respectively (d^{-1}). The kinetic coefficients of step1 ($k_{hyd,}$) and step 2 (K_m) were estimated using the regression-based model introduced in Chapter 3. The parameters for the regression-based model are provided in Appendix B. The gas flow rate (Q_{ad_gas}) is calculated based on total gas transfer, shown in Appendix B. The parameters used in the anaerobic co-digestion was listed in Table 4.4.

Table 4.4 Summary of model parameters for the anaerobic co-digestion.

Parameters		Values
Liquid flow rate (m^3 d^{-1})	Q_{ad}	87-90
Liquid volume of the digester (m^3)	V_{ad}	1,750-1,800
Gas volume of the digester (m^3)	V_{ad_gas}	900-950
Kinetic constant for hydrolysis (d^{-1})	$k_{hyd,}$	0.12
Non-degradable fraction	β	0.72
Kinetic constant for methanogenesis (d^{-1})	K_m	3.3
Conversion factor for methane gas (m^3 CH_4 g^{-1}SCOD)	α_{ad_m}	0.013
Conversion factor for biogas (m^3 CO_2 g^{-1}SCOD)	α_{ad_C}	0.006

4.2.3 Model Performance

The series of mass balance equations (Eq. 4.3-4.14) were solved using Matlab R2014a software. The microalgae biomass, aqueous CO_2, and NH_4-N concentrations for cultivation were solved using the finite element method with Taylor series expansion and the Euler method solver (ode15s), while the simplified anaerobic co-digestion model was solved by the Euler method solver (ode15s). The sensitivity of model outputs to model parameters were evaluated by varying CO_2 sparging area (10%, 25% (base case), 50%, and 80% of the pond area) and NH_4-N concentrations (200, 397 (base case) and 500 g m^{-3}). The initial conditions of this study are shown in Table 4.5.

Table 4.5 Input parameters for the process modeling.

Parameters	Value
Initial microalgae concentration (g m^{-3})	100
Initial NH_4-N concentration (g m^{-3})	40
Initial CO_2 concentration (g m^{-3})	20
Gas phase concentration of CO_2 at air-water interface of the fine bubble (g m^{-3})	205
Gas phase concentration of CO_2 at air-water interface of the pond surface (g m^{-3})	0.57
Flow rate of waste sludge (m^3 d^{-1})	87
PCOD concentration of waste sludge (kg m^{-3})	49
SCOD concentration of waste sludge (kg m^{-3})	4.8

4.3 Results and Discussion

4.3.1 Performance of Integrated Process Model

Since there is no pilot or full-scale data for the integrated system, the model was validated by comparing the simulation results with published data for microalgae cultivation and anaerobic digestion of wasted sludge. For cultivation, the data were obtained from Passos et al. (2015). In their study, cultivation was performed in a pilot-scale High Rate Algae Pond (HRAP) with 1.54 m^2 (0.3 m depth, 8 day hydrualic retention time). Wastewater was continuously pumped to the pond with 0.06 m^3 d^{-1} flow rate. The system was operated over a year, from July 2012 to July 2013

in Barcelona, Spain. Figure 4.4 (a) shows the microalgae areal productivity predicted from the cultivation model developed in this study compared with the data from their pilot system. The model was able to predict quite well microalgal biomass productivity in the HRAP. For anaerobic digestion, the data was obtained from Ozkan-Yucel and Gökçay (2010). The system is the full scale anaerobic digester of Ankara Central Wastewater Treatment Plant (Turkey) operated at mesophilic condition (35°C) with 10,800 m^3 working volume (23-day hydraulic retention time). The influent sludge was 1:1 mixture of primary and secondary sludge (2.44±0.39% VS). The organic loading was varied between 0.71-2.16 kg COD m^{-3} d^{-1} (average 1.4 kg COD m^{-3} d^{-1}). Figure 4.4(b) shows the CH_4 production predicted from the anaerobic digestion model developed in this study compared to the data from the full scale digester. The predicted CH_4 productions were close to the measured CH_4 production from the existing study (Ozkan-Yucel and Gökçay, 2010). The results were statistically tested, which is shown in Table 4.7. As shown in Table 4.7, the means of simulated results and actual data are not significantly different using a 0.05 of significance level.

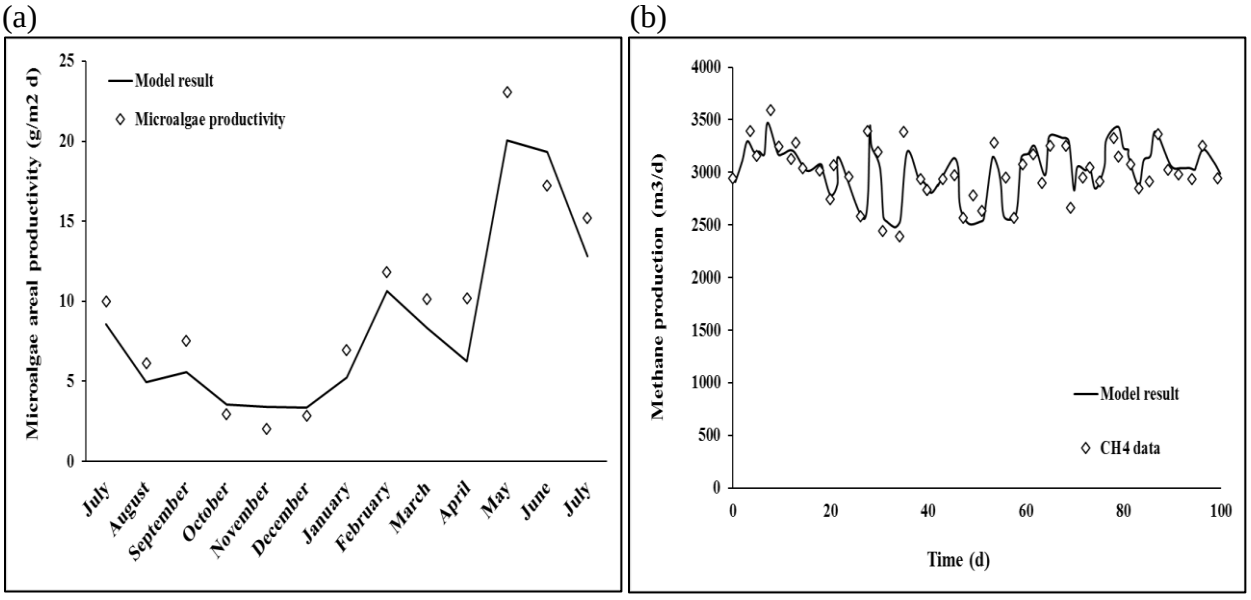


Figure 4.4 Existing data compared to model simulations: (a) Microalgae productivity and (b) Methane production.

Table 4.6 T-test results for mean differences between the model simulation data and existing data from the microalgae cultivation and anaerobic digestion. Note: Null hypothesis: Existing data=Model result, Significance level (α) =0.05.

Source	Mean	Standard Deviation	t	P value
Existing data for the productivity	9.698	6.104	0.4658	0.6456
Model results for productivity	8.621	5.678		
Exiting data for CH_4 production	3006	263	-0.2926	0.7705
Model result for CH_4 production	3020	249		

The validated process model was used to simulate the integrated system for 365 days. The simulation results presented the dynamics of the microalgae cultivation and anaerobic co-digestion systems for 25% CO_2 sparging area in the open pond, which was shown in Figures 4.5 and 4.6. Microalgae areal productivity increased with increased light intensity and temperature (Figure 4.5). Between 128-276 days (May-Oct.), the high productivities were achieved due to high incident light intensity and temperature (average light intensity: 2,130 μmol photon m^{-2} s^{-1}; Average Temperature: 27°C). According to Ogata et al. (1987), both light intensity and temperature significantly influenced the microalgae productivity. Yoder (1979) found high correlations among growth rate, temperature and light intensity (> 0.97). Also, it was observed that high microalgae areal productivity resulted in increased aqueous CO_2 concentration due to released respiratory CO_2 during the night (Červený et al., 2009) (Figure 4.5 (a)).

After 276 days, the microalgae productivity decreased due to decreased temperature. In addition, high concentrations of microalgae in the cultivation system reduced the average light intensity so that the productivity was steadily reduced. The average productivity was about 41 g $m^{-2}d^{-1}$ for 365 days, and the productivity was about 77 g $m^{-2}d^{-1}$ during the summer season. During the cultivation, the light/dark cycle resulted in variation of the microalgae productivity. According to Chisti (2016), the microalgae productivity for the raceway pond system varied between 25-50 g m^{-2} d^{-1}. The productivity of this study was within the range

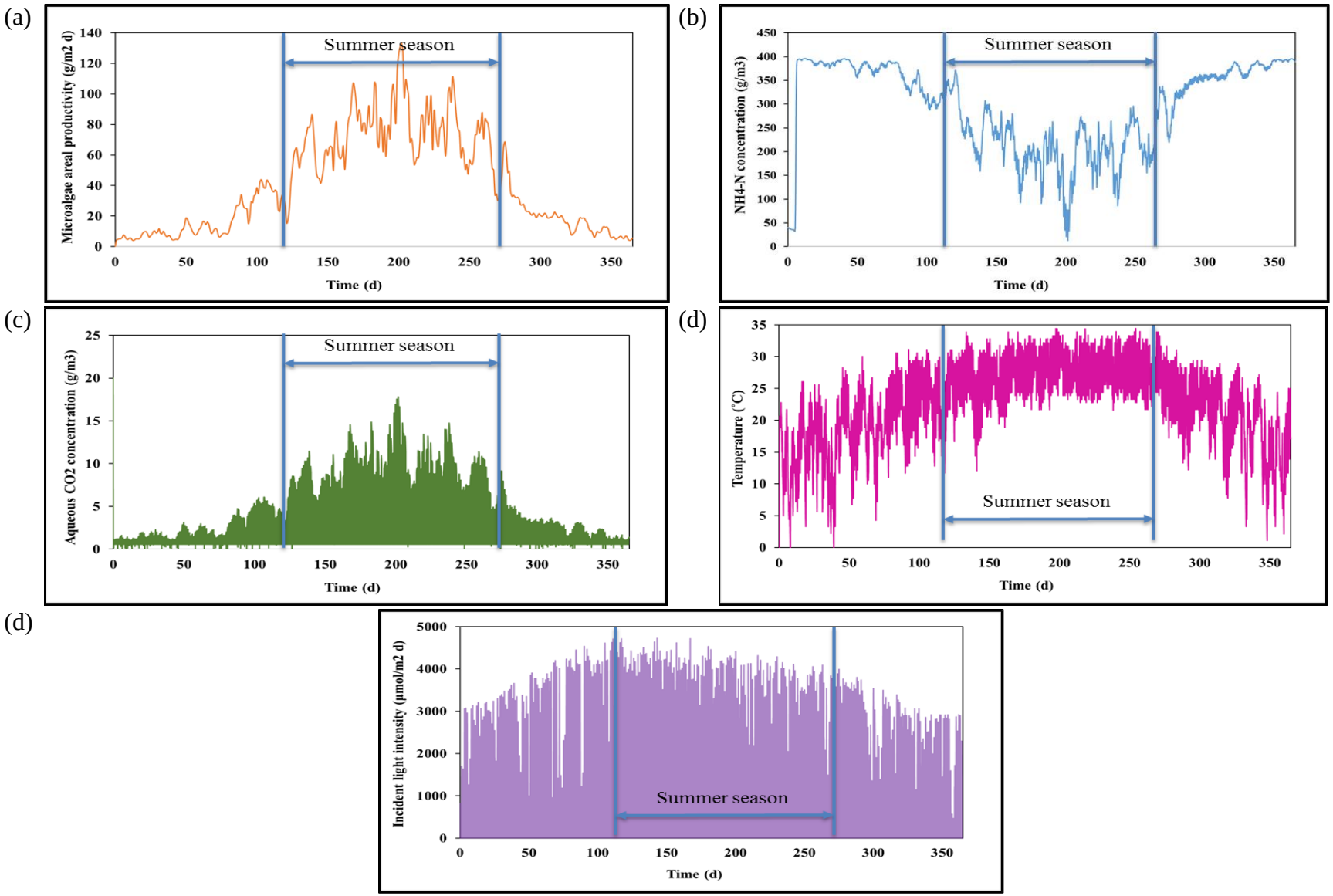
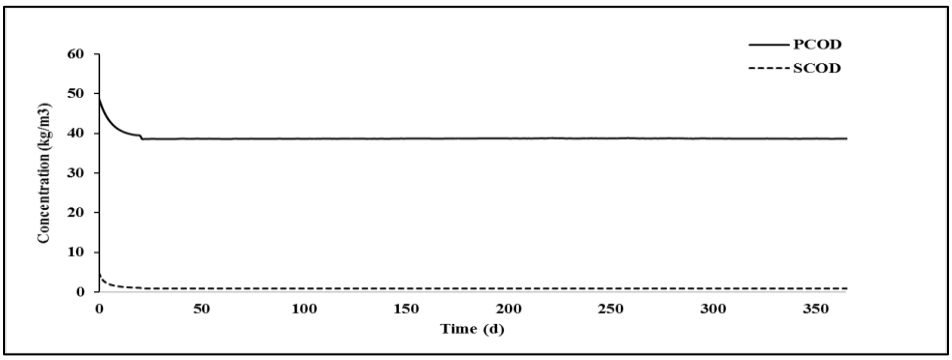


Figure 4.5 Simulation results for microalgae cultivation at 365-day operation: (a) Microalgae areal production, (b) Effluent concentration of NH_4-N, (c) Aqueous CO_2 concentration, (d) Temperature, and (e) Incident light intensity.

