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# Nutrients Recycling Strategy for Microalgae-based CO2 Mitigation System

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Dr. Donald Colliver, Director of Graduate Studies

# NUTRIENTS RECYCLING STRATEGY FOR MICROALGAE-BASED $CO_2$ MITIGATION SYSTEM

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DISSERTATION

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Engineering at the University of Kentucky

By
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# ABSTRACT OF DISSERTATION

## NUTRIENTS RECYCLING STRATEGY FOR MICROALGAE-BASED $CO_2$ MITIGATION SYSTEM


Coal-fired electricity production is the major emitter of $CO_2$ and other greenhouse gases including $NO_x$ and $SO_x$. Microalgae-based $CO_2$ mitigation systems have been proposed to reduce the net $CO_2$ emission from coal-fired power plants. This study focused on developing an optimum culture media and exploring the possibilities for recycling nutrients, which were added as commercial mineralized chemicals at the beginning of cultivation.

In order to release the nutrients embedded in the cells so that they can be used as a nutrient source for new cells, *Scenedesmus* biomass was digested by anaerobic bacteria. Results showed that thermal pretreatment enhanced the methane production rate for the first 7 days of digestion. Three operational factors were tested: heating temperature, heating duration and NaOH dosage. The combination of 10 min heating with 3~6% NaOH at 50 °C gave the highest cell wall destruction for all samples except oven-dried algae.

The anaerobic digestate, rich in mineralized nutrients including ammonium and phosphate, potassium and magnesium ions, was tested as a possible nutrient source for the algae cultivation. To cope with the high solid content of the digestates, the dosage of the digestates was reduced or the solid particles were removed prior to addition to the microalgae. Both approaches worked well in terms of providing nutrients with minimal effect on light penetration. Using digestates without any sterilization did not cause contamination or other deleterious effects on the *Scenedesmus* growth rate.

Harvesting microalgae cells was critical to ensure a continuous and robust growth rate. The used media could be recycled at least four times without altering the algae growth. Nutrient replenishment was the key for a healthy culture when used media was incorporated. The combination of used media and digestates can sustain a normal algae growth.

Life cycle assessment was conducted on the system including the photobioreactor, the anaerobic digester, the biomass settling and dewatering and used media and nutrient recycling. Considering methane as the energy source, the overall energy return of the system was 2.4. $CO_2$ mitigation rate was about 39% under current mitigation system.

KEYWORDS: *Scenedesmus*, urea, anaerobic digestion, used media, life cycle assessment

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# NUTRIENTS RECYCLING STRATEGY FOR MICROALGAE-BASED $CO_2$ MITIGATION SYSTEM

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# Chapter 1: Introduction

Greenhouse gases have a major impact on the Earth's climate. Typical greenhouse gases include $CO_2$, water vapor, ozone and methane. They reflect incoming radiation from the sun and trap outgoing infrared radiation (Karl and Trenberth 2003). Since the beginning of the industrial revolution, anthropogenic emission of greenhouse gases by burning fossil fuels has imposed a growing threat to the global climate, and this situation is likely to continue for many decades (Karl and Trenberth 2003). $CO_2$ is the key player in this greenhouse gases crisis. The average atmospheric $CO_2$ increased from 280 ppm when the industrial revolution started to 381 ppm in 2006 (Canadell et al. 2007). The current $CO_2$ concentration in the atmosphere is believed to be the highest level in the past 650,000 years (Canadell et al. 2007). One of the major industrial sectors responsible for the $CO_2$ emissions is the coal-fired power plant (EIA 2009). In the U.S., coal-fired power plants emitted approximately 2.1 billion metric tons of $CO_2$ annually, which accounted for 36.5% of $CO_2$ emissions (EIA 2009).

Driven by the Kyoto Protocol and solid evidence for climate change, many countries have started taking actions on reducing greenhouse gas emissions. A few strategies have been proposed to capture the $CO_2$ from coal-fired power plants, and a number of them have been tested at a pilot scale (EIA 2009 and Yamasaki 2003). $CO_2$ mitigation can simply rely on natural sequestration processes. Terrestrial and aquatic vegetation has the potential to fix $CO_2$ into carbohydrates by photosynthesis. Meanwhile chemical weathering of rocks, soil and sands also take $CO_2$ out of atmosphere. On the other hand, researchers have proposed the artificial direct injection of $CO_2$ into ocean or underground reservoirs to mitigate $CO_2$ emissions (Yamasaki 2003). $CO_2$ is captured by physical or chemical reactions, and then it has to be separated out and pumped into the reservoir. Not only is the process very energy-intensive, but also raises environmental concerns. Introducing large amount of $CO_2$ to the ocean can alter the pH, which imposes unknown consequences to the marine ecosystem. Underground storage has less detrimental impact on the ecosystem but massive $CO_2$ leakage is possible (Yamasaki 2003).

Microalgae-based $CO_2$ mitigation systems have been proposed to alleviate greenhouse gas emissions (Wang et al. 2008, Ho et al. 2011). Microalgae are fast growing autotrophic cells, which generate two to ten times more biomass per unit land area than the best terrestrial plants (Wang et al. 2008). Cultivation of microalgae in an open or closed reactor resembles the natural $CO_2$ sequestration by photosynthesis, but in a more efficient way. The fundamental concept is to develop a microalgae $CO_2$ mitigation system that utilizes strain selection, photobioreactor design and biomass downstream processing.

Microalgae exist and thrive almost everywhere on the planet, but not all microalgae are suitable for fixation of $CO_2$ from flue gases. Flue gases from coal-fired power plant are normally high temperature and contain 10~15% $CO_2$. The photobioreactors are often installed in an open area, where temperature fluctuation inside the reactor is inevitable due to the cyclic temperature of the environment. Therefore two primary properties of the ideal algal strains should be tolerance to high $CO_2$ concentrations and ability to grow over wide temperature fluctuations. With this in mind, scientists isolated and selected various species from warm natural carbonate springs (Sakai et al. 1995 and Westerhoff et al. 2010), wastewater treatment facility (de-Bashan et al. 2008) or artificial extreme conditions (Morais and Costa 2007). Historically chlorophyta, such as *Chlorella* and *Scenedesmus* are one of the most studied microalgae for $CO_2$ bio-mitigation and biofuel production (Graham and Wilcox 2000). *Chlorella* occurs as 2~12 μm spherical or ellipsoidal unicells thriving in fresh or marine waters (Graham and Wilcox 2000). *Scenedesmus* typically appears as a flat colony of 4, 8 or 16 linearly arranged cells growing in fresh water and occasionally brackish water (Graham and Wilcox 2000).

In order to identify an organism suitable for warm weather cultivation, an initial screening was performed in collaboration with Dr. James Dawson of Pittsburg State University. One hundred and fifty candidate strains were identified from the literature, and were screened for growth at pH 5.5 and a temperature of 37 °C, corresponding to the conditions anticipated for a culture system fed by flue gas from a coal-fired power plant. Cultures were obtained from the University of Texas Culture Collection (UTEX) and adapted strains from the University of Kentucky CAER's photobioreactors. Only three strains (*Chlorella sorokiniana*, UTEX 246 and adapted *Scenedesmus* grown in CAER photobioreactors) exhibited excellent growth.

Downstream processing of algal biomass can balance the construction and operation cost of the $CO_2$ bio-mitigation system (Wang et al. 2008 and Ho et al. 2011). Algal biomass can be converted into energy rich products via biochemical, thermochemical or chemical reactions, or can be directly combusted to provide energy (Wang et al. 2008). Specifically algal biomass can be the potential substrate for fermentation, anaerobic digestion, gasification, pyrolysis, liquefaction or biodiesel production.

## 1.1. Objectives for the dissertation

The current project focused on recycling the nutrients from the algal cultivation through the anaerobic digestion of algal biomass. Microalgae are packed with organic compounds and trace nutrients. Anaerobic bacteria can digest organic material into biogas (mixture of $CO_2$, $CH_4$ and other minor gases), ammonium and trace nutrients (K, P, Ca, Mg and S). Biogas is the

potential fuel source and ammonium is the preferred nitrogen source for algae growth. Essentially by anaerobically digesting the algal biomass, mineralized nutrients can be recycled for algae growth and carbon can convert into methane for fuel use. This effort will reduce the fertilizer input in the PBR system. Meanwhile, waste media generated from algal biomass harvesting process should be appropriately allocated to minimize the scale of wastewater treatment required.

The specific research objectives were:

1. Optimize the medium formula using urea as the nitrogen source for the growth of *Scenedesmus* (Chapter 3).
2. Investigate the feasibility of recycling the waste media, and how the potential inhibitors influence the growth of *Scenedesmus* (Chapter 5).
3. Study the efficiency of anaerobic digestion when algae biomass is used as substrates. Evaluate the effects of pretreatment on the digestion efficiency (Chapter 4).
4. Incorporate the nutrients from anaerobic digestate into algal growth media and evaluate the effect of growth (Chapter 5).
5. Conduct a LCA study on the algae-based $CO_2$ mitigation system with special attention to nutrient recycling (Chapter 6).

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# Chapter 2: Literature review

## 2.1. Definition of microalgae

From a morphological standpoint, algae can be defined as microalgae or macroalgae. Microalgae occur as single cells with micrometers diameter (*Chlorella* 2~12 μm), while macroalgae like seaweed appear more like terrestrial plants (Graham and Wilcox 2000). Summarizing those versatile characteristics in habitat, phylogenesis, metabolism and morphology, algae are defined as aquatic organisms (with numerous exceptions) that are photosynthetic, oxygenic autotrophs (with frequent exceptions) that are smaller and less structurally complex than land plants (except for kelps) (Graham and Wilcox 2000).
From an algal nutrition point view, algae can be grouped into three categories: autotrophic, heterotrophic and mixotrophic (Graham and Wilcox 2000). Autotrophic algae possess chlorophylls along with accessory pigments and are capable of performing photosynthesis; heterotrophic species use organic carbons (e.g. glucose and glycerol) as sole energy source and thus do not rely on photosynthesis; mixotrophic algae are able to not only perform photosynthesis, but also take up and utilize organic nutrients, and both organic and inorganic carbon are essential (Graham and Wilcox 2000, Chojnacka and Marquez-Rocha 2004). Mixotrophic algae exist in most major algal groups.

Nine algal phyla have been recognized and defined: cyanobacteria, glaucophyta, euglenophyta, cryptophyta, haptophyta, dinophyta, ochrophyta, rhodophyta and chlorophyta (Graham and Wilcox 2000). Cyanobacteria are well known as a group of eubacteria with presence of chlorophylls and photosynthesis. Glaucophyta occur in fresh water, and have blue-green plastids containing chlorophyll alpha, phycobilins and carotenoids. Euglenophyta are a group of euglenoid flagellates having colorless plastids, green plastids or no plastids, and synthesize ß-1, 3-linked glucan instead of starch as storage material. Cryptophyta contain unicellular cryptomonad flagellates usually recognized by their flattened asymmetrical shape and two anterior, slightly unequal flagella. Haptophya are primarily marine unicells or colonies mostly coated with calcium carbonate-rich scales. Dinophyta include colorless heterotrophs as well as golden brown autotrophs with unique pigment accessory xanthophyll peridinin. Ochrophyta, also known as chromophytes, contain number of species (to name a few, diatoms, chromophytes, phaeophyceans) and range in size from microscopic unicells to giant kelps. Rhodophyta are primarily red marine algae favoring warm tropical water. Last, but not least, is chlorophyta known as green algae. Chlorophyta, especially *Chlorella* and *Scenedesmus* have been intensively studied in $CO_2$ mitigation, wastewater treatment and algal biofuel researches.

## 2.2. Photosynthesis

The underlying principle of algal $CO_2$ mitigation is to take advantage of the photosynthesis driven by solar energy and ultimately converting $CO_2$ to organic carbon (Figure 2.1). Photosynthesis is one of the most indispensable chemical reactions and fuels the Earth. Foyer (1984) defined it as a light-driven anabolism of carbon dioxide. Organisms that are able to carry out this process are photoautotrophs. It is believed that photosynthesis consist of four essential events: light absorption, charge separation, electron transport and energy storage (Foyer 1984). Considering the light dependence, photosynthesis is also recognized as a two-step process with a light reaction and a dark reaction (also referred as reductive pentose phosphate cycle, $C_3$ path way, photosynthetic carbon reduction cycle or Calvin cycle) (Horton et al. 2002).

In eukaryotes, photosynthesis takes place in specialized organelles named chloroplasts (Horton et al. 2002). Chloroplasts are typically composed of a double membrane permeable to $CO_2$, a highly folded membrane network called thylakoid membrane and the supporting aqueous matrix referred as stroma. Series pigments and enzymes are embedded in thylakoid membranes and complete light-dependent reactions that yield significant amounts of ATP and NADPH.

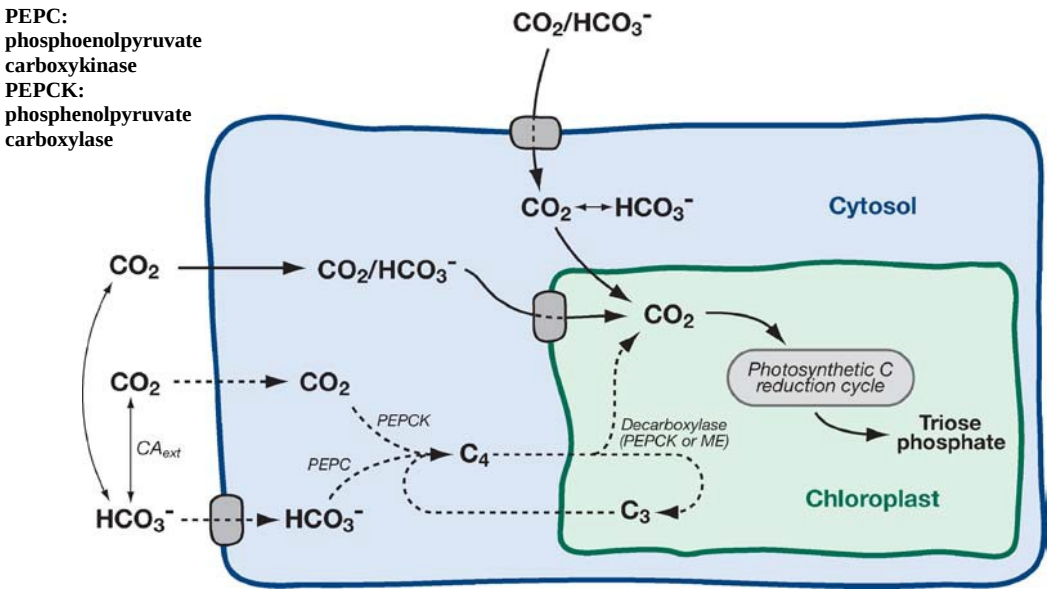


**Figure 2.1 A model for inorganic carbon transport and $CO_2$ accumulation process in eukaryotic algal cells. Adapted from Giordano et al. (2005).**

As a phototrophic species, algae can capture light and convert it to chemical energy. Pigments (mainly chlorophylls), two photosystems and an electron transport chain are responsible for this transformation (Nugent 1995, Horton et al. 2002, Renger 2010). Chlorophylls are a group of noncovalent protein-bound pigments locating in thylakoid membranes. Algae contain four

chlorophyll species (chlorophyll-a, c, d and e) (Foyer 1984). The active reaction site of chlorophyll is a tetrapyrrole ring containing $Mg^{2+}$ chelated by the nitrogen atoms of the ring (Horton et al. 2002). The light-harvesting function is fulfilled by several accessory pigments in addition to chlorophylls especially in marine organisms (Foyer 1984, Horton et al. 2002). Two major classes of accessory pigments are carotenoids and phycobiliproteins, which facilitate chlorophylls to capture light by broadening the range of light energy that can be absorbed by phototrophs (Foyer 1984 and Horton et al. 2002). In addition, carotenoids as efficient scavengers of singlet oxygen are able to protect chlorophylls from photooxidation (Foyer 1984). Antenna pigments can rapidly transfer photon to adjacent enzyme complexes known as photosystem I (PSI) and photosystem II (PSII) (Horton et al. 2002, Renger 2010). Each photosystem contains number of proteins and pigments embedded in the thylakoid membrane. These two photosystems cooperating with cytochrome *bf* complex, ferredoxin and ferredoxin-$NADP^+$ oxidoreductase operate in series to transfer electrons from $H_2O$ to $NADP^+$. The photochemical reaction center of each photosystem consists of proteins, electron-transporting cofactors and two chlorophyll molecules called the special pair (Horton et al. 2002 and Renger 2010). The maximum absorption of the special pair of PSI is at 700 nm, so the special pair pigments are named P700; in contrast, the special pair of PSII at 680 nm shows the maximum absorption, thus pigments are referred as P680. Electrons are transferred from more negative reduction potential to more positive reduction potential, which is known as the Z-scheme (Nugent 1995 and Horton et al. 2002). The initial reaction for electron flow is the oxidation of $H_2O$ in lumen by oxygen-evolving complex. Two electrons are stripped off from each $H_2O$ molecule and accepted by $P680^+$, the oxidized form of P680. P680 has the most positive reduction potential along the electron flow. Once photochemical reaction occurs, P680 becomes excited ($P680^+$) and has a more negative reduction potential than the original P680. Therefore electrons flow from $P680^+$ to the next series of carriers, pheophytin *a*, plastoquinone and plastoquinol, cytochrome *bf* complex, plastocyanin and finally P700 which has the most positive reduction potential in PSI. The light excites P700 to $P700^+$ which is the strongest reducing agent among those electron carriers. Once again electrons are transferred along with the increase of reduction potential. $P700^+$ donates electrons to chlorophyll *a*, known as $A_0$. Then electrons are transferred to phylloquinone (vitamin $K_1$ or $A_1$), and further to a series of membrane-bound iron-sulfur clusters ($F_X$, $F_A$ and $F_B$). The last iron-sulfur cluster, $F_B$, donates electrons to the iron-sulfur protein coenzyme ferredoxin (Fd) which is located in the chloroplast stroma. Catalyzed by Fd-$NADP^+$ oxidoreductase, $NADP^+$ accepts electrons from Fd and is reduced to NADPH. This noncyclic electron transport produces NADPH as energy units for inorganic carbon assimilation. The uptake of proton from stroma during the

reduction of $NADP^+$ and the release of proton to lumen during the oxidation of $H_2O$ induce a transmembrane proton concentration gradient, which facilitates the formation of ATPs in stoma. Due to light dependence, the process of ATP generation is called photophosphorylation, which provides another crucial chemical energy source for the dark reaction. Note that exposed to high light intensity, cells are able to adjust the ratio of stromal NADPH to $NADP^+$ by operating a cyclic electron transport in order to balance the energetics of light and dark reactions. In cyclic electron transport, ferredoxin donates its electrons to the PQ pool (made of plastoquinone and platoquinol) instead of $NADP^+$ via the cytochrome *bf* complex, and then electrons flow back to P700 (Horton et al. 2002).