For the cultivation, 50% NH_4-N removal efficiency was achieved during the summer season. During the winter season, however, the cultivation system had low microalgae productivity due to low temperature (0-80 days and 325-365 days, average temperature 16.8°C), which resulted in low NH_4-N removal. The removal efficiency of NH_4-N was lower than that of Bello et al. (2015) (88%), because different cultivation conditions, such as temperature and light intensity as well as influent NH_4-N concentrations, resulted in different microalgae concentrations in the cultivation system. In their study, microalgae system was modelled under 20°C, 77.8 MJ m^{-2} d^{-1} (4,698 μmol m^{-2} d^{-1}), and 90 g NH_4-N m^{-3}. Previous studies reported that NH_4-N removal efficiency in open ponds using wastewater with moderate NH_4-N concentrations (<100 g NH_4-N m^{-3}) ranged from 60 to 99.5% (Batista et al., 2015; Posadas et al., 2015). In the pond system, ammonia stripping had played an important role as a NH_4-N removal mechanism (Passos et al., 2015). In this study, because the ammonia stripping was excluded, the NH_4-N removal efficiency was lower than the previous studies. To obtain better simulated results for NH_4-N concentrations, ammonia stripping needs to be considered in the process modeling in the future. Also, since the simulated results were based on the fixed design parameters, other design parameters, such as the retention time (solid and hydraulic), cross-sectional area of the system, internal recycling, and depth of the pond system, need to be optimized to improve NH_4-N removal of the integrated system.

Microalgae biomass was harvested from two-stage harvesting process. The percentage of the microalgae for the co-digestion (by VS) was in the range of 1-5. Based on microalgae biomass obtained from the cultivation system (25% CO_2 sparging area) and waste sludge produced from the main wastewater treatment process, the biogas production and COD for the integrated system were estimated using the integrated process model. Figure 4.6 shows the simulated co-digestion results for biogas productions and COD concentrations.

(a)



(b)

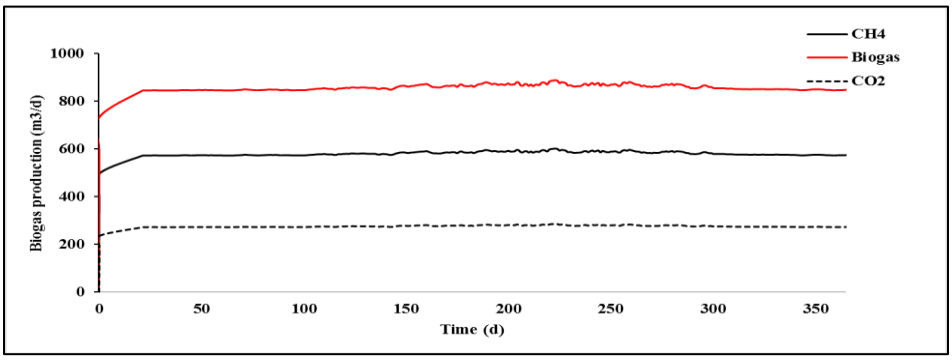


Figure 4.6 Simulation results of the anaerobic co-digestion for 365 days: (a) COD concentrations and (b) Biogas production.

The system was able to produce about average 858 m^3 d^{-1} biogas that includes 582 m^3 d^{-1} CH_4 and 276 m^3 d^{-1} CO_2 (68% CH_4, 32% CO_2), and 26% COD removal (about 54% solid reduction) was achieved. The biogas production was stable over a year, because microalgae biomass was relatively small amount compared to waste sludge. The percentage of the CH_4 content in this study was within the typical range for anaerobic digestion of sewage sludge, which is 60-70% CH_4 (Wong, 2011). Compared to the anaerobic digestion system for a 4 MGD WWTP (CoW, 2010), the biogas production in this study was higher than that from their system (221-538 m^3 d^{-1} with 60-65 % CH_4) due to high amount of biosolids from waste sludge and microalgae biomass.

In their system, approximately 45% VS content was reduced, which was lower than our system. This result is consistent with the previous finding from the study of Wang et al. (2013) that volatile solid reduction of anaerobic digestion for waste activated sludge alone was lower than

the co-digestion of microalga and waste activated sludge. Thus, additional biomass from microalgae improved the biogas production as well as solid reduction due to the synergetic effects of the co-digestion (Mata-Alvarez et al, 2014).

4.3.2 Results from Sensitivity Analysis

The integrated system with 25% CO_2 sparging area and 397 g m^{-3} NH_4-N concentration in influent of the cultivation system was selected as a base case for the sensitive analysis. Figure 4.7 shows the results (microalgae areal productivity, NH_4-N concentrations in effluent of the cultivation system, CH_4 production, and biosolids production) for changing of CO_2 sparging areas and influent NH_4-N concentrations. The results show that the productivity increased with increasing CO_2 sparging areas, because increasing CO_2 sparging areas improved aqueous CO_2 concentrations in the culture, which directly affected microalgae growth rates. Thus, the highest productivity was achieved in the integrated system with 80% CO_2 sparging area. In addition, because of high sparging area with the relatively shallow raceway pond (0.3 m depth), the cultivation system has similar performance as a photobioreactor. The productivity (91 g $m^{-2}d^{-1}$) in this study was comparable to productivity of photobioreactor (60-1,148 g m^{-2} d^{-1} (0.2-3.8 g L^{-1} d^{-1})) (Kumar et al., 2015). The largest increase in productivity occurred when the sparging area for the cultivation increased to 50% CO_2 due to high adsorption of CO_2 with low emission of CO_2 from liquid (culture medium) to the atmosphere (Yang, 2011). The removal of NH_4-N was not sensitive to CO_2 sparging areas due to low temperature during winter seasons, as shown in Figure 4.7 (a). The NH_4-N removal of 38% was achieved in the integrated system with 50% CO_2 sparging area. The changes in CO_2 sparging areas have small impact on CH_4 and biosolids production as shown in Figure 4.7 (b), because relatively small amount of the harvested microalgae biomass used in the co-digestion (about 5% of the feeding for the digestion by VS).

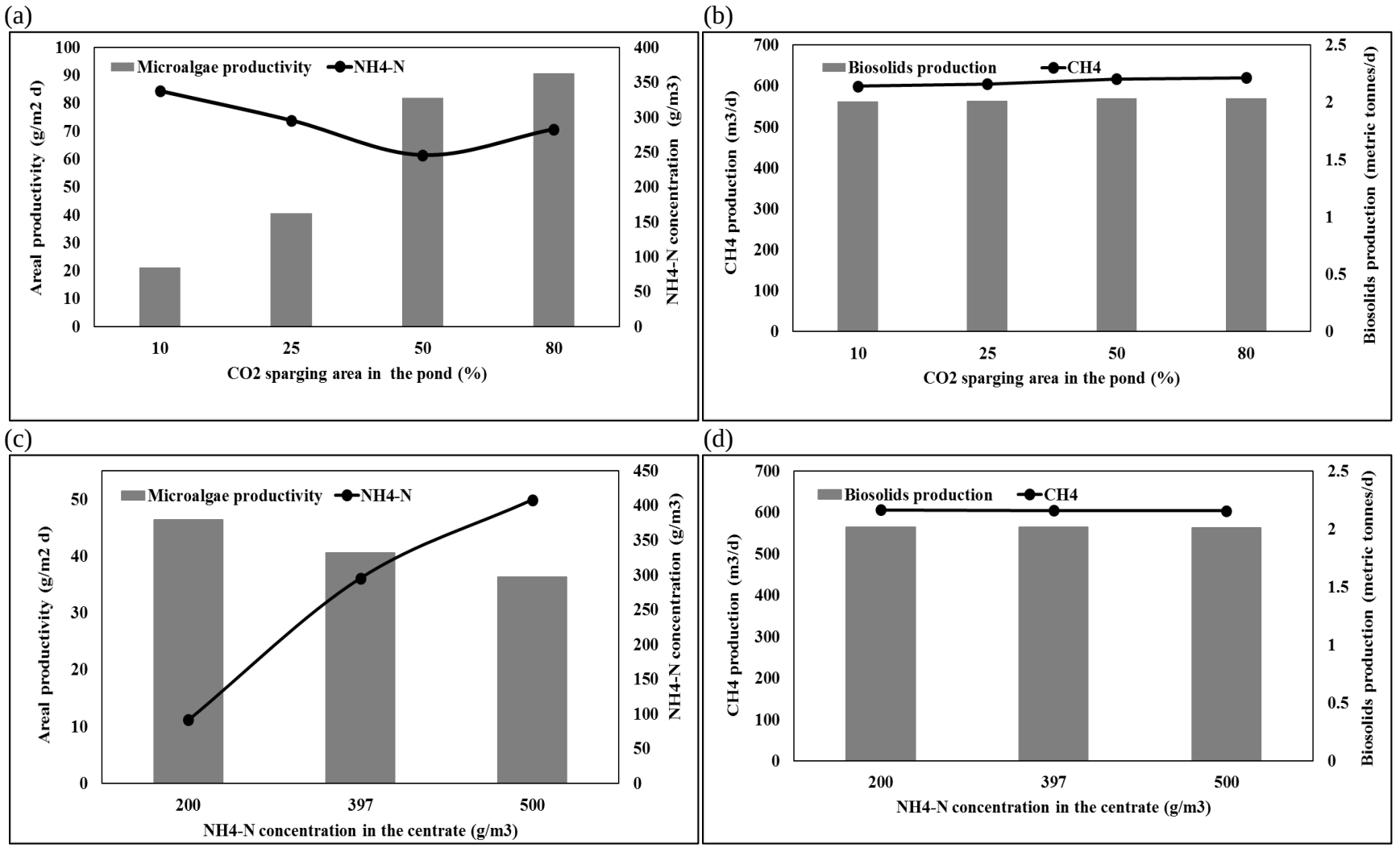


Figure 4.7 Simulation results of the integrated system with varying CO_2 sparging area and NH_4-N concentration: (a) Microalgae areal productivity and NH_4-N concentrations for different CO_2 sparging areas in the cultivation system, (b) CH_4 and Biosolids productions for different CO_2 sparging areas in the cultivation system, (c) Microalgae areal productivity and effluent NH_4-N concentrations for different influent NH_4-N concentrations, and (d) CH_4 and Biosolids productions for different influent NH_4-N concentrations.

For variations of influent NH_4-N concentration, the highest microalgae productivity (46 g m^{-2} d^{-1}) with 54% N removal efficiency was observed in the integrated system with influent NH_4-N concentration of 200 g m^{-3} (Figure 4.7 (c)). When NH_4-N concentration was higher than 250 g m^{-3}, the microalgae growth was inhibited by NH_4-N (Lee and Zhang, 2016). Thus, the productivity decreased with increasing NH_4-N concentrations. Because of microalgae experienced N inhibition during cultivation with 397 and 500 g NH_4-N m^{-3}, the system had low N removal efficiency. For co-digestion, the impact of microalgae biomass on CH_4 and biosolids production was insignificant, because relatively small amounts of microalgae was used in the co-digestion.

4.3.3 Mass Balance on N and P in the Integrated System

Mass balance on N and P was conducted based on their concentrations from the simulated results (the integrated system with 25% CO_2 sparging area with 397 g N m^{-3}) and experimental data (real centrate data (Chapter 2) and waste sludge characteristics obtained from Fountoulakis et al. (2010)). In this analysis, outgassing of NH_3 gas was not considered. Also, only microalgae assimilation was considered as the N and P removal mechanisms during the cultivation (Denitrification was not considered). In addition, it was assumed that there is N and P accumulation in the integrated system.

Figure 4.8 shows the N and P mass balances in the integrated system. The anaerobic digester daily received approximately 15 kg N and 3 kg P from harvested microalgae and 89 kg N and 72 kg P from waste sludge. After dewatering of anaerobically digested sludge, N and P recovered in the biosolids (digestates) are 71 kg N d^{-1} and 57 kg P d^{-1}, respectively. 18 kg N d^{-1} and 15 kg P d^{-1} are released with the treated water, which flows back to the secondary treatment process.