During light reactions, cells capture light and convert it into chemical energy. Algae cells use this chemical energy to fix inorganic carbon ($CO_2$ or bicarbonate) and store it as organic compounds (e.g. sucrose and starch). In dark reactions, $CO_2$ is assimilated by the reductive pentose phosphate cycle (RPP cycle) and eventually synthesized into carbohydrates (Foyer 1984, Horton et al. 2002). RPP cycle is catalyzed by series of enzymes including ribulose 1,5-*bi*phosphate carboxylase-oxygenase (RuBisCo), fructose 1,6-*bi*phosphatase, sedohetulose 1,7-*bi*phosphatase, phosphoglycerate kinase, phosphoribulokinase and glyceraldehyde 3-phosphate dehydrogenase. RuBisCo in the stroma matrix catalyzes the first step of CO2 fixation. Thus RuBisCo, as one of the most abundant enzymes in nature, accounts for the rate of $CO_2$ assimilation (Schneider et al. 1992). Initially one molecule of gaseous $CO_2$ is reacted with one molecule of ribulose 1,5-*bis*phosphate and this reaction yields two molecules of 3-phosphoglycerate. Then the two-step reduction of 3-phosphoglycerate by ATP and NADPH converts former intermediate into glyceraldehpde 3-phosphate which later is either removed from RPP cycle and used for carbohydrate synthesis, or involved in the regeneration of ribulose 1,5-*bi*phosphate in order to complete the cycle (Horton et al. 2002). Once leaving RPP cycle, glyceraldehyde 3-phosphate initiates number of carbohydrate synthesis pathways (Foyer 1984). The net formulation of RPP cycle is

$$3CO_2 + 9ATP + 6NADPH + 5H_2O \rightarrow 9ATP + 8P_i + 6NADP^+ + G3P \tag{2.1}$$

Focusing on solar energy conversion, photosynthesis can be simplified as the following equation (Melis 2009):

$$CO_2 + H_2O + 9.5h\upsilon \rightarrow \frac{1}{6}C_6H_{12}O_6 + O_2 \tag{2.2}$$

This formula indicates that 9.5 mol photons ($h\nu$) accounts for 1 mol $CO_2$ conversion. For the sake of estimating theoretically maximum productivity of photosynthesis, several assumptions have to be made. A daily average delivery of photons is 35 mol/$m^2$ (in the US); cellular

respiration consumes 10% of the primary biomass; photorespiration and other metabolisms account for another 20% loss of primary biomass. Accordingly, the theoretical maximum productivity of photosynthesis is calculated to be 77±5 g dry cell weight per square meter cultivation area per day (Melis 2009).

### 2.3. $CO_2$ availability and fixation by microalgae

$CO_2$ is the major inorganic carbon source for microalgae, especially for freshwater species. How efficient $CO_2$ can be mitigated by microalgae partially depends on the solubility of $CO_2$ in the water based cultivation media. $CO_2$ solubility has been reported as either direct measurements or predicted using models (Wiegran et al. 1999, Duan et al. 2003, Duan et al. 2006). The process of dissolving $CO_2$ in water involves a series of pH-dependent chemical reactions and three species of carbonates (Wiegran et al. 1999).

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^- \quad (2.3)$$

$$HCO_3^- + OH^+ \leftrightarrow CO_2^{2-} + H_2O \quad (2.4)$$

A massive amount of $CO_2$ solubility data taken between 1939 and 1994 were analyzed by Duan et al. (2003). According to Duan's model (2003), at 1 bar and 303.15 K, $CO_2$ solubility is 0.0286, 0.0238, 0.0200, 0.0147 mol/kg water at 0 M, 1 M, 2 M or 4 M NaCl solutions, respectively. Duan's later model (2006) predicts, at 1 bar and 293.15 K, $CO_2$ solubility in 35‰ seawater is 0.0330 mol/kg water. Apparently, $CO_2$ solubility is affected by ionic strength. $CO_2$ concentration in the bubbling gas (vol%) was plotted against dissolved inorganic carbon (mg/kg water) by Ota et al. (2009) in an experiment aimed to discover the effect of inorganic carbon on growth of *Chlorococcum littorale*. It was shown that dissolved inorganic carbon concentration is proportional to $CO_2$ concentration in the gas flux, and dissolved inorganic carbon concentration is 1370 mg/kg water (0.0311 mol/kg water) at $CO_2$ concentration of 100% (Ota et al. 2009). The solubility of $CO_2$ in freshwater is unaffected by the pH and maintained about 10~15 μM at 18 °C, but the exchange rate of dissolved $CO_2$ and atmospheric $CO_2$ increases at low pH (Gross 2000). Considering the poor availability of gaseous $CO_2$ or bicarbonate, algae as well as higher plants develop $CO_2$ concentration mechanisms (CCM) to cope with the shortage of $CO_2$ and maintain a relatively high $CO_2$ concentration around RuBisCo active sites. In eukaryotic algal cells, $CO_2$ crosses membranes by diffusion or active transport, whereas $HCO_3^-$ is converted to $CO_2$ via carbonic anhydrase (CA) outside cells, or actively transferred into cells (Giordano et al. 2005). Internal carbonic anhydrase has been found to convert $HCO_3^-$ to $CO_2$ in cytosol. By direct and indirect transport, $CO_2$ is accumulated inside chloroplast, which favors photosynthesis.

CA is believed to catalyze the interconversion of $CO_2$ and $HCO_3^-$, and facilitate $CO_2$ supply to RuBisCo (Moroney et al. 2001). CA has been found in most organisms including animals, plants, archaebacteria as well as eubaceria (Mornoey et al. 2001). In a recent study, CA has been identified as four independent classes, termed α-, β-, γ-, and δ-CAs (Badger 2003). However based on amino acid sequencing and molecular configuration, it has been realized that these four classes have no significant differences to each other (Badger 2003). All CAs are $Zn^{2+}$ metalloenzymes, which employ a two-step enzymatic mechanism (Equation 2-5, 2-6 and 2-7) (Badger 2003).

$$E\text{-}Zn^{2+} - OH + CO_2 \leftrightarrow E\text{-}Zn^{2+} + HCO_3^- \quad (2.5)$$

$$E\text{-}Zn^{2+} + H_2O \leftrightarrow E\text{-}Zn^{2+} - OH + H^+ \quad (2.6)$$

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^- \quad (2.7)$$

Due to the widespread occurrence of CAs, their function related to carbon dioxide concentrating mechanism (CCM) in algal photosynthesis has been well investigated. Uncatalyzed interconversion between $CO_2$ and $HCO_3^-$ is $10^4$ times slower than the flux of $CO_2$ in photosynthesis (Badger 2003). Therefore CA is an absolute requirement to ensure a sufficient $CO_2$ supply.

Algal photosynthesis fixes inorganic carbon and produces a very significant quantity (~50% of the 111-117 petagrams C $y^{-1}$ global primary productivity) of organic carbon (Giordano 2005). However $CO_2$ has been found not to be the only source of dissolved inorganic carbon (DIC) for algae. Availability and transport of $HCO_3^-$ into algae has drawn intensive investigations. Many algae were shown to have multiple inorganic carbon uptake systems, and external CA is usually expressed to facilitate $HCO_3^-$ uptake (Colman et al. 2002). Colman et al. (2002) reviewed 20 papers and grouped eukaryotic microalgae into two categories based on different inorganic carbon acquisition mechanisms. Most marine and freshwater algae are reported to have multiple inorganic carbon (Ci) uptake mechanisms, and external CA is present particularly under low $CO_2$ concentration (atmospheric). The list of these algae includes some species of Chlorophyta (e.g. *Scenedesmus obliquus*), Bacillariophyta, Rhodophyta, Haptophyta and Eustigmatophyta. Evidences of kinetics of Ci uptake and direct measurement of $CO_2$ uptake by mass spectrometry illustrate that these algae can actively take up $CO_2$ and $HCO_3^-$. In addition, some algae like *C. ellipsoidea* and *C. kesslerii* have been found to express no external CA, so they are able to directly uptake $HCO_3^-$ from media. On the other hand, some algae are believed to have the single Ci acquisition mechanism, and are usually absent of external CA (Colman et al. 2002). This category includes some species of Chlorophyta, Dinophyceae and

Eustigmatophyceae. Several algae have been shown to lack active transport of $HCO_3^-$ but can take up $CO_2$ via diffusion and/or transporters. Commonly these species are found to be restricted to acidic media and develop a mechanism of Ci acquisition to adapt their environment. Two marine eustigmatophyte algae, *N. gaditana* and *N. oculata*, are documented as the only species to have an energy-dependent uptake of $HCO_3^-$ and no $CO_2$ utilization (Colman et al. 2002). Seawater with a pH value around 8 contains sufficient $HCO_3^-$ as Ci source, so it is feasible for these two marine algae to maintain equilibrium internal $CO_2$ concentration by taking up medium $HCO_3^-$ and enzymatic converting it to $CO_2$ (Amoroso et al. 1998 and Colman et al. 2002). Inorganic carbon accumulation had been proved as an energy dependent process (Badger et al. 1980). After adding certain energy inhibitors (e.g. methyl viologen) into cultural medium of *Chlamydomonas*, internal Ci concentrations of cells were significantly less than the control.