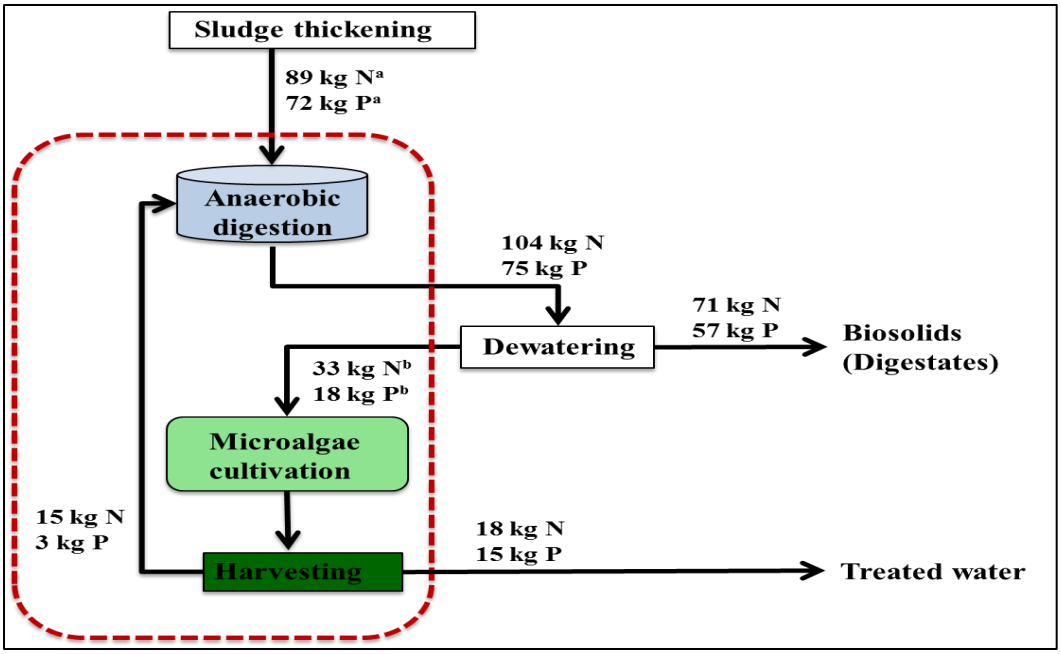


Figure 4.8 Nutrient mass balance on daily basis for the integrated system. Note: red box: system boundary, [a]: data obtained from Fountoulakis et al. (2010) and [b]: data obtain from actual centrate of anaerobic digestion.

4.4 Conclusions

A process model has been developed in this study to simulate the dynamic behavior of the integrated microalgae system with wastewater treatment. The microalgae system with 25% CO_2 sparging area achieved the areal productivity of average 41 g m^{-2} d^{-1} and average effluent NH_4-N concentration of 295 g m^{-3}. For this integrated system, removal efficiency of NH_4-N by microalgae was increased with addition of CO_2 gas supply. The areal productivity was improved with increasing CO_2 sparging area in the cultivation system. Changing NH_4-N concentration in the influent of the cultivation system affected the areal productivity and the effluent NH_4-N concentration. The integrated system with 200 g NH_4-N m^{-3} achieved the high productivity (46 g m^{-2} d^{-1}) and NH_4-N removal due to no inhibitory effect of NH_4-N. For anaerobic co-digestion, as the microalgae productivity increased, the CH_4 and biosolids production increased as a result of the increased amount of the substrates from the harvested microalgae biomass. The increase of CH_4 and biosolids productions, however, was minor because of small amount of microalgae

biomass for the co-digestion, which accounts for only 5% of the substrate by mass. Since there is no implementation of the integrated system, the model cannot be validated using the pilot or full-scale data for the co-digestion. Future pilot studies have to be conducted to validate the model and improve the accuracy of the model prediction for the integrated system.

CHAPTER 5: LIFE CYCLE ASSESSMENT OF MICROALGAE BIOENERGY PRODUCTION COUPLED WITH WASTEWATER TREATMENT

5.1 Introduction

Wastewater treatment plants (WWTPs) require a large amount of energy to remove nutrients from influents. In 2012, 14,748 WWTPs existed in the U.S. and they consumed about 30.2 billion kWh per year. This accounts for 3-4% of total electricity use in the U.S. (USEPA, 2016; Electric Power Research Institute, 2013). In WWTPs, 60-80% of total energy was used in biological nutrient removal (BNR) processes to remove chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP) from the wastewater (Selvaratnam et al., 2015). As a result of electricity consumption, WWTPs indirectly emitted around 21 million metric tons of greenhouse gases (GHG) per year, which may contribute to global climate change (Shen et al., 2015). Because regulations for nutrient discharges have become more stringent over time, increasing energy consumption associated with BNR would be inevitable in WWTPs. Thus, it is critical for WWTPs to operate in a sustainable way so they can efficiently recover nutrients from wastewater while minimizing external energy consumption as well as reducing carbon footprints.

Many studies have investigated process modifications and technology innovations to reduce GHG emissions as well as to recover energy and nutrients in WWTPs (Wang et al., 2016). For example, use of reclaimed wastewater for landscape irrigation has proven a sustainable method to save freshwater and energy from additional treatment (Levine and Asano, 2004). Anaerobic Digestion (AD) has widely applied to produce biogas from the waste to offset a portion of energy

requirements in WWTPs (Shen et al., 2015). According to Mo and Zhang (2013), application of microalgae in wastewater treatment is a promising option to achieve nutrient and energy recovery with carbon dioxide reduction and mitigation. Systems using microalgae for wastewater treatment have shown high N and P removal efficiency (Wang et al., 2010). Unlike the traditional BNR processes, this system does not require an energy for vigorous aeration. In addition, autotrophic microalgae have an ability to mitigate carbon dioxide (CO_2) emission and convert it to energy-rich organic carbon through photosynthesis. The harvested microalgae biomass can be applied as energy feedstock to produce biogas through existing anaerobic digesters in the WWTPs (Craggs et al., 2013). Due to these benefits, integrating microalgae cultivation with wastewater treatment has been suggested (Menger-Krug et al., 2012).

To understand potential environmental impacts and energy return of microalgae system integrated with different wastewater sources, Life Cycle Assessment (LCA) studies have been conducted (Lardon et al., 2009; Clarens et al., 2010). Since secondary or primary municipal wastewaters are the main flows in the WWTPs, use of these wastewaters for an integrated system has been explored (Beal et al., 2012; Medeiros et al., 2015). A few studies (Li et al., 2011; Wu et al., 2014) used nutrient-rich municipal wastewater, such as centrates from dewatering of anaerobically digested sludge, in integrated system since such wastewater could enhance productivity of microalgae due to high N and P concentrations.

The majority of previous studies focused on energy production such as biodiesel or syngas as end-products (Drexler et al., 2014; Mu et al., 2014; Sturm et al., 2011). These studies concluded that energy production from microalgae was promising, but additional infrastructures were needed for energy conversion in the WWTPs. Instead of using harvested microalgae for biodiesel or syngas production, biogas production from AD is a preferred option because of existing

infrastructures (i.e., digesters) in many advanced WWTPs for waste sludge treatment (Chen and Chen, 2013).

There are several studies that evaluated biogas production from anaerobic digestion of microalgae (Menger-Krug, 2012). For example, Menger-Krug et al. (2012) evaluated energy balances and nutrient emissions of the overall integrated system based on theoretical assumptions and published experimental results. However, the life cycle impacts associated with nutrients were not addressed in their study. From a holistic perspective, it is important to consider carbon, nutrient, and energy balances to avoid shifting between global environmental impacts (e.g. carbon footprint) and local environmental impacts (e.g. eutrophication) (Foley et al., 2010). To understand the sustainability of the integrated system implemented in WWTPs, it is necessary to evaluate environmental impacts associated with energy, carbon, and nutrient in the system (Fang et al., 2016).

Therefore, this study analyzed the potential life cycle impacts of energy, carbon, and nutrient from the integrated system using microalgae cultivation as side-stream treatment processes for energy production. Also, economic impacts of the integrated system were assessed to better understand the benefits of the integrated system. Unlike previous studies, this study used the integrated process model developed in Chapter 4 to simulate microalgae cultivation and anaerobic digestion and linked with life cycle assessment to investigate the CO_2 supply strategies.

5.2 Materials and Methods

5.2.1 Goal and Scope Definition

This study was performed following the guidelines of ISO 14040 (International Standardization Organization (ISO), 2006). The goals of the LCA were to evaluate environmental impacts and benefits of the proposed system (integrated system) as well as compare the integrated

systems with different CO_2 supply areas (CO_2 supplied with 0%, 10%, 25%, 50%, and 80% areas in the cultivation system) to the conventional wastewater treatment system alone. To improve microalgae productivity related to the increased bioenergy production, there are several variables, such as the retention time, depth of the raceway pond, and cross-sectional area of the raceway pond, for the integrated system. Since microalgae growth (autotrophic microalgae) is usually limited by inorganic carbon source in the raceway pond, the increased CO_2 supply areas in the cultivation system improve the increasing inorganic carbon concentrations during the cultivation, which results in improved microalgae productivity (Chisti, 2016; Yang, 2011). In this research, therefore, the CO_2 supply area in the cultivation system was selected as a variable to improve microalgae productivity.

Based on the EPA 2007 report (EPA, 2007), wastewater treatment plants with capacities of less than 5 million gallons per day (MGD) (18,900 m3/d) do not produce enough biogas to make electricity generation feasible or cost-effective. For this reason, this analysis was based on an advanced wastewater treatment facility with a 5 MGD capacity. The facility consists of pretreatment (grit removal, bar screens), primary treatment, secondary treatment with BNR (A^2/O process), filtration, disinfection, waste sludge thickening (rotary-drum thickener), anaerobic digestion, digested sludge dewatering (centrifuge), and Combined Heat and Power (CHP) units. For waste sludge thickening, the waste primary sludge is thickened by the gravity thickening (6% solids concentration), while the waste secondary sludge is thickened by the rotary-drum thickener with addition of polymer (4% solids concentration). For digested sludge (biosolids), the digested sludge is dewatered by centrifugation with addition of polymer. It was assumed that biosolids have a 25% solid concentration. The system boundary of the integrated system and conventional wastewater treatment system are shown in Figure 5.1.

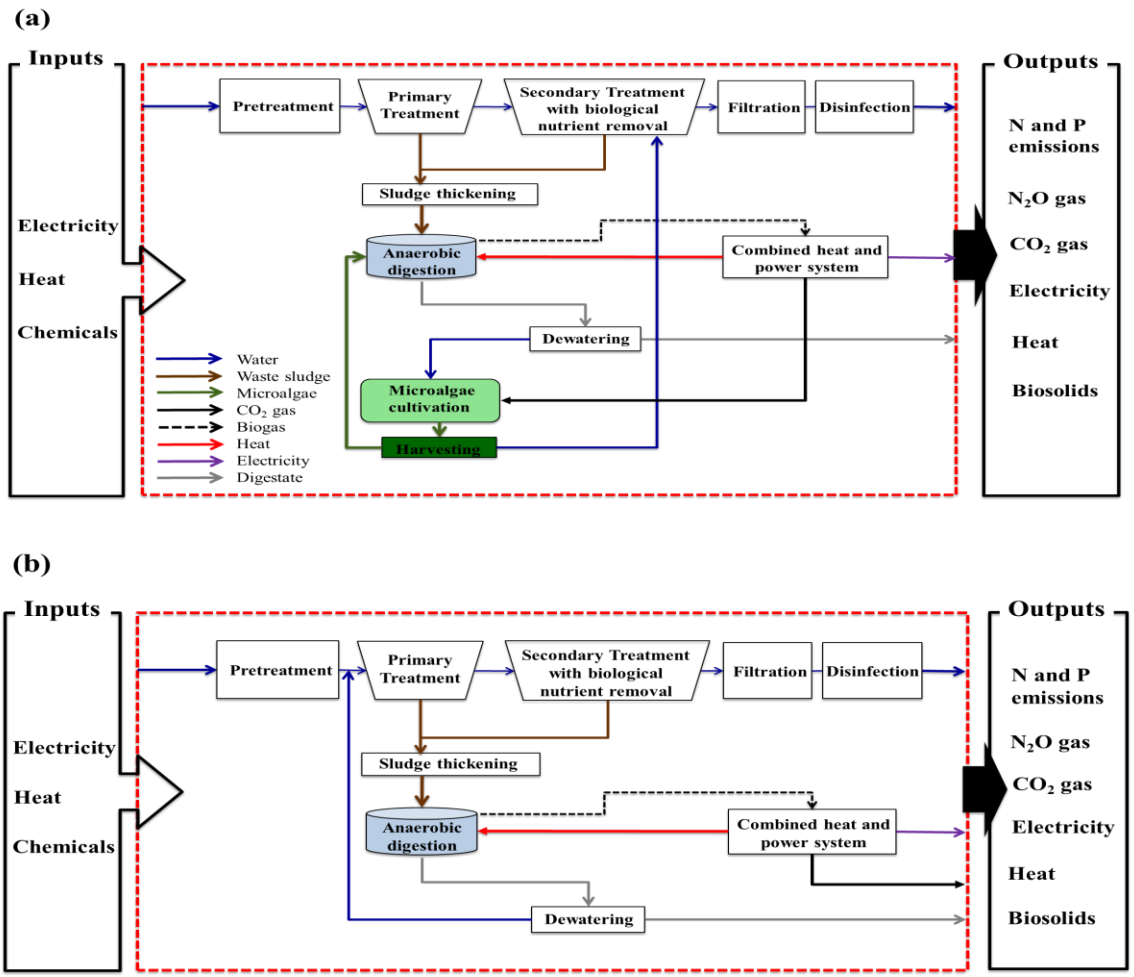


Figure 5.1 Process flow diagram of the 5 MGD wastewater treatment plant: (a) Integrated system and (b) Conventional system.