Both marine and freshwater algae can effectively utilize $HCO_3^-$ along with $CO_2$ as Ci sources. However, assimilation efficiency and algal mass productivity are significantly different between these two Ci sources. An experiment aimed to distinguish the quality of $HCO_3^-$ and $CO_2$ in terms of Ci assimilation efficiency and algal mass productivity was conducted on one marine diatom (*Phaeodactylum tricornutum*) and three fresh water green algae (*Chlorella vulgaris*, *Scenedesmus obliquus* and *Selenastrum capricorutum*) (Goldman et al. 1981). At steady state, Ci assimilation efficiency is defined as comparison of dissolved $HCO_3^-$ mass flux in or $CO_2$ mass flux in with algal carbon mass flux out. When $HCO_3^-$ is provided as the sole Ci source, carbon assimilation efficiencies are 100 and 72% for *Phaeodactylum tricornutum* and the freshwater algae, respectively. Maximum concentrations of added $HCO_3^-$ lead to linear responses in algal productivity with the maximum rate of 28 mg C/day. By contrast, when 1% $CO_2$ is supplied as the sole Ci source in the freshwater media, carbon assimilation efficiency drops to 61% for small bubbles and 38% for large bubbles, which correspond to a maximum Ci input flux of 350 mg C/day. In conclusion, $HCO_3^-$ is an excellent source of Ci in terms of carbon assimilation efficiency; however higher $HCO_3^-$ levels can induce chemical precipitation and an increase in pH, which directly deteriorate culture media.

Does high concentration of dissolved inorganic carbon always lead to high algae productivity or high rate of photosynthesis? The relationship between external dissolved inorganic carbon (DIC) concentration and rate of photosynthesis is revealed in Figure 2.2 (Miyachi et al. 2003).

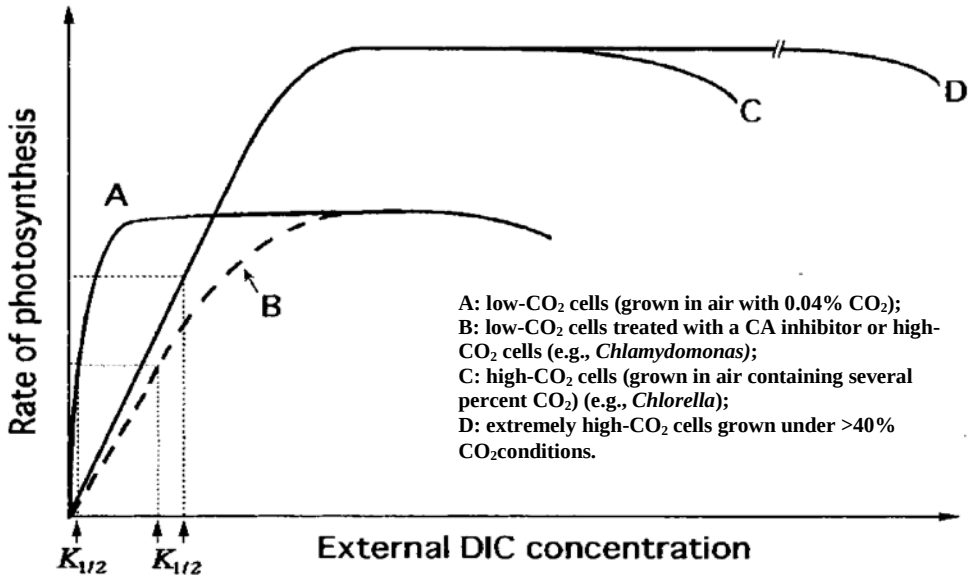


**Figure 2.2 Relationship between rate of photosynthesis and external DIC concentration in microalgae grown under low, high and extremely high-$CO_2$ conditions**. **Adapted from Miyachi et al. (2003).**

It is apparent that regardless of cell type, photosynthesis is eventually saturated by elevated external DIC concentrations. Low-$CO_2$ cells or low-$CO_2$ acclimated cells represent those algae and cyanobacteria that are transferred from high to low-$CO_2$ conditions, and their photosynthetic activity is increased at a $CO_2$ limited condition (Miyachi et al. 2003). By contrast, some cells are grown in high-$CO_2$ medium, and after transferring to low-$CO_2$ environments, their ability to efficiently fix $CO_2$ is limited; but under high-$CO_2$ condition, maximum photosynthesis is significantly higher than low-$CO_2$ cells. These algae or cyanobacteria are referred to as high-$CO_2$ cells or high-$CO_2$ acclimated cells (Miyachi et al. 2003). Thus one alga can be either low-$CO_2$ or high-$CO_2$ cell based on different growth histories. Microalgae are generally considered to susceptible to a very high $CO_2$ concentration. Despite this, it has been reported that a number of algae (e.g. *Chlorococcum littorale*) can grow rapidly at 40% $CO_2$ environment (Miyachi et al. 2003). It has also been shown that extremely high concentration (≥50%) of $CO_2$ slows down photosynthesis and algal growth (Maeda et al. 1995, Ota et al. 2009, Ho et al. 2010).

After transferring to an extreme high-$CO_2$ condition, low-$CO_2$ *C. littorale* experiences a lag period before growth starts (Miyachi et al. 2003). In the initial lag period, $CO_2$ fixation and $O_2$ evolution are suppressed and then increase; cyclic electron transfer in PSI is greatly enhanced; CA activities decline to zero; RuBisCo, which governs photosynthesis rate, is inactivated due to the decrease of pH in stroma (Miyachi et al., 2003). By contrast, high-$CO_2$ cells, especially those tolerant to extremely high $CO_2$ condition (e.g. *Stichococcus bacillaris*), show no significant state transition after being transferred from low to extremely high $CO_2$ conditions (Miyachi et al. 2003). During high-$CO_2$ acclimation, low-$CO_2$ cells must generate extra ATP to power proton

pumps in order to maintain intracellular pH constant, especially in the lag phase. Several observations suggest that extra ATPs are produced by the cyclic electron transfer in PSI (Miyachi et al. 2003). *Chlorococcum littorale* was incubated under a range of $CO_2$ concentrations (5, 20, 35 and 50%), and growth rates decrease with increasing $CO_2$ concentrations (Ota et al. 2009). By analyzing $CO_2$ and $HCO_3^-$ distributions in media, it has been shown that specific growth rates of *C. littorale* decrease when $CO_2$ concentrations are greater than $HCO_3^-$ concentrations, and start increasing when $HCO_3^-$ is becoming predominant (Ota et al. 2009). Ota et al. (2009) concluded that $HCO_3^-$ appears, as a promoter for *C. littorale* cell growth and overdosed $CO_2$ is a potential inhibitor. This hypothesis may explain the inhibition of *C. littorale* growth by high $CO_2$ concentrations.

Low-$CO_2$ *Chlorella emersonii* has been shown to have a higher net $CO_2$ uptake than its high-$CO_2$ counterpart at limiting $CO_2$ concentration ($CO_2$ < 10 µM) (Beardall and Raven 1981). *Chlamydomonas reinhardtii* has been studied for evidences of $CO_2$ concentration mechanism (Badger et al. 1980). When photosynthesis is stable under 100 µM external Ci in low $CO_2$ cells, internal Ci is approximately 10-fold higher than external Ci, whereas for high $CO_2$ cells, internal Ci is only approximately 5-fold higher than external Ci (Badger et al. 1980). In another study, *Chlamydomonas reinhardtii* and *Dunaliella tertiolecta* were studied to understand $HCO_3^-$ and $CO_2$ utilization in low-$CO_2$ and high-$CO_2$ cells (Amoroso et al. 1998). Similarly to the previous results, for both algae, low-$CO_2$ cells show a higher Ci uptake than high-$CO_2$ cells under a limiting concentration of DIC ($CO_2$ < 80 µM, $HCO_3^-$< 4 mM). However under a saturating DIC condition, both high and low-$CO_2$ cells reach the maximum photosynthesis rates, and high-$CO_2$ cells show a higher photosynthesis rate compared to low-$CO_2$ cells (Miyachi et al. 2003). Preferences of $CO_2$ and $HCO_3^-$ vary among different algal species. It appears that both high- and low-$CO_2$ cells of *C. reinhardtii* take up almost same percentage of $CO_2$ and $HCO_3^-$, but high- and low-$CO_2$ cells of *D. tertiolecta* utilize more $HCO_3^-$, with $CO_2$ and $HCO_3^-$ contributing to about 20 and 80% of Ci uptake respectively (Amoroso et al. 1998). Kinetic evidences and direct measurements of Ci uptake in low-and high- $CO_2$ cells indicate that the increase in Ci transport efficiency after acclimation to low $CO_2$ conditions is attributed to adjustments of the transport mechanism rather than increment of the number of transporters (Amoroso et al. 1998). It was postulated that growth environment (pH 8 and 1 M NaCl) and lower external CA activity lead to a predominant $HCO_3^-$ transport system in *D. tertiolecta*, which seems to be typical for marine algae (Amoroso et al. 1998).

Although external pH does not significantly affect algal internal pH, mechanism of $CO_2$ acquisition in acid-tolerant algae seems to vary from algae growing at neutral or slightly basic

pH. The solubility of $CO_2$ in freshwater is unaffected by the pH and maintained about 10~15 μM at 18 °C, but the exchange rate of dissolved $CO_2$ and atmospheric $CO_2$ is increased at low pH (Gross 2000). Therefore, a great amount of dissolved $CO_2$ is provided to acid-tolerant algae. When pH value is less than 4.5, the high $CO_2$ medium of acid-tolerant *Chlamydomonas* almost completely represses external CA but not internal CA (Balkos and Colman 2007). Consequently no active $HCO_3^-$ uptake could be detected in this situation (Balkos and Colman 2007). In the absence of active transport of inorganic carbon, acid-tolerant *Chlamydomonas* take up $CO_2$ by diffusion (Balkos and Colman 2007).