Both systems considered treated water (e.g. N and P emissions to surface water), N_2O emissions during the BNR process, CO_2 emissions from the CHP, electricity, heat, and biosolids (digestates) as outputs. For the integrated system, the CO_2 emissions from the CHP were avoided during the day because the CO_2 was used for microalgae cultivation during the daytime. The water qualities of influent and effluent in the wastewater treatment plant are summarized in Table 5.1.

Table 5.1 Influent and effluent water qualities in the 5 MGD wastewater treatment plant.

Items	BOD_5	TSS	TN	TP	References
Influent wastewater quality (mg L^{-1})	230	260	53	13	Seiple et al. (2017) Spellman (2013)
Treated effluent quality (mg L^{-1})	≤ 5	≤ 5	≤ 3	≤ 1	Florida Senate (n.d.)

The functional unit (FU) for the analysis was 5 mega gallon (MG) wastewater treated for 25-year lifespan. For this analysis, some phases were excluded, such as infrastructure construction, repair and maintenance of the infrastructure and equipment, because impacts of these phases were relatively small compared to the use phase (Lardon et al., 2009). The integrated system was modeled based on the simulation results using the integrated process model (discussed in Chapter 4), while the conventional system was modeled based on literature (Tchobanoglous et al., 2003).

5.2.2 Life Cycle Inventory

The inventory analysis compiled the data for chemical use (e.g., alum, polymer, and sodium hypochlorite), energy consumption (e.g., electricity, heat), direct emissions (e.g., N_2O from wastewater treatment, CO_2 from the CHP), nutrient emissions (e.g., N, P), resource recovery offsets (e.g., biosolids, electricity, and heat offsets) across the system during the use phase, shown on Appendix C.

Nitrous oxide emissions (N_2O) is released during biological nitrogen removal in the secondary treatment system. N_2O is an intermediate in the heterotrophic denitrification pathway (Law et al., 2012). Also, N_2O is produced by autotrophic nitrifying bacteria, such as nitrite-oxidizing bacteria and ammonia-oxidizing bacteria, in the autotrophic nitrification (Law et al., 2012). N_2O emissions during treatment were calculated based on an EPA method (USEPA, 2010), while CO_2 emissions for combustion gas from the CHP was calculated based on the complete combustion of methane (from biogas) (shown in Appendix C). However, based on IPCC guidelines (IPCC, 2006), biogenic CO_2 during treatment was not considered in this analysis.

The waste sludge produced from the primary treatment and secondary treatment systems was theoretically calculated based on Tchobanoglous et al. (2003). Biogas and biosolids (digestate)

productions for both the integrated and conventional systems were estimated using the integrated process model presented in the previous chapter (Chapter 4).

For both systems, the energy consumptions for pretreatment, primary treatment, sludge thickening of the secondary treatment were derived from Burton (1996) and Pabi et al. (2013). The energy consumptions of the chemical addition, chlorination, sludge dewatering, and disinfection were estimated based on Beal et al. (2012). The energy and chemical consumptions of the secondary treatment system were theoretically calculated based on Tchobanoglous et al. (2003). The energy demands for microalgae system and anaerobic digestion with biosolids dewatering in the integrated system were explained in the following section.

The information for the electricity and chemicals were obtained from existing inventory databases, such as Ecoinvent and the US LCI database. In this analysis, a U.S. Florida energy mix (23.65% coal, 4.42% oil, 54.83% gas, 0.63% other fossil, 1.74% biomass, 0.01% hydro, 14% nuclear, 0.005% solar, 0.7% unknown/other purchased fuel) was used to calculate the environmental impacts. Since there is no heat energy data for U.S. region in the existing inventory database, the global heat energy mix was used to calculate the environmental impacts.

5.2.2.1 Microalgae Cultivation

This study applied the indigenous green microalgae *Chlorella* sp. It was assumed that microalgae were cultivated in the open raceway pond with a water depth of 0.3 m. The facility was located in south Florida (Tampa Bay). The weather condition of Florida can be suitable for microalgae cultivation including rich sunlight and high average temperature. The microalgae production was modeled based on the integrated process model as described in the previous chapter (Chapter 4). The centrate from the anaerobic co-digestion was provided as nutrient medium for microalgae growth (average 397 mg/L NH_4-N, 238 mg/L TP, and pH of 7.81). The

detailed cultivation conditions were discussed in the Chapter 4. The energy demand of blowers for CO_2 supply was estimated by using Eq. (5.1) (Tchobanoglous et al., 2003):

$$P_{blower} = \left(\frac{Q_{gas}\, R\, T_{air}}{MW\, n_a\, e_b}\right)\left[\left(\frac{P_{in}}{P_{out}}\right)^{0.283} - 1\right] \quad (5.1)$$

where P_{blower} is the power requirement for blowers, kW; Q_{gas} is the mass flow rate of the combustion gas, kg/s; R is the universal gas constant, 8.314J/mol K; T_{air} is the absolute temperature of the combustion gas, K; MW is molecular weight of the combustion gas, 29.7 g/mol; n_a is the constant used in determining blower power, (k_{CO2}-1)/k_{CO2}; k_{CO2} is the ratio of specific heat for CO_2, 1.28; e_b is the blower net efficiency, 0.7; P_{in} is the inlet air pressure in diffuser, 8.5 atm ; and P_{out} is the outlet pressure, 1 atm.

A single paddle wheel with a velocity of 20 m/s was used for the culture mixing and circulation. The power requirement for the paddle wheel was calculated according to Chisti (2013):

$$P_{paddle} = \frac{1.59\, A_P\, \rho\, g\, v^3 f_M^2}{e_m\, d_h^{0.33}} \quad (5.2)$$

where P_{paddle} is the power requirement for the paddle wheel, W; A_P is the surface area of the pond, m^2; ρ is the density of the culture, kg/m^3; g is the gravitational acceleration, 9.81 m/s^2; v is the mixing velocity, m/s; f_M is the Manning channel roughness factor, 0.015 $s/m^{0.33}$ for an unfinished concrete surface; e_m is the efficiency of the paddle wheel system, 0.17; and d_h is the hydraulic diameter, 1 m.

5.2.2.2 Microalgae Harvesting

Microalgae harvesting was done in two steps, gravity setting and centrifugation. The setting velocity was assumed to be 3.575 m/d, which allowed microalgae biomass (*Chlorella* sp.) reaching a concentration 20 times higher than the culture concentration (Las et al, 2011). The centrifugation was done through disc-stack centrifuge (ALFA LAVAL ALSYS 20), which

concentrated microalgae biomass to the concentration 5 times higher than that from the gravity-settling step. The energy requirement for microalgae harvesting was calculated as follows (Sazdanoff, 2006):

$$P_{centrifuge} = \frac{V_s C_{centri}}{Q_h} \quad (5.3)$$

where $P_{centrifuge}$ is the power required by centrifuge, kWh; V_s is the volume of slurry that goes through the centrifuge by daily basis, m^3; C_{centri} is the centrifuge motor power depending on the capacity, 25 kW; and Q_h is the nozzle flow rate depending on the capacity, 5 m^3/h.

5.2.2.3 Anaerobic Co-Digestion and Energy Generation

The anaerobic co-digester was designed as a completely mixed flow reactor with a hydraulic retention time (HRT) of 20 days. The energy requirement for mixing was estimated by using the following equation (Tchobanoglous et al., 2003):

$$P_{mixing} = G^2 \mu V_{ad} \quad (5.4)$$

where P_{mixing} is the power requirement for mixing, W; G is the average velocity gradient, 70 s^{-1}; μ is the dynamic viscosity, N s/m^2; and V_{ad} is the working volume of the digestion, m^3.

It was assumed that the digester is able to treat an average flow of 89 m^3/ d with 5% Total Solid (TS). This leads to an organic loading rate (OLR) of 29310 g COD/m^3 d. It was assumed that the digestion was performed under mesophilic conditions (35°C). The heat power required for the digestion was calculated based on Wang et al. (2016):

$$P_{heat} = \rho Q \gamma (T_R - T_{in}) + 0.024\, kA(T_R - T_{in}) \quad (5.5)$$

where P_{heat} is heat requirement for the digester, kWh/d; γ is the specific heat for the water, 0.0012 kWh/kg °C; T_R is the temperature in the digester, °C; T_{in} is the temperature of influent (Detailed temperature information is showed in supplementary data), °C; k is the heat transfer coefficient, 0.7 W/m^2 °C; and A is the surface area of the digester wall, m^2. The heat required for operating the

digester was provided by the Combined Heat and Power (CHP) unit (Efficiency of heat 51%, Efficiency of electricity 27%). If additional heat is required, a biogas boiler with the efficiency of 88% was used. Based on the methane production from the digester, the energy production was estimated according to (Wang et al., 2016):

$$P_{Production} = Y_{CH4}\delta n_i \quad (5.6)$$

where $P_{production}$ is the energy production, kWh/d; Y_{CH4} is the methane yield, m^3/d; δ is the low heating value of methane, 9.94 kWh/m^3; and n_i is the energy conversion efficiency in terms of *i*, such as heat or electricity.

5.2.2.4 Dewatering of Anaerobically Digested Sludge

Stabilized biosolids from anaerobic co-digestion were assumed to be dewatered to a solids content of 25% using a centrifuge, with polymer added as a coagulant to increase the dewatering efficiency. The energy requirement for dewatering was calculated based on the Eq. 5.3.

5.2.3 Life Cycle Impact Assessment and Interpretation

The life cycle impact assessment was conducted through SimaPro 8 (PhD version) using the TRACI (Tool for the Reduction and Assessment of Chemical and other environmental Impacts) method. The TRACI is a midpoint impact assessment method that is based on U.S. data. Among the impact categories, the eutrophication and global warming potential were selected to represent the potential environmental impacts regarding carbon and nutrient, while the embodied energy was estimated by using the cumulative energy demand method (expressed as MJ/5 MG). In addition, the energy balance was assessed over 25 years and expressed as kWh/5 MG.

5.2.4 Life Cycle Cost Analysis

A cost analysis was conducted on the integrated and conventional systems using the present value (PV) method. Microalgae cultivation and harvesting processes are additional systems in the

existing WWTPs, which requires additional infrastructures. For this reason, this analysis includes operation costs for the integrated and convention systems and capital costs for microalgae systems (cultivation and harvesting). Cost of labor was not included in the scope of the analysis. All cost calculations are based on 2017 dollars. The life cycle cost (LCC, $/5 MG) was computed as follows:

$$LCC = C_P + C_{O,E} \times UPV^* + C_{O,C\&H} \times UPV - (C_{R,H\&B} \times UPV + C_{R,E} \times UPV^*)/FU \quad (5.7)$$

where C_P is the capital cost for microalgae system including cultivation and harvesting, $C_{O,E}$ is the operation cost for electricity consumption, $C_{O,C\&H}$ is the operation costs for heat and chemical use, $C_{R,H\&B}$ is the revenues from biosolids and heat sales, and $C_{R,E}$ is the revenue from electricity sale. UPV is a uniform PV factor, and UPV* is a non-uniform PV factor. The parameters for the life cycle cost analysis are shown in Table 5.2.

Table 5.2 Parameters for the life cycle cost analysis.

Item		Value	Reference
Operation cost	Interest rate	0.05	Amini et al. (2015)
	Escalation rate	0.01	Amini et al. (2015)
	Biosolids price ($/metric tonne)	11.2	Schwarzenegger (2010)
	Electricity price ($/kWh)	0.08	EIA (2017)
	Heat rate ($/kWh)	0.01	Moriarty (2013)
	Chlorine ($/kg)	0.4	Baresel et al. (2015)
	Polymer($/kg)	4	Baresel et al. (2015)
	Alum ($/kg)	0.16	Jiang et al. (2005)
Capital cost for microalgae cultivation and harvesting systems	Earthworks ($/m^2)	1.67	Gao et al. (2012)
	Walls and structural($/m^2)	1.36	
	Mixing system ($/m^2)	0.81	
	Instrumentation ($/m^2)	0.08	
	Settling ponds ($/m^2)	1.23	
	Centrifuges ($/m^2)	0.65	
	Water supply/distribution system ($/m^2)	0.73	
	CO_2 distribution ($/m^2)	0.04	
	Electricity distribution & supply ($/m^2)	0.32	
	Engineering/Construction contingency ($/m^2)	1.92	

5.3 Results and Discussion

5.3.1 Impact of Nutrients

Emissions of nutrients to surface water (discharge of the treated water) led to local environmental impacts such as eutrophication. The nutrient impacts were represented as eutrophication potential (expressed as kg N eq./5 MG). Figure 5.2 (a) shows that there is no significant difference between the integrated and conventional systems for the eutrophication potential. This is mainly because the direct nutrient emissions (e.g., nutrient discharged directly to the environment) greatly contributed to the eutrophication potential for all systems, which accounts for >95% of the eutrophication potential. For the direct emission to water, both systems have the same eutrophication potential because the systems have the same effluent quality in terms of nutrients (e.g. N and P).