## 2.4. RuBisCo – primary enzyme for carbon fixation

RuBisCo catalyzes the first step of carbon fixation in Calvin cycle. From a molecular point of view, RuBisCo consists of eight large subunits (50-55 kDa each) and eight small subunits (12-18 kDa each) for a total molecular weight of about 560 kDa (Schneider et al. 1992, Horton et al. 2002, Andersson 2008). The majority of RuBisCo consist of a core of four L2 dimers arranged around a 4-fold axis, and small subunits capped at the end of each dimer (Schneider et al. 1992 and Andersson 2008).

RuBisCo has an affinity for both $CO_2$ and $O_2$. The oxygenation reaction of RuBisCo involves one molecule of ribulose 1,5-*bi*phosphate and one $O_2$, and yields one molecule of 3-phosphoglycerate entering the RPP cycle and one molecule of 2-phosphoglycerate (Foyer 1984 and Horton et al. 2002). It is believed that 2-phosphoglycolate is further metabolized in peroxisomes and mitochondria via an oxidative pathway and modified into 3-phosphoglycerate, which also enters the RPP cycle (Horton et al. 2002). Besides the dual role of carboxylation and oxygenation of RuBP, RuBisCo has several other characteristics. First, the enzyme shows poor catalytic efficiency. It has been calculated that RuBisCo account for 50% of leaf proteins in plants, and has a turnover number of 3 $s^{-1}$ (Schneider et al. 1992). Secondly, RuBisCo cycles between active and inactive states (Portis 1992 and Andersson 2008). To possess catalytic potential, RuBisCo needs to be activated by carbamylation of the active site Lys201. This reaction is initiated by gaseous $CO_2$, which is distinct from the RPP cycle substrate $CO_2$, and the carbamylated Lys201 is stabilized by binding to $Mg^{2+}$ (Portis 1992, Andersson 2008). Overall the activated RuBisCo is a ternary complex of enzyme, $CO_2$ and $Mg^{2+}$. The requirement of $Mg^{2+}$ indicates light as a regulation factor. Under illumination, protons move from stroma to lumen along with the migration of $Mg^{2+}$ from lumen into storma, which enhances the activated RuBisCo pool (Horton et al. 2002, Andersson 2008). Once activated, the active site is available for either RuBP or other sugar phosphates (Portis 1992). When bound by other sugar phosphates (e.g.

phosphoglycerate), RuBisCo becomes inactive. Moreover de-activation and activation of RuBisCo is modulated by RuBisCo activase, which is recognized as a member of ATPases (Portis 1992, Houtz and Portis 2003). The affinity of the sugar phosphate to the active site is reduced at the presence of RuBisCo activase (Portis 1992, Houtz and Portis 2003). The activity of RuBisCo is also affected by environmental factors (Salvucci and Crafts-Brandner 2004). At moderate high temperatures, the pool of activated RuBisCo shrinks, because the ability of RuBisCo activase to maintain RuBisCo in an active state cannot keep pace with a faster rate of de-activation. Due to the sensitivity of protein to environmental stress, RuBisCo has been found in degradation forms when exposed to UV radiation, ozone or high light intensity (Houtz and Portis 2003).

The oxygenation of RuBP consumes $O_2$ and NADPH or ATP produced by light reaction and yields phosphoglycolate. The metabolism of phosphoglycolate releases $CO_2$. This process resembles the mitochondrial respiration, but is light dependent; therefore it is called photorespiration (Foyer 1984). The reactions of photorespiratory cycle occur in chloroplasts, cytosol, peroxisomes and mitochondria, and recover carbon from phosphoglycoate ultimately back to phosphoglycerate (Foyer 1984). Photorespiration seems to be a waste of RuBP and energy, but indeed protect plant against photooxidative damage and $CO_2$ limitation (Foyer 1984, Horton et al. 2002). Especially reactive oxygen species induced by high light intensity can be scavenged by the oxygenation of RuBP.

However high photorespiration rate offsets the productivity of photosynthesis with respect to $CO_2$ mitigation, $O_2$ evolution and biomass yields. Therefore optimal growth condition should balance the rate of two processes. The competition between $CO_2$ and $O_2$ to the active site of RuBisCo is related to the partial pressure of each gas around the RuBisCo reaction center (Giordano et al. 2005).

## 2.5. Nitrogen assimilation in algae cells

Several inorganic forms of nitrogen including nitrate, nitrite, ammonium and urea, have been found to serve as N sources for algae growth (Syrett 1988, Graham and Wilcox 2000, Glass et al. 2009). In addition, cyanobacteria can perform $N_2$ fixation catalyzed by nitrogenase. However, only the most reduced form ($NH_4^+$) is incorporated in metabolic pathways and synthesized into amino acids, nucleotides, chlorophylls and phospholipids (Horton et al. 2002 and Glass et al. 2009). Therefore, electrons must be donated to $N_2$, $NO_3^-$ and $NO_2^-$ to reduce to $NH_4^+$. It is believed that nitrogen assimilation in algae is facilitated by series of enzymes: urease, nitrate

reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) and glutamate synthase (GltS) (Syrett 1988, Horton et al. 2002, Inokuchi et al. 2002).

Cyanobacteria are the only known oxygenic photosynthetic prokaryotes, which perform $N_2$ assimilation (Glass et al. 2009). Reducing $N_2$ to $NH_4^+$ requires nitrogenase (Nif) and 16 molecules of ATP for each $N_2$:

$$N_2 + 8e^- + 8H^+ + 16ATP \xrightarrow{\text{nitrogenase}} 16ADP + 16P_i + 2NH_3 + H_2 \quad (2.8)$$

When $NO_3^-$ is supplied to algae cells as nitrogen source, it has to be reduced to $NO_2^-$ first:

$$NO_3^- + 2e^- + 2H^+ \xrightarrow{\text{nitrate reductase}} NO_2^- + H_2O \quad (2.9)$$

In algae, nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) acts as electron donor; whereas in cyanobacteria, electrons are directly donated by ferredoxin (Glass et al. 2009). Nitrate reductase (NR) is responsible to catalyze this reaction (Inokuchi et al. 2002 and Glass et al. 2009). Prokaryotic NR is composed of one [4Fe-4S] cluster and one molybdopterin cofactor with cysteines; and its eukaryotic counterpart contains four identical subunits, and each consists of flavin adenine dinucleutide (FAD), cytochrome$b_{557}$ containing Fe atoms, and molybdenum-molybdopterin (Mo-MPT) (Inokuchi et al. 2002, Glass et al. 2009). Therefore Fe and Mo are required for NR function.

In the following step, catalyzed by nitrite reductase (NiR), $NO_2^-$ accepts six electrons and is reduced to $NH_4^+$:

$$NO_2^- + 6e^- + 8H^+ \xrightarrow{\text{nitrite reductase}} NH_4^- + 2H_2O \quad (2.10)$$

NiR, as a monomer, contains one [4Fe-4S] cluster and one siroheme. It is believed that [4Fe-4S] cluster transfer electrons from ferredoxin to Fe of siroheme, where $NO_2^-$ obtains electrons and transforms into $NH_4^+$ (Glass et al. 2009).

Besides inorganic N, algae can also take up dissolved organic nitrogen well known as urea and amino acid. Urea can be used as nitrogen source for many algae, including dinoflagellates, *Chlorella* and *Chlamydomonas* (Healey 1973, Syrett 1988, Solomon and Glibert 2008). Urea is transported into the cell via either passive or active transport (Syrett 1988). Inside the cell, urea is further decomposed by urease, and converted to ammonium and carbon dioxide.

$$CO(NH_2)_2 + H_2O + 2H^+ \xrightarrow{\text{urease}} 2NH_4^+ + CO_2 \quad (2.11)$$

However *Chlorella* and *Chlamydomonas* have been found lack of urease (Healey 1973, Syrett 1988). These algae use urea amidohydrolase coupling with one molecule of ATP to break down one urea molecule (Syrett 1988):

$$CO(NH_2)_2 + HCO_3^- + 3H^+ + ATP \xrightarrow{\text{ureaamidohydrolase}} 2NH_4^+ + 2CO_2 + ADP + P_i \quad (2.12)$$

Another theory is that there are two enzymes involved in the hydrolysis: urea carboxylase coupled with ATP converts urea into allophanate, which is later hydrolyzed into ammonium and bicarbonate by allophanate hydrolase (Thompson and Muenster 1971).

Amino acid can be taken up directly or deaminated first and then assimilated as $NH_3$ in algae (Healey 1973). *Chlorella pyrenoidosa, Platymonas subcordiformis, Nitzschia ovalis, Melosira nummuloids, and Coscinodiscus asteromphalus* have been reported to perform energy dependent amino acid uptake which is not affected by the presence of ammonia and nitrate (Healey 1973). On the other hand, some red algae (including coccolithophorids and dinoflagellates) can deaminate amino acid outside the cell by a Cu-containing cell-surface amine oxidase (GuAO), and then take up $NH_3$ (Glass et al. 2009). Amino acids are oxidized by the following reaction:

$$RCH_2NH_2 + O_2 \xrightarrow{GuAO} H_2O_2 + RCHO + NH_3 \quad (2.13)$$

Once N compounds are converted into $NH_4^+$, $NH_4^+$ enters the glutamine synthetase-glutamate synthase (GS-GltS) pathway, and then constitutes protein, chlorophyll, nucleotide and phospholipid (Glass et al. 2009). The initial step of this pathway is to generate glutamine:

$$\text{Glutamate} + ATP + NH_4^+ \xrightarrow{\text{Glutamine synthetase}} \text{Glutamine} + ADP + P_i \quad (2.14)$$

GS is catalyzed the formation of glutamine, and requires Mg or Mn ions for activation (Glass et al. 2009).