Figure 5.2 (b) shows the eutrophication potential without considering the direct emission to water for the integrated and conventional systems. The integrated system had the lower eutrophication potential than the conventional system, due to the reduced demands from chemical, heat, and electricity. Compared to the conventional system, the biosolids offset for the integrated system was reduced, due to lower waste sludge produced from the integrated system. For the integrated systems, the eutrophication potential slightly increased with increasing CO_2 supply areas due to the increase in the electricity demand from microalgae cultivation. Because the sum of the offsets from biosolids, heat, and electricity was similar to the sum of the demands, the net eutrophication potential for the integrated systems was similar to the conventional system.

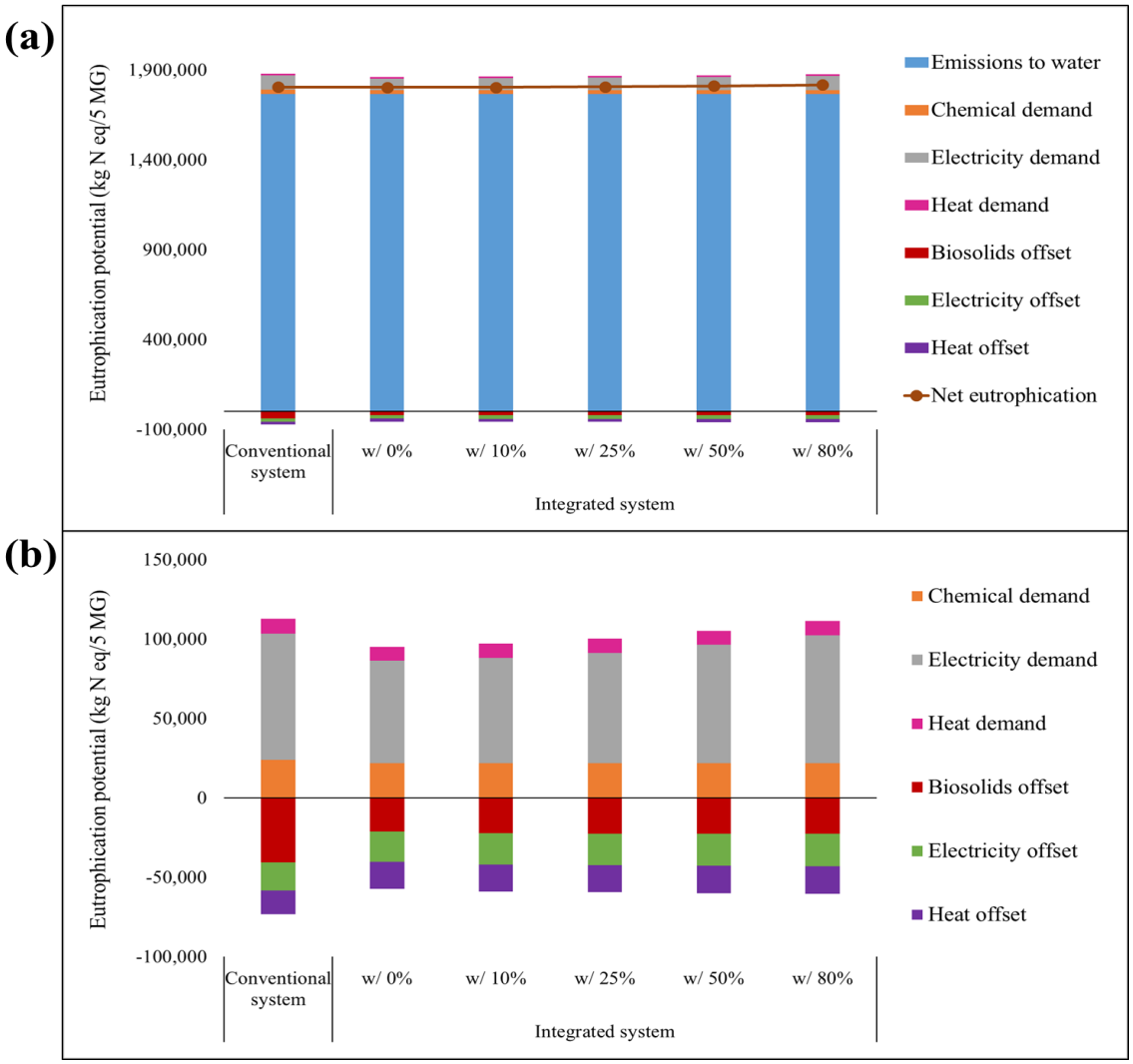


Figure 5.2 Eutrophication potential of the conventional system and integrated systems with different CO_2 supply areas (0, 10, 25, 50, and 80% CO_2 supply area); (a) Eutrophication potential considering all factors and (b) Eutrophication potential without considering the emission to water.

5.3.2 Carbon Footprint

In this study, the carbon footprint was represented by global warming potential (expressed as kg CO_2 eq./5 MG). Figure 5.3 shows the results of carbon footprint for the integrated and conventional systems. The integrated systems had the lower carbon footprint than the conventional system. For all systems, the major contributor to carbon footprint was the electricity demand followed by emissions to air (N_2O from wastewater treatment and CO_2 gas from the CHP). Considering the integrated systems with changing CO_2 supply areas in the microalgae cultivation

system (10, 25, 50, and 80% CO_2 supply areas), the overall carbon footprint increased with the increase in CO_2 supply areas. This is mainly because the increased CO_2 supply areas resulted in the increasing electricity demand for blower used in the microalgae cultivation system. It was found that the biosolids, electricity, and heat offsets from the integrated systems increased with the increase in CO_2 supply areas due to an improvement of the microalgae biomass production.

The integrated system without CO_2 addition was able to reduce the overall carbon footprint as a result of the decreased electricity demand for wastewater treatment as well as the increased offsets from electricity and heat, compared to the conventional system. In the integrated systems, diverting the recirculation flow of the centrate resulted in a reduction of the electricity demand for extensive aeration. In the typical conventional system, the extensive aeration is required for treating the centrate containing high N loading (up to 30% for N) recirculated to the headwork of the treatment plant in order to meet stringent limits of the effluent discharge (Kotay et al., 2013). It was found that the carbon footprint reduction from wastewater treatment was greater than the carbon offsets associated with beneficial products provided by the integrated system. This indicated that the integrated system provided more benefits from the reduced electricity use for wastewater treatment than from beneficial products (electricity and heat).

As shown in Figure 5.3, the integrated systems were not able to achieve the carbon neutrality, but the carbon footprint was greatly reduced for the integrated systems with 10% CO_2 supply area, compared with the conventional system. This is mainly due to the decreased electricity demands for wastewater treatment and avoided direct CO_2 emissions from the CHP. Compared to the integrated system without CO_2 addition, the integrated systems with CO_2 addition were able to reduce the direct CO_2 emissions. In this integrated system, the CO_2 from the combustion of methane in the CHP was used for microalgae cultivation. Through photosynthesis, microalgae was

able to uptake CO_2 and convert to organic carbon. This led to the large reduction of the direct CO_2 emissions to air.

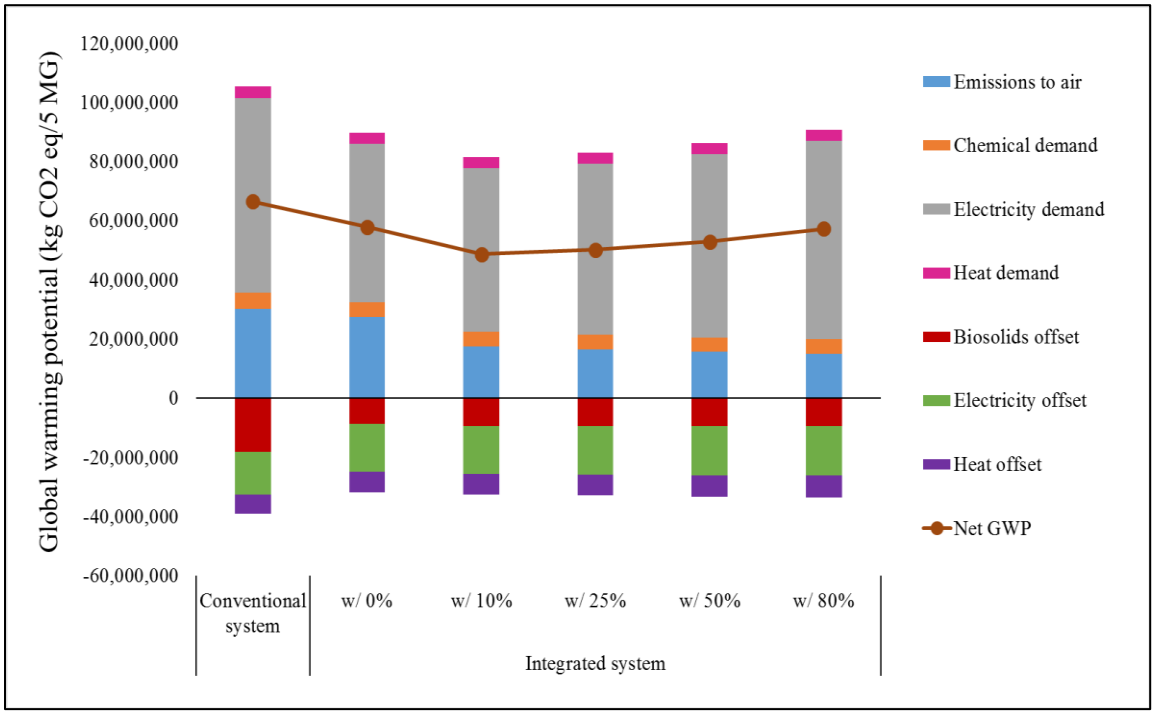


Figure 5.3 Global warming potential (GWP) of the conventional system and integrated systems with different CO_2 supply areas (0, 10, 25, 50, and 80% CO_2 supply areas) in microalgae cultivation systems.

5.3.3 Impact of Energy

The energy balance for the integrated and conventional systems are shown in Figure 5.4. The energy demand for the integrated systems was lower than the conventional system, even though the integrated systems required additional energy for the microalgae cultivation and harvesting systems. The lower energy demand for the integrated system was attributed to the reduced energy demand for the secondary treatment system, because the integrated system reduced the N loading in the mainstream of wastewater treatment by diverting the centrate to the microalgae cultivation. The reduction of N loading resulted in reduction of aeration energy, which accounts for 48% of total energy demand in the conventional system. Similar results were also found in Menger-Krug et al. (2012). The second largest contributor for energy demand was anaerobic

digestion, because the anaerobic digestion required a high energy input for heating. For all options, the heat produced from the CHP was able to meet the heat requirement for the anaerobic digestion, because of the warm weather condition in the Tampa area. In terms of energy production, the energy offsets from the integrated systems was higher than the offsets from the conventional system due to the high methane production of the integrated system.

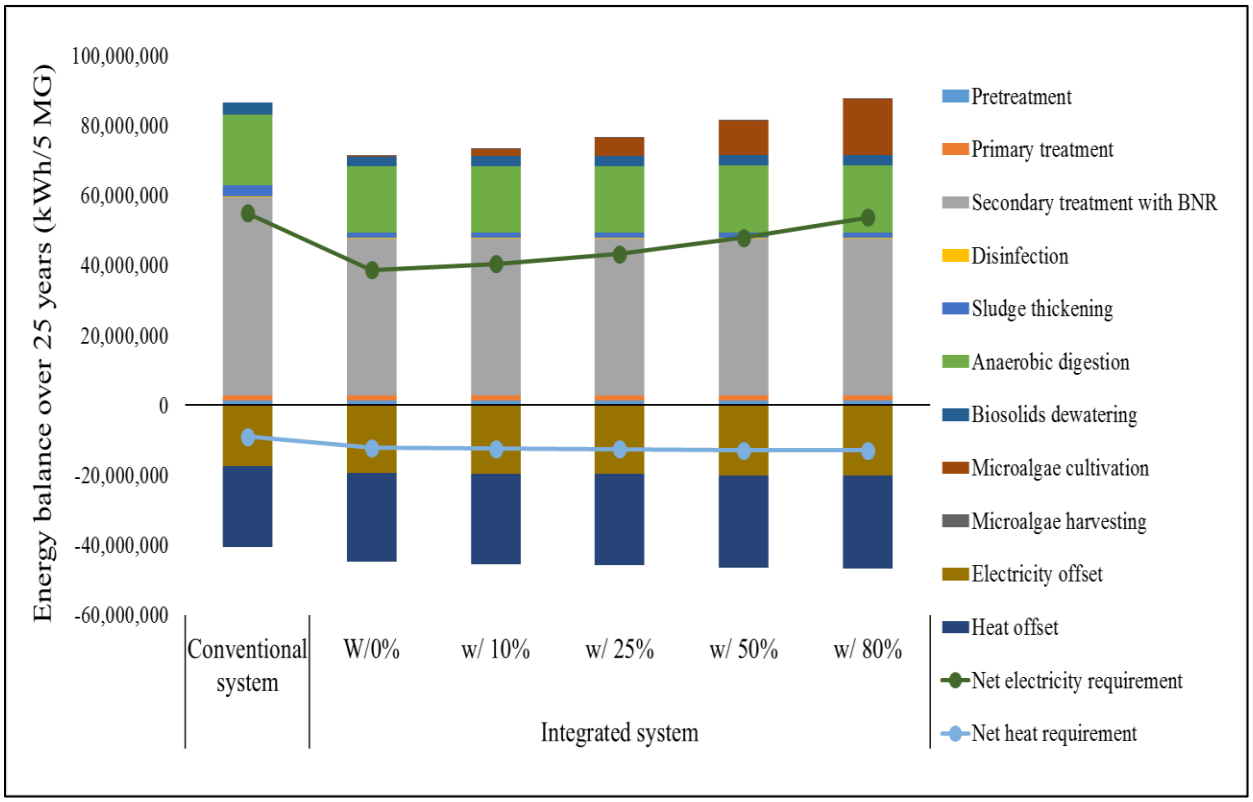


Figure 5.4 Energy balance of the conventional and integrated systems with different CO_2 supply areas. Note: Net electricity requirement=total electricity demand-electricity offset, and Net heat requirement=total heat demand-heat offset.