## 2.6. Nutrient requirement for *Scenedesmus* cultivation

Four major components constituting algae biomass are carbon, oxygen, hydrogen and nitrogen, where oxygen and hydrogen originate from water molecules, and carbon is assimilated from $CO_2$ (Table 2.1). Nitrogen is not freely available to microalgae cells, but can be provided in the media. Besides the four key elements, 5% of *Scenedesmus* dry weight is composed of phosphorus, potassium, magnesium, sulfur, iron, calcium, zinc, copper and manganese.

**Table 2.1 Elemental composition of *Scenedesmus* used in this study (by weight)[a].**

| Element | % |
|---|---|
| Carbon (C) | 50.02 |
| Oxygen (O) | 25.98 |
| Hydrogen (H) | 7.43 |
| Nitrogen (N) | 7.80 |
| Phosphorus (P) | 2.20 |
| Potassium (K) | 0.82 |
| Magnesium (Mg) | 0.39 |
| Sulfur (S) | 0.69 |
| Iron (Fe) | 0.43 |
| Calcium (Ca) | 1.3 |
| Manganese (Mn) | 0.0023 |
| Copper (Cu) | 0.0021 |
| Zinc (Zn) | 0.0053 |

[a]Data were measured in this study and methods are detailed in Chapter 5.

Nitrogen is often supplied in forms of ammonium, nitrate or urea; phosphorus is added as phosphate, which also functions as a buffer to maintain a neutral pH. Micronutrients are critical for enzymes formation and activity, which directly links to $CO_2$ fixation and biomass production. As aforementioned, PBRs are aquatic system where nutrients suspend around microalgae cells to ensure a healthy growth of microalgae. To make the system sustainable, input from commercial fertilizers should be minimized, and mineralized nutrients from AD digestate could be reused instead. Organic macromolecules are mineralized through anaerobic digestion (Moller and Muller 2012) (Figure 2.3). Anaerobic bacteria break down carbohydrate and fatty acids and generate biogas (a mixture primarily of $CH_4$ and $CO_2$). Nitrogen in proteins is mineralized to $NH_3$ in gas form, or $NH_4^+$ in solution. Phosphate as essential building blocks for nucleic acid is released from the macromolecules, and available for anaerobic bacteria to assimilate. Sulfur existing in cysteine or methionine to form disulfide bonds and is mineralized to $H_2S$, which can dissolve in the liquid digestate or vent out as a gas.

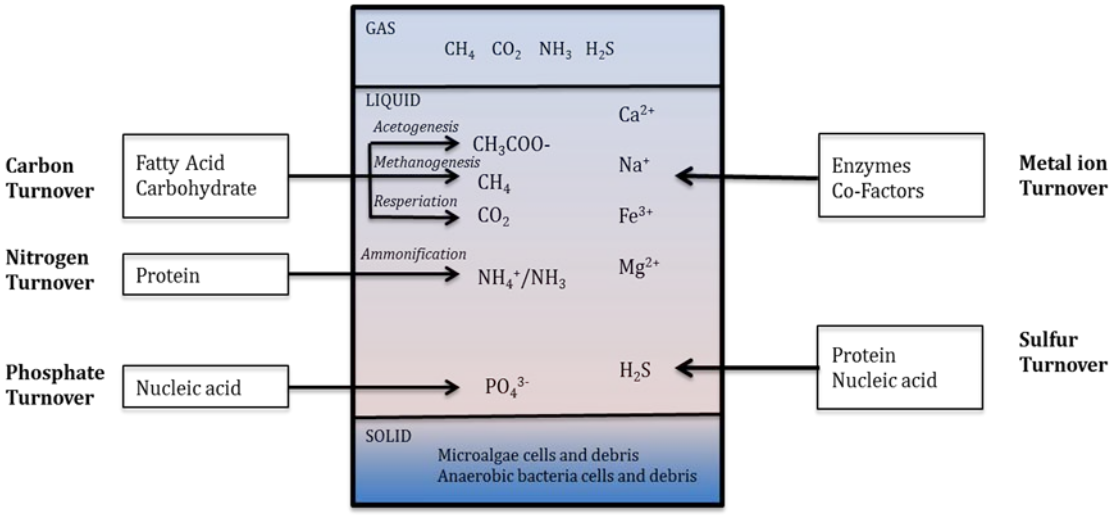


**Figure 2.3 Mineralization of macromolecules by anaerobic digestion.**

Using digestates as fertilizer is not a new concept. Researchers have been studying the impacts of digestates to soil fertility and crop phytotoxicity. Farm manures are common substrates for anaerobic digesters, so digested manure slurry or co-digested crop residue and manure slurry have been extensively studied.

Digestate is best known as the rich source of mineralized nutrients (N and P), which also plays a vital role in determining the suitability of the digestate as fertilizer (Alburquerque et al. 2012). Alburquerque et al. (2012) investigated the phytotoxicity of anaerobically digested manure slurries in lettuce and cress hydroponics. Directly using the digestate without any dilution caused no germination or biomass accumulation, which was explained by the high salinity of the digestate. Diluting to 20% of concentration, seeds germinated, and 1% digestate concentration gave the best germination and highest biomass accumulation. Researchers linked the fertilizing performance of various dilutions of digestate to organic matter content, and concluded that when dissolved organic carbon (DOC) and biochemical oxygen demand ($BOD_5$) were less than 1.5 or 2.5 g/L respectively, the digestate was suitable for lettuce and cress hydroponics. Nishikawa et al. (2012) conducted a six-year experiment on the effects of digested cattle manure in a rice paddy in the warmer region of Japan. The digestates were compared to commercial fertilizers in terms of grain yield and nitrogen efficiency, which was the normalized nitrogen uptake (the difference of control and varying nitrogen dosage). Regardless of the dosage and application method, first three years' result showed commercial fertilizer yielded higher N efficiency, but the second three years' result indicated a superior effect of digestates on N efficiency. In terms of grain yield, two kinds of fertilizers rendered similar rice production rates. Manure digestate was an appropriate alternative to commercial fertilizers in rice cropping since rice plants preferred ammonia N rather

than nitrate N. Bachmann et al. (2011) tested the fertilizing effects of codigested slurry of dairy slurry, maize silage and wheat grain on two crops species: *Zea mays* L. and *Amaranthus cruentus* L.. They concluded that dairy slurry and codigested dairy slurry were as effective as the highly soluble mineral P source with respect to the plant P uptake rate. In particular for amaranth growing on the sandy soil, digestates offered even higher plant P uptake. However an opposite pattern occurred for N uptake, organic fertilizers caused more than 50% less plant N uptake than commercial fertilizers. Interestingly the dry matter yields did not drop dramatically with the lowing N uptake for both *Zea mays* L. and *Amaranthus cruentus* L.. They also studied the microbial activity in the soils fertilized by digestates and commercial fertilizers. It turns out significant high microbial activity has been maintained in the soils fertilized by digestates, which can benefit the organic matter turnover and the plant growth.

Studies have shown anaerobic digestates can potentially benefit crop growth as well as nitrogen uptake, however this application should be carefully monitored to ensure a constant soil quality at the early stages (Arthurson 2009). Due to the high content of organic matter (especially organic C), the soil can undertake a different route to adjust to the addition of digestates compared to commercial fertilizers. Overlooking the short-term alternation of soil quality can be risky to maintain the sustainability of soil quality in the long run.

## 2.7. Anaerobic digestion of algae

Anaerobic digestion has been studied for decades as a practical approach to decompose microalgae biomass and produce methane as fuel gas (Golueke et al. 1956, Foree and McCarty 1970, Chen 1987, Ras et al. 2011). In fact, anaerobic decomposition of microalgae takes place in the natural aquatic system. Because of gravitational sedimentation, microalgae eventually sink to the anoxic and aphotic zones, and are broken down by anaerobic bacteria (Foree and McCarty 1970). If this anaerobic digestion takes place in a bioreactor, microalgae can be degraded into inorganic elements, which can be transferred back to the photobioreactor and support microalgae proliferation. Thus there is a strong motivation to design an anaerobic digester, and optimize its operational parameters to decompose microalgae in the most cost effective way.

The most important environmental factors for a successful digester are temperature, pH, alkalinity, nutrients, and mass transfer efficiency. pH is an important parameter because (1). methanogens are more sensitive to pH variation than other microorganisms; (2). digester stability is greatly affected by the operational pH; (3). microbial metabolites (like volatile fatty acids (VFAs) and $NH_4^+$) can drastically change the pH value. Optimum pH for acidogens is 5.5~6.5, and for methanogens is 7.8~8.2. For the combined cultures, the optimum pH range is from 6.8 to

7.4 (Chen 1987, Khanal 2009, Ras et al. 2011, Ehimen et al. 2011). As mentioned previously, lipid and protein are hydrolyzed into fatty acids and amino acids, meanwhile acidogens and acetogens convert the hydrolysate into simple organic acids like acetic acid or carbon dioxide. If methogens are not able to consume the acetic acid or carbon dioxide produced by other bacteria, the acidic environment cannot be effectively neutralized and eventually it results in decreased pH (Khanal 2009). Chen (1987) had proposed several measures to cope with the pH decrease: (1) decreasing the C/N ratio of the feed, (2) extending the retention time, and (3) decreasing the substrate loading. A novel method to boost the pH is to periodically dose oxygen into the anaerobic digester (Khanal 2009). It is believed with limited oxygen some facultative microbes quickly consume the excess VFAs (Khanal 2009). Besides artificial interventions, the pH of an anaerobic system can be mediated by self-produced alkalinity (Khanal 2009). The decomposition of protein leads to the production of inorganic nitrogen, and in anaerobic systems, ammonia is the predominant final product (Khanal 2009):

$$RCHNH_2COOH + H_2O \rightarrow RCOOH + NH_3 + CO_2 + 2H_2 \quad (2.8)$$

$$NH_3 + H_2O + CO_2 \rightarrow NH_4^+ + HCO_3^- \quad (2.9)$$

Alkalinity is the ability of buffer to neutralize acids. Since alkalinity is mainly contributed by the decomposition of organic nitrogen (e.g. proteins), only protein-rich biomass can contribute to the alkalinity. If using the carbohydrate-rich substrates, one has to consider the alkalinity supplementation using chemicals such as sodium bicarbonate, sodium carbonate, ammonium hydroxide, gaseous ammonia, lime, and sodium and potassium hydroxide. Alkalis captures carbon dioxides and form alkalinity:

$$Ca(OH)_2 + 2CO_2 \leftrightarrow Ca^{2+} + 2HCO_3^- \quad (2.10)$$

$$Na_2CO_3 + H_2O + CO_2 \leftrightarrow 2Na^+ + 2HCO_3^- \quad (2.11)$$

Among all chemicals, sodium bicarbonate is the typical choice due to its high solubility, long-lasting impact and low toxicity (Khanal 2009).