For the integrated systems, total energy demand increased with increasing the CO_2 supply areas due to energy requirement for CO_2 sparging. For instance, the energy demand for CO_2 sparging accounts for 21% of the total energy demand in the integrated system with 80% CO_2 supply area, which became the second largest contributor to total energy consumption. Thus, increasing CO_2 supply area had a negatively impact on the energy balance. For the integrated

systems with 0, 10, and 25% CO_2 supply areas, the net electricity requirement (electricity demand – electricity offset) was much lower than that of the conventional system. The integrated systems can have energy benefits from reduced energy consumption for wastewater treatment as well as increased energy offsets.

The cumulative energy demand (CED) for the integrated and conventional systems are shown in Figure 5.5. The electricity demand for the integrated systems (with 0%, 10%, and 25% CO_2 sparging areas) were lower than the conventional system, and the similar result was found in the energy balance result. In fact, the integrated systems required additional electricity for microalgae system, but the systems required much less electricity for wastewater treatment than the conventional system. This is because the integrated system reduced the N loading in the mainstream of wastewater treatment.

The second largest contributor for the CED was the chemical demand. The integrated systems slightly reduced the CED associated the chemical demand compared to the conventional system. Because of nutrient removal by microalgae assimilation, the waste sludge production in the integrated system was reduced. For this reason, the chemical demand was reduced in the integrated system. The heat demand was relatively small among others due to the warm weather condition in the Tampa area. Thus, heat produced from the CHP can meet the heat requirement for anaerobic digestion. As shown in Figure 5.5, the energy offsets from the integrated systems was larger than the energy offsets from the conventional system due to the increased methane production. Overall, the addition of microalgae system in wastewater treatment system contributed to a reduction of the total energy demand indicated as CED.

Similar to energy balance results, the CED increased with increasing the CO_2 supply areas for the integrated systems, due to higher energy requirement for CO_2 sparging. The integrated

system was not able to achieve energy neutrality from a life cycle perspective, but they could reduce high electricity demand for nutrient removal. As shown in Figure 5.4 and 5.5, energy reduction from wastewater treatment was greater than improvement in energy offsets in the integrated system, compared to the conventional system. Therefore, the addition of microalgae system in wastewater treatment plant greatly contributed to a reduction of the total energy demand.

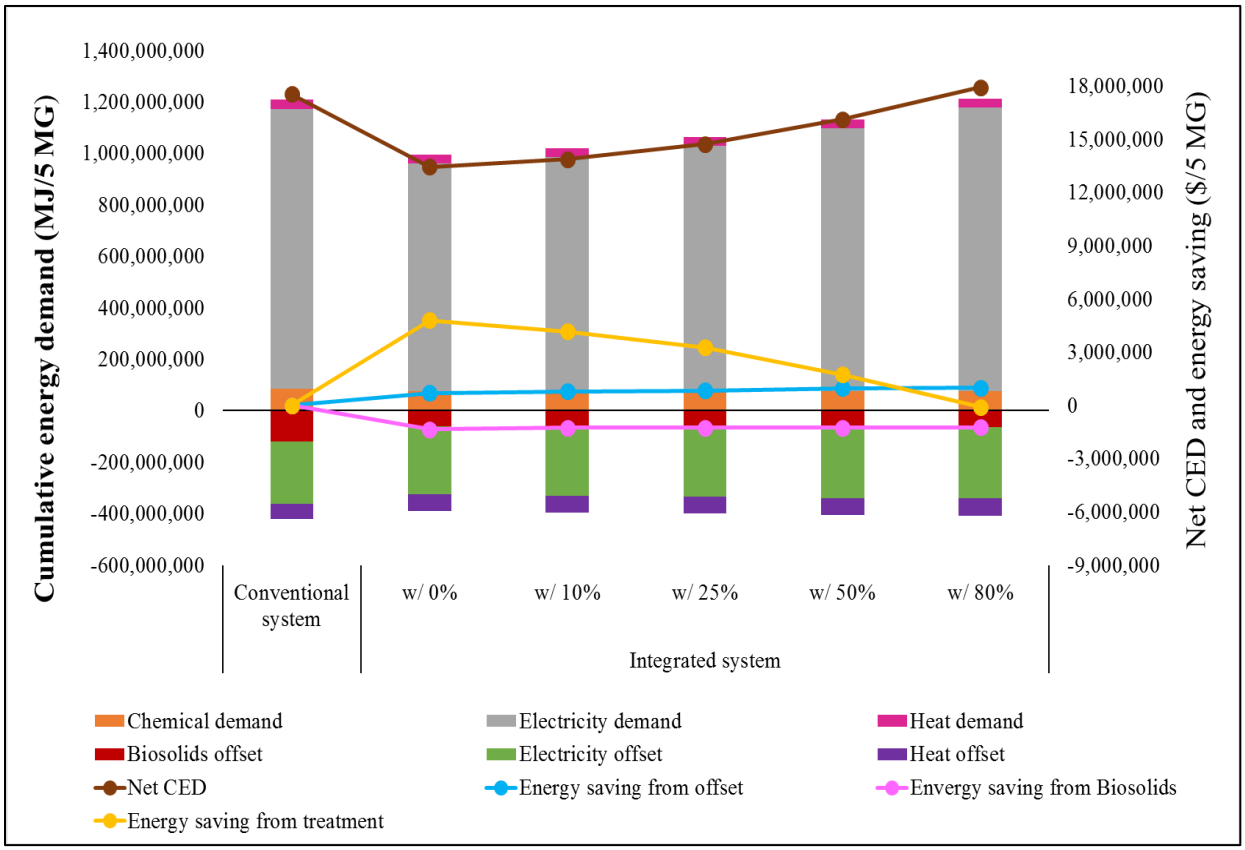


Figure 5.5 Cumulative energy demands (CED) of the conventional and integrated systems with different CO_2 supply areas.

5.3.4 Life Cycle Costs

Table 5.3 shows life cycle costs for conventional and integrated systems. It was observed that the most significant cost contributor was the secondary treatment with BNR and anaerobic digestion. The life cycle cost results show that the integrated systems have lower costs per 5 MG of wastewater treated than the conventional system, because the secondary treatment cost for the conventional system is much greater the integrated systems. In integrated systems, addition of

microalgae biomass in anaerobic digestion process was able to achieve higher methane production, which resulted in higher revenues from offsets for electricity and heat compared to the conventional system. In addition, for the integrated system, the cost reduction for secondary treatment is greater than increased revenues from the offsets, compared to the conventional system. In the life cycle cost of the integrated system, the contribution of the capital cost for microalgae system is small, which accounts for less than 1%.

Table 5.3 Life cycle costs for the integrated and conventional systems. Unit: $/5 GM.

Treatment stage	Conventional system	Integrated system				
		w/ 0%	w/ 10%	w/ 25%	w/ 50%	w/ 80%
Operation cost						
Pretreatment	71,100	71,100	71,100	71,100	71,100	71,100
Primary treatment	61,800	61,800	61,800	61,800	61,800	61,800
Secondary treatment with BNR	2,654,200	2,099,300	2,097,700	2,094,900	2,091,000	2,088,600
Disinfection	84,000	84,000	84,000	84,000	84,000	84,000
Sludge thickening	349,900	260,100	260,100	260,100	260,100	260,100
Anaerobic digestion	980,300	916,200	918,800	923,300	929,900	933,900
Biosolids dewatering	582,100	342,700	342,700	343,100	344,400	345,300
Microalgae cultivation	-	9,200	104,600	247,200	485,700	771,400
Microalgae harvesting	-	3,100	3,100	3,100	3,100	3,100
Electricity offset	-846,300	-933,200	-945,300	-952,500	-967,800	-971,100
Heat offset	-115,400	-127,300	-128,900	-129,900	-132,000	-132,400
Biosolids offset	-229,500	-108,900	-115,600	-116,100	-117,100	-117,400
Capital cost						
Microalgae system	-	20,800	20,800	20,800	20,800	20,800
Life cycle cost	3,592,200	2,698,900	2,774,900	2,910,900	3,135,000	3,419,200

5.4 Conclusions

This study used the life cycle assessment and life cycle cost analysis to evaluate both the environmental impacts (carbon, nutrient, and energy balances) and the economic impacts of the integrated microalgae system with wastewater treatment considering different CO_2 supply areas. The impacts of the integrated systems were also compared to the conventional system. The integrated systems reduced the impacts on carbon footprint and cumulative energy demand compared to the conventional system, due to the reduction of direct carbon emissions as well as electricity demand for secondary treatment system with BNR. However, there is no significant difference between the integrated and conventional systems for eutrophication potential. It was found that the integrated systems have lower life cycle costs per 5 MG of wastewater treated than the conventional system. The cumulative energy demand and life cycle cost per 5 MG for the integrated systems were found to decrease as CO_2 supply area decreases, likely due to CO_2 sparging energy. Among integrated systems with different CO_2 supply areas investigated, the system with 10% CO_2 sparging area was able to achieve the lowest carbon footprint, while the system without CO_2 supply area had the lowest energy balance and life cycle cost. In this study, the integrated system was not able to achieve carbon, nutrient, or energy neutralities, but the system considerably reduced the energy and cost for wastewater treatment via reduction of electricity demand from nutrient removal processes.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

This goal of this dissertation study investigated the environmental impacts and benefits of the integrated system using microalgae cultivation as side-stream wastewater treatment processes with energy production in order to provide a holistic understanding of energy, carbon, and nutrient balances in the integrated system. Through this study, three stated hypotheses were proposed:

- Hypothesis 1: The integrated system is a net energy producer, and carbon and nutrient neutral from a life cycle perspective.
- Hypothesis 2: The combination of threshold and multiplicative relationships will be an appropriate structure of the rate expression (model predictions with R^2>0.8) for microalgae cultivation using wastewater as the nutrient medium.
- Hypothesis 3: Anaerobic co-digestion of waste-activated sludge and a certain percentage of microalgae will improve methane production rates in the anaerobic digestion step compared to conventional anaerobic digestion with the sludge only.

To understand microalgae growth in the centrate from dewatering of anaerobically digested sludge, the integrated co-limitation kinetic model was developed (Chapter 2). Nitrogen (N), dissolved carbon dioxide (CO_2) concentrations, light intensity, and temperature were considered as major growth factors in the model. The model framework was constructed by combining threshold and multiplicative structures to explain the relationship among these factors. For each factor, two alternative rate expressions were provided in the model structure, which are the representative rate expressions of limitation and inhibition conditions of nutrients and light. These

expressions include the Andrews and Monod models for both CO_2 and N, and the Chalker and Muller-Fuega models combined with the Arrhenius equation for light intensity and temperature. Depending on culture conditions for each factor, the rate expression was selected based on criteria (e.g. limitation condition: NH_4-N $\leq$ 150 mg L^{-1}, aqueous $CO_2 \leq$ 50 mg L^{-1}, and light intensity $\leq$ 90 μmol photon m^{-2} s^{-1}; inhibition condition: outside these ranges). The model was calibrated and validated not only using batch studies with anaerobically digested municipal sludge centrate but also using published data from the studies that applied the centrates as a growth medium. The model was shown to have a reasonable growth rate prediction of *Chlorella* sp. under different nutrient levels of the centrate (R^2>0.8), which supported the hypothesis 1. Thus, the model was able to predict the microalgae growth rate in wastewater, especially centrate. This model can be applied for photobioreactor design as well as process control and optimization of microalgae cultivation systems integrated with wastewater.