Anaerobic digesters should be operated under a well-controlled temperature range. Based on different optimum growing temperatures, there are three types of methanogens: psychrophile (5~15 °C), mesophile (35~40 °C), and thermophile (about 55 °C) (Khanal 2009). Chen (1987) found that at 40 °C there is a peak for methane specific gas production by mesophiles and at 55 °C there is another peak for methane specific gas production by thermophiles. However over 65 °C, methanogens become inactive and methane production decreases. In another study of anaerobic digesting microalgae (Ehimen et al. 2011), it was found that the highest specific $CH_4$

yield occurred at 35 °C for substrate C/N ratio of 12.44. But for substrate C/N ratio of 8.53, the highest specific $CH_4$ yield was shown at both 35 and 40 °C.

Some nutrients should be provided to the anaerobic digester if they are not supplied by the organic substrates. The empirical formula for an anaerobic bacteria cell is $C_5H_7O_2N$ (Khanal 2009). The nitrogen required for biomass synthesis can be calculated by the empirical formula. Phosphorus demand is about 1/7 to 1/5 of nitrogen demand. The typical nitrogen and phosphorus sources are urea and aqueous ammonia, and phosphate salt. In the current study microalgae will be tested as the sole nitrogen and phosphorus source. Trace elements including iron, cobalt, molybdenum, selenium, calcium, magnesium, sulfide zinc, copper, manganese, tungsten, boron, nickel and $V_{B12}$. The growth of methanogens requires nickel, because it is a constituent of factor F430, which is only found in methanogens.

On the other hand, some chemicals adversely affect the anaerobic biogas production, and are even toxic to microbes. It has been indicated that 1500 mg /L was a practical upper limit for ammonia concentration in algal anaerobic digesters (Chen 1987). A study of anaerobic digestion of cattle manure showed that 4000 mg N/L ammonia inhibited the anaerobic digestion (Angelidaki and Ahring 1994). In addition, thermophiles are more susceptible to ammonia than mesophiles (Khahal 2009). Sodium chloride also has toxicity to anaerobic bacteria. Chen (1987) found that 0.5 M or more NaCl severely depressed methane production.

## 2.8. Thermal chemical pretreatment of algal biomass

Anaerobic digestion is potentially suitable for *Scenedesmus* degradation, however living algal cells are reported to resist bacterial attack, due to their rigid cell wall structure. Golueke et al. (1956) compared the degradation rate between raw sewage and microalgae, and it showed that after 35 °C mesophilic digestion, 60% of raw sewage was degraded, but only 40% of microalgae was destructed. Chen (1987) studied anaerobic digestion of microalgae collected from the effluent of high-rate sewage stabilization ponds. Prior to thermal pretreatment, the methane production was 0.14 L/g VS; by contrast, the methane production increased to 0.20 L/g VS, when the microalgae substrate was heated to 50 °C for 30 min. Apparently heating improved microalgae biodegradability.

To further explore ways to improve microalgae biodegradability, algal cell structure needs to be understood. Cell wall structures of *Scenedesmus* and *Chlamydomonas* are significantly different. Based on the observations under light and electron microscopy, Bisalputra et al. (1963) described the cell wall of *Scenedesmus quadricauda* as a sandwich-like structure consisting of cellulosic layer, middle layer, and pectic layer. Given the sugar composition of cell

walls isolated from array of *Scenedesmus* species, Takeda (1996) concluded that glucose was the major sugar component in the rigid wall, along with galactose and mannose. The isolated cell wall from *Scenedesmus communis* was examined by $^{13}$C-NMR (solid state $^{13}$C-nuclear magnetic resonance spectroscopy) and FTIR (Fourier transform infrared spectroscopy), which revealed to compose of a highly aliphatic material; meanwhile pyrolysis-GC/MS's measurement confirmed the dominance of *n*-alkene/*n*-alkane doublets in the aliphatic molecules, which were cross-linked via ether-linkages (Blokker et al. 1998). On the other hand, *Chlamydomonas* has a distinct type of cell wall structure. *Chlamydomonas* was firstly considered to contain mainly hydroxyproline heterooligosaccharides in its cell wall, which was linked to the fact that the *Chlamydomonas* cell walls are more susceptible to mechanical rupture than *Chlorella* walls are (Miller et al. 1972). Shortly after this discovery, the chemical composition of cell walls of *C. gymnogama* was determined, and arabinose (24.8% w/w) and galactose (19.6% w/w) were the predominant sugars, and amino acids accounted for about 10.2% w/w of the wall (Miller et al. 1974). Sugars were bound to amino acids and formed glycoprotein structure, which was estimated to be at least 32% of the cell wall weight (Miller et al. 1974, Catt et al. 1976). The cell wall of *Chlamydomonas reinhardtii* is a multilayered membrane composed of carbohydrates and 20-25 polypeptides, meanwhile it lacks cellulose in the structure (Imam et al. 1985). Mussgnug (2010) carried out anaerobic digestion on six common microalgae species including *Scenedesmus obliquus* and *Chlamydomonas reinhardtii* without any pretreatment. The methane production rates for *S. obliquus* and *C. reinhardtii* were 287 and 587 mL/g VS respectively. The light microscopic images of microalgal cells indicated after 28 days mesophilic (38 °C) anaerobic digestion, intact cells of *Scenedesmus* or *Chlamydomonas* still existed in the sludge.

To improve the cell degradation and methane production, pretreatment should be carried out prior to the anaerobic digestion. Cell wall disruption can be implemented with thermal, chemical, enzymatic, or mechanical pretreatment (Gonzalez-Fernandez et al. 2011). Thermal pretreatment is the most researched method. Chen (1987) studied the thermal pretreatment on the methane production efficiency from the microalgae collected from the effluent of high-rate sewage stabilization ponds. When heating duration was 30 min, high temperature pretreatment appeared to significantly improve the methane production, and especially the highest yield (0.25 L/g VS) was carried out by the microalgae subjected to 100 °C pretreatment. Meanwhile moderate temperature (40 °C) also benefited the methane yield, but only less increase (0.17 vs. 0.14 L/g VS). When temperature rose to 120 °C, the methane yield was the same as pretreatment at 100 °C. Heating duration was also studied. Comparing 15 min to 4 hours, latter had 0.04 L/g VS more methane being produced. Besides temperature and duration, he also tested the effects of

substrate loading rates. Interestingly increasing the substrate concentration from 3.7 to 22.5% made the methane yield plunged by 18%. Another study carried out by Gonzalez-Fernandez et al. (2012) revealed the relationship of heating temperature (70 vs. 90 °C), soluble COD of the substrate and the methane yield. After 180 min of heating, soluble COD of *Scenedesmus* sludge was increased 4 or 5 fold when pretreated at 70 and 90 °C, respectively. The methane production over 33 days showed that raw microalgae and microalgae pretreated at 70 °C resulted in a similar quantity of methane produced. If the microalgae was pretreated at 90 °C, the gas production was 2 fold higher. However after the first 10 days, pretreated microalgae showed significantly higher methane yield than did raw microalgae, and especially for the one treated at 90 °C. Appels et al. (2010) and co-workers studied the pretreatment of wastewater sludge and demonstrated similar trends but with more significant differences. Pretreatments at 80 and 70 °C rendered the same amount of methane when treatment duration was 15 min. Pretreatment at 80 °C showed slightly higher biogas yield when the duration was prolonged to 60 min. By contrast, pretreatment at 90 °C gave superior results at all three heating duration (15, 30 and 60 min). Thermal pretreatment often is companioned by high pressure if the process is implemented in an autoclave (Mendes-Pinto et al. 2001, Barjenbruch and Kopplow 2003, Miranda et al. 2012). Barjenbruch and co-workers (2003) investigated the thermal pretreatment of sewage sludge by autoclave under three temperatures (80, 90 and 121 °C), and it turned out 90 °C carried out the highest improvement.