In anaerobic co-digestion, hydrolysis and methanogenesis can be considered as rate limiting steps. The rates of hydrolysis and methanogenesis, which affect methane production rate, are commonly described by the first order and Monod-type kinetics, respectively. Due to limited kinetic information under the co-digestion conditions, however, extensive experimentations were required. To estimate the kinetic parameters for anaerobic co-digestion of microalgae and waste activated sludge (WAS), regression-based models were introduced (Chapter 3). The models were developed using the ratios of co-substrates and the kinetic parameters for the single substrate as indicators. It was found that for anaerobic co-digestion of WAS and microalgae, the best combinations for hydrolysis and methanogenesis were 10% microalgae with 90% WAS and 60% microalgae with 40% WAS, respectively. The results indicated these combination improved the methane production rate, which supported the hypothesis 2. For model application, it was shown

that the model using a hyperbola function was better for the estimation of the first-order kinetic coefficients, while the model using inverse tangent function closely estimated the Monod kinetic parameters. The models can be used for estimating kinetic parameters for not only microalgae-WAS co-digestion but also other substrates' co-digestion such as microalgae-swine manure and WAS-aquatic plants. Using the estimation models presented, the kinetic parameters for co-digestion can be determined for different ratios of co-substrates with limited experiments.

In Chapter 4, an integrated process model was developed to simulate the dynamic behavior of the integrated system. Unlike previous process modeling studies, the model included microalgae cultivation, harvesting, and anaerobic co-digestion processes in the integrated system. Also, this research investigated the effects of different sparging CO_2 areas and influent N concentrations on the integrated system. The integrated system achieved the average areal microalgae productivity of 41 g m^{-2} d^{-1}. For the integrated system, removal of NH_4-N by microalgae was not effective. In particular, the NH_4-N removal was minimal during the winter season due to low microalgae growth. The areal productivity was improved with increasing CO_2 sparging areas in the cultivation system, but the highest increment was found at microalgae cultivation with 50% CO_2 sparging area. Changing NH_4-N concentrations in influent affected the areal productivity as well as effluent quality. As NH_4-N concentrations increased, the effluent quality and productivity decreased due to the NH_4-N inhibition. As the microalgae productivity increased, the CH_4 and biosolids production increased as a result of the increased amount of the substrates from the harvested microalgae biomass. The increase of CH_4 and biosolids productions, however, was minor because of small amount of microalgae biomass for the co-digestion, which accounts for only 5% of the substrate by mass. The present model could be used for simulating various conditions and further refinement of design and operating procedures for the integrated system.

In Chapter 5, based on simulated data, the life cycle environmental impacts (carbon, nutrient, and energy balances) and the economic impacts of the integrated system were evaluated and compared to the conventional wastewater treatment system. The integrated systems, except the integrated system with 80% CO_2 area, reduced the impacts on carbon footprint, cumulative energy demand and life cycle cost compared to the conventional system, due to the reduction of direct GHG emissions as well as electricity demand for secondary treatment system with BNR. However, there was no significant difference between the integrated and conventional system for eutrophication potential due to the equal effluent qualities of those systems. On the other hand, the carbon footprint and cumulative energy demand as well as life cycle cost increased as the CO_2 supply areas increased because of additional energy requirements for the microalgae system. Among integrated systems with different CO_2 supply areas investigated, the system with 10% CO_2 sparging area was able to achieve the lowest carbon footprint, while the system without CO_2 sparging area had the lowest life cycle energy and cost. In terms of an energy saving with the integrated systems, the benefit of energy reduction for the wastewater treatment was greater than the energy production from the anaerobic co-digestion, compared to the conventional system. The system was not able to achieve carbon, nutrient, and energy neutralities as stated in the hypothesis 3, but the system improved the carbon and energy balances for the wastewater treatment.

6.2 Research Limitations and Recommendations

6.2.1 Integrated Co-Limitation Kinetic Model

The integrated co-limitation kinetic model developed in this study is useful to predict microalgae growth in wastewater. However, there are several limitations for broad application of the kinetic model.

First, the kinetic parameters of the model for different cultivation conditions, such as light-dark cycles, wastewater sources, microalgae species, need to be assessed in order to increase the usability of the model as well as to improve the model prediction. Depending on the cultivation conditions, the estimated kinetic parameters for microalgae growth may be different. The parameters provided in this study were obtained from the experiments using the centrate and indigenous *Chlorella* sp. under 24:0 h light dark cycle. Application of the parameters from this study results in poor predictions if the cultivation conditions do not agree with the tested conditions (Liu and Zachara, 2001).

Second, appropriate rate expressions for P in the integrated model need to be investigated. In this study, microalgae growth in the centrate was not limited by P so that P was not considered as a major factor in the final growth kinetic model. Thus, developing the robust integrated model would require suitable rate expressions of P for future applications. In addition, organic carbon needs to be considered as a growth factor in order to explain microalgae growth in wastewater containing organic carbon (such as effluent of primary wastewater treatment), since microalgae (e.g., *Chlorella* sp.) have an ability to use organic carbon as a carbon source for their growth. Future research may focus on these limitations to improve the applicability of the model to various types of wastewaters.

6.2.2 Regression Based Parameter Estimation Models

In Chapter 3, it is important to obtain accurate kinetic parameter values for single substrates from the experiments, because those are the important factors in the co-digestion kinetic parameter estimation model developed in this study. In order to improve reliability and predictability of the model, appropriate data points from experimentation are needed to accurately determine the kinetic parameter for single substrates (La Du and Tanaka, 1989). Another important factor that affected

the model estimation was the constant "*a"*. The constant "*a"* accounts for synergetic effects of co-digestion in the model, and the synergetic effect is different depending on types of co-substrates (Esposito et al., 2012). In this study, the model was developed to estimate the kinetic parameters for the co-digestion of microalgae and waste activated sludge. When the model applied for the other substrates, it was observed that the model predictions were underestimated or overestimated within the acceptable range (NRMSE < 30%, shown in Chapter 3), because the equal synergetic effect was assumed for all tested substrates in this study. In order to obtain accurate kinetic parameters for other co-digestion cases, therefore, the constant "*a"* also needs to be determined based on substrates characteristics, such as particle size and C/N ratios. Future research may focus on these limitations to improve the applicability of the model to various substrates for the co-digestion.

6.2.3 Integrated Process Model

Since detailed experimental results for the integrated system are hard to find in the literature, the validation of the present model was performed to simulate microalgae cultivation in wastewater and anaerobic co-digestion of microalgae and wasted activated sludge using literature studies. For cultivation, the existing data was not available for the open pond system using wastewater with different CO_2 sparging area. For anaerobic co-digestion, there was no pilot scale data for the co-digestion of microalgae and waste sludge. Thus, future pilot studies are required to validate the model and improve the accuracy of the model prediction for the integrated system.

For performance of the integrated system, additional N treatment is needed to meet effluent discharge limits because the integrated system did not achieve high NH_4-N removal by microalgae (shown in Chapter 4). To obtain better simulated results for NH_4-N concentrations, ammonia stripping and optimization of other design parameters need to be considered in the process

modeling. In particular, the ammonia stripping was shown to an important role as a NH_4-N removal mechanism in open pond systems (Batista et al., 2015; Posadas et al., 2015).

An algal-bacterial consortium, which combines microalgae production system with shortcut N removal via nitritation/denitritation, might be a potential option to improve NH_4-N removal in the centrate (Wang et al., 2015). In prior studies, the algal-bacterial consortium reported improvement of organic carbon, N and P removal without aeration in wastewater treatment due to the synergetic activity: photosynthesis from microalgae produces oxygen for nitrification, resulting in reduced aeration demands; N removal is achieved through assimilation by microalgal and bacterial biomass and nitritation/denitritation process (He et al., 2013; Subashchandrabose et al., 2011). In order to consider this process in the integrated system, the process model should be extended to consider kinetics and mass balance of bacteria.

6.2.4 Life Cycle Assessment for the Integrated System

The current research was based on the data for an integrated system located in Tampa, FL. Thus, LCA considering different local conditions (e.g. geographical locations) is needed for more comprehensive understanding of carbon, nutrient, and energy balances of the integrated system. For example, it would be beneficial to evaluate influences of geographical locations on environmental sustainability of the integrated system, because microalgae cultivation in the integrated system is significantly affected by temperature (Ras et al., 2013). Thus, this can provide useful information for appropriate geographic locations in order to apply the integrated system.

This study focused on embodied energy, carbon footprint and eutrophication potentials to evaluate the environmental impact of the integrated system. However, LCA tools can be used to investigate a wide range of environmental impact categories (e.g. ozone depletion, acidification, ecotoxicity). Other categories are also important and needed to be investigated to identify impacts

of systems over the life cycle. The conclusions of this study were based on the use phase. According to existing study for microalgae-based bioenergy system (Lardon et al, 2009), the use phase is a dominant phase for the environmental impacts. However, considering other phases (e.g. construction, end of life) are equally important to evaluate overall environmental impacts of the integrated system for understanding of sustainability of the integrated system. Understanding of environmental impact for seasonal variation in nutrient discharge to water bodies and electricity use is important for successful implementation of the integrated system. Thus, sensitivity or uncertainty analysis for LCA is needed for future studies.

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APPENDIX A: MONOD KINETICS FOR PUBLISHED DATA

Table A.1 Monod kinetic parameter for Gordon (2015). Note: WAS: Waste activated sludge.

Substrate compositions	k_m	K'	R^2
100% WAS	17.3	0.093	0.90
25% *Chlorella sp.* + 75% WAS	24.0	0.333	0.88
50% *Chlorella sp.* + 50% WAS	15.2	0.107	0.90
75% *Chlorella sp.* + 25% WAS	17.3	0.407	0.84
100% *Chlorella sp.*	12.1	0.021	0.84

Table A.2 Monod kinetic parameter for Lu and Zhang (2016). Note: SS: Septic Sludge.

Substrate compositions	k_m	K'	R^2
100% SS	36.0	0.566	0.88
25% *Chlorella sp.* + 75% SS	30.6	0.257	0.93
50% *Chlorella sp.* + 50% SS	19.9	0.001	0.97
75% *Chlorella sp.* + 25% SS	15.1	4E-05	0.99
100% *Chlorella sp.*	4.8	0.001	0.73

Table A.3 Monod kinetic parameter for Wang et al. (2013). Note: WAS: Waste activated sludge.

Substrate compositions	k_m	K'	R^2
100% WAS	23.5	0.01	0.99
25% *Chlorella sp.* + 75% WAS	34.8	0.07	0.96
25% *Micractinium sp.* +75% WAS	32.5	0.06	0.96
100% *Chlorella sp.*	29.4	0.02	0.94
100% *Micractinium sp.*	26.8	0.02	0.95

Table A.4 Monod kinetic parameter for Kim and Kang (2015). Note: RS: Raw sludge from sewage wastewater treatment plant.

Substrate compositions	k_m	K'	R^2
100% RS	3.29	0.001	0.69
50% RS+50% *Chlorella sp.*	2.41	0.001	0.91
100% *Chlorella sp.*	1.32	0.001	0.84

APPENDIX B: CALCULATIONS OF PARAMETERS

B.1 CO_2 Concentration in Fine Bubbles

$$y_s = \frac{V_{CO2}}{V_{air}} \frac{P_{air}}{R \cdot T} \frac{1}{H} = 205\ g\ m^{-3} \tag{B.1}$$

where y_s is the gas phase concentration of the CO_2 fine bubble at air-water interface; H is the Henry's law constant (H=0.8766 for CO_2 at 23°C); R is an ideal gas constant (0.0821 L atm mol^{-1} K^{-1}); P is the CO_2 gas pressure (2 atm); T is temperature (296 °K).

CO_2 concentration in atmosphere is 400 ppm (mole fraction: 400×10^{-6} mole/mole). Since the mole fraction is equal to the partial pressure, P_{CO2} is 400×10^{-6} atm.

$$C_s = \frac{P_{CO_2}}{H_A} = 0.57\ g\ m^{-3} \tag{B.2}$$

where H_A is the Henry's law constant at 23°C which is 31 L atm $mole^{-1}$.

B.2 CO_2 Mass Transfer Coefficient Rate for Fine Bubble

The CO_2 mass transfer coefficient rate and volume faction of gas holdup were calculated by the methods suggested by Yang (2011). Specific equations for the mass transfer were listed in Table B.1.

Table B.1 Equations for CO_2 mass transfer.

Items	Equations
Mass transfer area for the bubble (α_b)	$\alpha_b = \frac{6}{3.23 \cdot (4 \cdot Q_0/\pi \cdot d_{b0} \cdot \mu_L)^{-0.1} \cdot (Q_0^2/d_{b0}^5 \cdot g)^{0.21} \cdot d_{b0}}$
Gas volumetric flow rate per diffuser (Q_0)	$Q_0 = \frac{Q}{n \cdot A_g}$
Mass transfer coefficient for bubble (K_L)	$K_L = \frac{D_{CO2} \cdot (2 + A * B)}{3.23 \cdot (4 \cdot Q_0/\pi \cdot d_{b0} \cdot \mu_L)^{-0.1} \cdot (Q_0^2/d_{b0}^5 \cdot g)^{0.21} \cdot d_{b0}}$ $A = 0.015(4 \cdot Q_0/\pi \cdot d_{b0} \cdot \mu_L)^{0.89}$ $B = (\mu_L/\rho \cdot D_{CO2})^{0.7}$

Table B.1 (Continued)

Items	Equations
Volume fraction of gas holdup (Ɛ)	$\varepsilon = \frac{n \cdot Q}{u_{Gb}}$
Bubble ascending velocity (u_{Gb})	$u_{Gb} = \sqrt{\frac{4 \cdot 3.23 \cdot (4 \cdot Q_0/\pi \cdot d_{b0} \cdot \mu_L)^{-0.1} \cdot (Q_0^2/d_{b0}^5 \cdot g)^{0.21} \cdot d_{b0} \cdot g}{3C_D}}$
Drag force coefficient (C_D)	$C_D = 18.5/(4 \cdot Q_0/\pi \cdot d_{b0} \cdot \mu_L)^{0.6}$
*__Nomenclature__: d_{b0}: the diameter of diffuser (0.05 m); μ_L: dynamic viscosity of liquid phase (0.0009321 Pa s); g: Acceleration of gravity (9.8 m s^{-2}); n: number of diffuser per unit area (250 ea m^{-2}); A_g: total aera utilized for introducing the gas flow (175 (5%), 438(25%), 875(50%), and 1400 (80%) m^2); D_{CO2}: diffusivity of CO_2 (1.97×10^{-9} m^2 s^{-1}); Q: total gas volumetric flow rate (0.2 vvm); ρ: liquid density (998 kg m^3)	

B.3 Evaporation Rate

In this study, the weather condition was based on Tampa, FL, and the weather data (e.g. temperature, wind speed, and dew point) were obtained from TMY3 database. Saturated vapor pressures (P_S) for the pond water and dew point temperature were calculated by Eq. B.3. Based on the saturated vapor pressures, an evaporation rate (R_E) was calculated by Eq. B.4 (Lam et al., 2001).

$$P_s = 0.6108 \times e^{\left(\frac{17.27\times T}{T+273.3}\right)} \tag{B.3}$$

$$R_E = (4.08 + 4.28v) \cdot \frac{P_{S,w} - P_{S,d}}{Y} \tag{B.4}$$

where T is either the pond water temperature or dew point (°C); v is the wind speed (m s^{-1}); Y is the latent heat for water (2257 KJ/kg); $P_{S,W}$ and $P_{S,d}$ are the saturated vapor pressures for the pond water temperature and dew point (*kPa*); and R_E is the evaporation rate (kg m^{-2} hr^{-1}). The evaporation rates from January to December were shown in Table B.2. An average evaporation rate was 0.12 kg m^{-2} d^{-1} (1.21×10^{-4} m^3 m^{-2} d^{-1}).

Table B.2 Evaporation rates in Tampa.

Time	Evaporation rate (R_E), kg/m^2 d
Jan	0.079
Feb	0.081
Mar	0.113
Apr	0.170
May	0.178
Jun	0.139
Jul	0.124
Aug	0.116
Sep	0.115
Oct	0.115
Nov	0.127
Dec	0.087

B.4 Gas Flow Rate for Anaerobic Co-Digestion

The gas flow can be calculated based on control loop in pressure of reactor. The gas pressure was calculated from partial pressures.

$$P_{gas} = P_{gas,CH4} + P_{gas,CO2} + P_{gas,H2O} \tag{B.5}$$

The reactor headspace was assumed to be water vapor saturated. The partial pressure of the gases were calculated based on the following equations.

$$P_{gas,H2O} = 0.0313 \cdot \exp\left(5290\left(\frac{1}{298} - \frac{1}{T}\right)\right) \tag{B.6}$$

$$P_{gas,CH4} = \frac{G_{CH4}}{\alpha_{ad\_m}} \frac{635.54}{16 \cdot V_{ad\_gas}} \cdot R \cdot T \tag{B.7}$$

$$P_{gas,CO2} = \frac{G_{CO2}}{\alpha_{ad\_c}} \frac{1748.9}{44 \cdot V_{ad\_gas}} \cdot R \cdot T \tag{B.8}$$

The gas flow was calculated to set it equal to total gas transfer with correction for water vapor. In this system, the total gas transfer rate equaled to the production rate of methane. Therefore, the gas flow was calculated by using Eq. (B.9):

$$Q_{ad\_gas} = k_p\left(P_{gas} - P_{atm}\right) \quad \text{(B.9)}$$

where P_{gas} is total pressure of gas (bar); $P_{gas,H2O}$, $P_{gas,CH4}$, and $P_{gas,CO2}$ are partial pressures of water, methane, and carbon dioxide (bar); T is the reactor temperature (308.15 K); G_{CH4} and G_{CO2} are methane gas and biogas productions (m^3); kp is the pipe resistance coefficient (5×10^4 m^3 d^{-1} bar^{-1}); P_{atm} is the external (atmospheric) pressure (1.01325 bar); and R is the gas constant (m^3 bar K^{-1} mol^{-1}).

B.5 Kinetic Coefficients for Anaerobic Co-Digestion

Kinetic coefficients for Step 1 and 2 were estimated based on the regression-based models (Eq. 3.6 and Eq. 3.7). For hydrolysis, the coefficient of the mixed sludge (primary and secondary sludges) was obtained from Costa et al. (2012), while the coefficient of the microalgae was obtained from Chapter 2. For methanogenesis, the coefficients of the mixed sludge and microalgae were obtained from Table A.4. The kinetic parameters for the regression-based models are shown in Table B.3.

Table B.3 Kinetic parameters for the regression-based models.

Substrate (% by VS)		K_{hyd} (1/d)	K_m (1/d)
Mixed sludge	Microalgae		
100	0	0.11	3.29
0	100	0.07	1.32

APPENDIX C: LIFE CYCLE INVENTORY

C.1 N_2O Emissions

N_2O emissions from the nutrient process were calculated as the following equation (Cornejo et al., 2016; USEPA, 2010):

$$N_2O = Q \times TKN \times EF_{N_2O} \times \left(\frac{44}{28}\right) \times (1 \times 10^{-3}) \quad \text{(C.1)}$$

where N_2O is the N_2O generated from the 5 MGD wastewater treatment plant (kg N_2O/yr) and Q is the wastewater influent flow rate (m^3/year). EF_{N2O} (0.005 g N emitted as N_2O per g TKN) (Chandran, 2010). The calculated N_2O emissions are shown in Table C.1.

C.2 CO_2 Emissions from CHP

It was assumed that methane was completely combusted in the CHP. The CO_2 from the CHP was calculated based on a stoichiometry-based equation for a complete combustion of methane:

$$CO_2 = Q_{CH4} \times D_{CH4} \times \frac{44}{16} \quad \text{(C.2)}$$

where CO_2 is the CO_2 generated from the CHP (kg CO_2/d) and Q_{CH4} is the methane production from the anaerobic digestion (m^3/d). D_{CH4} is the densities of methane (0.636 kg/m^3). For the integrated system, since CO_2 from the combustion gas was used in the cultivation system, it was assumed that there was no CO_2 emission from the CHP during the daytime.

C.3 Energy and Chemical Requirements for Secondary Treatment with BNR

Aeration energy demand was theoretically calculated based on Eq. 5.1 (Tchobanoglous et al., 2003). The power requirement for the pump was estimated using the following equation (Tchobanoglous et al., 2003):

$$P = \frac{\rho Q H g}{E} \tag{C.3}$$

where P is the power requirement (W), Q is the flow rate (m^3/s), ρ is the density of water (kg/m^3), H is the head loss (m), g is the acceleration of gravity (m/s^2), and E is the efficiency of the pump. Mixing energy was estimated based Eq. 5.4. For energy requirement for chemical addition, Beat et al. (2013) reported that the energy demand of the chemical addition was 52.5 J/L ($1.46*10^{-5}$ kWh/L). Thus, energy consumption for the chemical addition was calculated based on this factor. In WWTP, phosphorus (P) was typically removed by a chemical precipitation method. According to Tchobanoglous et al. (2003), P can be removed by alum, which is most widely used in WWTP:

$$Al^{3+} + H_n PO_4^{3-} \leftrightarrow AlPO_4 + nH^+ \tag{C.4}$$

Based on the above stoichiometric equation (Eq. (C.4)), 1 mole of Al can remove 1 mole of P, but the amount of Al cannot simply be calculated due to competing chemical reactions (to be determined in the lab-scale results for each case). Thus, It was assumed that 1.5 mole of Al removed 1 mole of P. It was assumed that liquid alum had 48% strength with density of 1.2 kg/L. In this condition, the consumption of alum was estimated.

C.4 Energy and Chemical Requirements for Disinfection

Beat et al. (2013) reported that the energy requirement of the disinfection was 2.57 J/L ($7.14*10^{-7}$ kWh/L). Thus, the energy consumption was calculated based on this factor, while the chlorine consumption was estimated based on 0.11 kg/m^3 treated waste water (Cornejo et al. 2016).

C.5 Fertilizer Offsets for Biosolids

Daily N and P mass flow rate was estimated based on Cornejo et al. (2016), shown in the following equations:

$$N\ fertilizer\ (g/d) = Biosolids\ (kg/d) \times \frac{1}{Density\ (kg/m^3)} \times 10.4(g\ N/m^3) \qquad \text{(C.5)}$$

$$P\ fertilizer\ (g/d) = Biosolids\ (kg/d) \times \frac{1}{Density\ (kg/m^3)} \times 4.6(g\ P/m^3) \qquad \text{(C.6)}$$

Table C.1 Daily energy and chemical input and output.

Treatment stage	Items	Unit	Reference	Conventional system	Integrated system_0%	Integrated system_10%	Integrated system_25%	Integrated system_50%	Integrated system_80%
Pretreatment	Bar screens	kWh/d	Pabi et al. (2013)	1	1	1	1	1	1
	Grit chamber	kWh/d	Pabi et al. (2013)	160	160	160	160	160	160
Primary treatment	Pimary setting	kWh/d	Pabi et al. (2013)	140	140	140	140	140	140
Secondary treatment with BNR	Aeration	kWh/d	This study	4,851	3,558	3,558	3,558	3,558	3,558
	Mixing	kWh/d	This study	868	863	863	863	863	863
	Secondary setting	kWh/d	Pabi et al. (2013)	350	350	350	350	350	350
	Pumping	kWh/d	This study	167	167	167.0	167.0	167.0	167.0
	Alum	kg/d	This study	631	612	612	612	612	612
	N2O emission	kg/d	This study	8.1	6.8	6.8	6.6	6.2	6.3
Disinfection	Chlorination	kWh/d	Beal et al. (2013)	14	14	14	14	14	14
	Chlorine	kg/d	Cornejo et al. (2016)	38	38	38	38	38	38
Waste sludge thickening	Pumping of primary solids	kWh/d	Beal et al. (2013)	0.50	0.50	0.50	0.50	0.50	0.50
	Thickening of secondary sludge	kWh/d	This study	332	160	160	160	160	160
	Pumping of secondary sludge	kWh/d	This study	17.2	9.0	9.0	9.0	9.0	9.0
	Ploymer	kg/d	This study	9.5	9.0	9.0	9.0	9.0	9.0
Anaerobic digestion	Mixing	kWh/d	This study	655	595	597	600	605	608
	Heat	kWh/d	This study	1,562	1,477	1,481	1,488	1,498	1,504
	Pumping	kWh/d	This study	3.07	2.80	2.80	2.80	2.80	2.90
Biosolids dewatering	Centrifugation	kWh/d	This study	390	310	310	311	314	316
	Polymer	kg/d	This study	20	10	10	10	10	10
Microalgae cultivation	CO2 sparging	kWh/d	This study	-	-	216	539	1,079	1,726
	Paddle wheel mixing	kWh/d	This study	-	20.00	20.00	20.00	20.00	20.00
	Pumping	kWh/d	This study	-	0.80	0.8	0.8	0.8	0.8
Microalgae harvesting	Centrifugation	kWh/d	This study	-	7.00	7	7	7	7
Total Energy requirements		kWh/d	This study	10,217	8,511	8,733	9,067	9,625	10,283

Table C.1 (Continued)

Treatment stage	Items	Unit	Reference	Conventional system	Integrated system_0%	Integrated system_10%	Integrated system_25%	Integrated system_50%	Integrated system_80%
Energy recovery	Methane production	m^3/d	This study	517	570	577	582	591	593
	Electricity offsets (27 % efficiency with LHV)	kWh/d	This study	1,916	2,113	2,141	2,157	2,192	2,199
	Heat offsets (51% efficiency with LHV)	kWh/d	This study	2,541	2,802	2,839	2,860	2,906	2,916
	CO_2 emission	kg/d	This study	904	997	101	102	103	104
Total Energy production		kWh/d	This study	4,458	4,916	4,979	5,017	5,098	5,115
Nutrient recovery	Biosolids production	tonnes/d	This study	3.98	1.89	2.01	2.01	2.03	2.04
	N fertilizer offsets-Biosolids	kg/d	This study	141	67	71	71	72	72
	P fertilizer offset-Biosolids	kg/d	This study	92	54	57	57	58	58

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