Alkaline or acid was also incorporated in the thermal pretreatment of microalgae to disrupt cell walls (Chen 1987, Mendes-Pinto et al. 2001, Halim et al. 2012, Miranda et al. 2012). Chen (1987) chose to heat the microalgae sludge at 100 °C, for 8 hours with various NaOH dosage (w NaOH/w dry microalgae %). Interestingly it showed that no addition of NaOH gave the highest methane yield, and increasing NaOH dosage from 2 to 20%, the methane yield plunged to close to zero, even though samples were neutralized to pH 7 after all pretreatments. The similar trend was revealed during a study on *Haematococcus pluvialis* which was considered as an important natural source of carotenoid astaxanthin for the aquaculture industry (Mendes-Pinto et al. 2001). The only difference to the previous study is that NaOH (0.1 M) or HCL (0.1 M) were applied at room temperature. Autoclave pretreatment had more significant effects on cell wall disruption than chemical pretreatment did. Sulfuric acid solution was also studied to aid the cell wall disruption (Halim et al. 2012). The most severe cell wall disruption took place when *Chlorococcum* sp. was subjected to 8 vol% sulfuric acid and heated to 160 °C for 45 min.

Ultrasonication and mechanical approaches have also been proposed to facilitate the cell wall disruption (Barjenbruch and Kopplow 2003, Mendes-Pinto et al. 2001, Halim et al. 2012). Scanning electron micrographs of *Haematococcus pluvialis* (Mendes-Pinto et al. 2001) illustrated

that both homogenization and autoclaving can effectively disrupt cell walls into fragments. But spray-drying did not alter the cell wall's structure very much, which agreed with another study comparing the biogas yield by fresh vs. dried microalgae (Mussgnug et al. 2010). In addition, one study on *Scenedesmus obliquus* drew the opposite conclusion (Miranda et al. 2012). Researchers compared the effects of thermal, chemical-thermal, sonication, bead-beating and homogenization pretreatments on cell disruption indicated by sugar extraction, and results showed acidic-thermal pretreatment had the superior effects on sugar extraction, and alkaline-thermal along with all three mechanical approaches had the least effects.

## 2.9. Life cycle assessment of the microalgae-based $CO_2$ mitigation system

LCAs have been employed to investigate and evaluate the environmental impacts of the production of microalgae based biofuel (Collet et al. 2011 and Hulatt et al. 2011). International standard stated: "LCA addresses the environmental aspects and potential environmental impacts (e.g. use of resources and the environmental consequences of releases) throughout a product's life cycle from raw material acquisition through production, use, end-of-life treatment, recycling and final disposal (i.e. cradle-to-grave)." (ISO 14040 2007). LCA is a powerful tool for making decisions. The priority for the design of a microalgae-based $CO_2$ mitigation system is sustainability. In this study, $CO_2$ emitted from coal-fired power plants is injected in the bio-mitigation system and fixed into algal biomass, and the net $CO_2$ should be negative. Therefore by conducting LCA, the net $CO_2$ emission can be estimated. LCA framework includes goal and scope definition, inventory analysis and impact assessment (ISO 14040 2007). The system boundary for this study enclosed the coal-fired power plant, photobioreactors (PBRs), anaerobic digesters (ADs), digestate recovery and nutrient recycling. The inventory analysis focused on the raw material acquisition, construction, and operation of the system. The environmental impact was centered on greenhouse gas emission and energy consumption.

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# Chapter 3: Influence of media composition on the growth rate of *Scenedesmus acutus* and *Chlorella vulgaris* utilized for $CO_2$ mitigation[1]

## 3.1. Summary

Atmospheric $CO_2$ levels have increased since the industrial revolution due to increasing combustion of fossil fuels. One possible $CO_2$ mitigation strategy is the use of microalgae. This paper focuses on the influence of media composition on the growth rate of *Scenedesmus acutus* and *Chlorella vulgaris*. The growth rate of *Scenedesmus* was highest in urea based medium. The nitrogen sources ($KNO_3$ or $CO(NH_2)_2$) did not affect the growth rate of *Chlorella*. This should result in an economic benefit in large-scale algae cultivation for $CO_2$ mitigation, as urea is typically less expensive than $KNO_3$.Additional media components were tested and it was found that EDTA, vitamin B and the addition of B, V and Mo do not result in a significant increase in algae growth rate under the process conditions investigated.

***Keywords:*** *Scenedesmus*, *Chlorella*, growth rate, urea.

## 3.2. Introduction

The current $CO_2$ concentration in the atmosphere is believed to be the highest it has been in the last 650,000 years (Canadell et al. 2007). $CO_2$ from energy-related activities represented 82% of total U.S. $CO_2$ emissions in 2008 (EIA 2008). Coal-fired power plants alone emit about 1.9 billion metric tons of $CO_2$ annually, representing 33% of these energy-related $CO_2$ emissions. One possible strategy for $CO_2$ mitigation is the use of microalgae (Sayre 2010, Wang et al. 2008, Ho et al. 2011). The fundamental concepts to develop a microalgal $CO_2$ mitigation system include strain selection, media development, cultivation system design, and downstream processing. Optimizing medium is to balance minimal amounts of nutrients to provide and maximum amounts of nutrients required by cells. Nutrient deficiency generally induces the decrease of $CO_2$ fixation rate and cell growth (Healey 1973). However it has been reported that nitrogen deficiency leads to accumulation of long chain fatty acids that would benefit biofuel production, which is another

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application of microalgae, but not the main focus of $CO_2$ bio-mitigation (Ho et al. 2010). On the other hand, over dosing of nutrients is a waste of resources and adds operational costs.

A number of fresh water based media have been investigated in the cultivation of Scenedesmus and Chlorella (Table 3.1). Nitrogen is often added to media in the form of potassium nitrate, sodium nitrate or calcium nitrate. Phosphorus can be in the form of monopotassium phosphate, dipotassium phosphate or disodium phosphate, which also functions as buffers to maintain the neutral pH of media. Iron and magnesium play critical roles in photosynthesis, so a sufficient supply of iron and magnesium directly benefits $CO_2$ fixation and biomass production. Iron can be provided as ferrous sulfate, Fe EDTA or ferric ammonium citrate ($C_6H_{5+4y}Fe_xN_yO_7$). Magnesium mainly comes from its sulfate form. Calcium is added as calcium chloride. Microalgae cells also contain trace amounts of zinc, copper and manganese, which are linked to cell division (Healey 1973). Therefore, manganese (II) chloride, copper (II) sulfate, and zinc sulfate are added as micronutrient supplements. Moreover, based on different observations regarding microalgae composition and metabolism, several other minerals are provided to the cells, for example, in Fitzgerald medium (Table 3.1), a total of eleven trace minerals are included.

Most marine and fresh water microalgae can utilize nitrate or urea as a nitrogen source. Both the nitrogen concentration and source in the medium can be responsible for important changes in the growth and biochemical composition of microalgal cells, chlorophylls, proteins, carbohydrates, and lipids (Fabregas et al. 1989). For practical applications at the pilot plant and industrial scale the choice of N-source of the medium should be based on algae metabolism. The nitrogen source for the media shown in Table 3.1 is $KNO_3$. However, there has been some work with Spirulina platensis that showed that replacing $KNO_3$ with urea achieved a biomass gain of 37% and consequently larger total amounts of chlorophyll at a lower cost as compared to cultures grown on $KNO_3$ (Danesi et al. 2002). Utilization of mixed N-sources, $KNO_3$ and urea, may involve inhibition or repression since $NO_3^-$ and $NH_4^+$ (hydrolysis of urea gives ammonium) are metabolized via a common pathway. Evolutionary optimal growth of bacteria, algae, and yeast on a mixture of C- or N-sources is achieved via control of uptake of a so-called “faster” substrate, which acts as a repressor to the uptake of the “slower” substrate. The use of a single compound with two different N-sources, such as $NH_4NO_3$, could also lead to inhibition.

Nutrient deficiency generally decreases cell growth rates and as a result lower $CO_2$ fixation rates (Healey 1973). On the other hand, oversupplying the medium with nutrients requires additional energy (embodied in the nutrients), adds operational costs, potentially results in additional $N_2O$ off gassing (a major greenhouse gas), and additional water treatment costs. Different media have varying nutrient quantities that can significantly change the quantity of cell

biomass produced during cultivation (Mandalam and Palsson 1998). Chlorella vulgaris has been cultivated in N-8 and M-8 media for over 500 hours. Due to lower amounts of nitrogen, iron, magnesium and sulfate compared with M-8 (Table 3.1), N-8 resulted in biomass accumulation during the first 250 hours, but not a steady accumulation of chlorophyll that is responsible for photosynthesis and continued cell growth. The extremely low synthesis rate of chlorophyll ultimately inhibits the cell growth. Because the consumption of nutrients synchronizes with cell growth, it is necessary to continuously monitor the composition of the medium and continuously replenish nutrients (Chen et al. 2011).

This paper focuses on the influence of media composition on the growth rate of Scenedesmus acutus and Chlorella vulgaris, which is considered suitable for the $CO_2$ mitigation purpose. Nitrogen source dependence was studied comparing $KNO_3$ (M-8 media) and urea (Urea media). Various urea loadings were tested to determine optimal levels of urea addition. Different EDTA sources were tested and compared to the growth rate without EDTA. Vitamin B and micronutrients (B, V and Mo) were added to determine if these additions could enhance growth. Other factors were held constant ($CO_2$ levels, temperature, light intensity and photobioreactor design).

**Table 3.1 Composition of fresh water-based media.**

| | Medium | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | N-8 | M-8 | BG-11 | Fitzgerald | MC | ASM-1* | Detmer | Urea** |
| Macronutrients (mg/L) | | | | | | | | |
| $KNO_3$ or $NaNO_3$ | 1000 | 3000 | 1500 | 496 | 1250 | 1000 | - | - |
| $KH_2PO_4$ | 740 | 740 | - | - | 1250 | 740 | 260 | 118.5 |
| $Na_2HPO_4 \cdot 2H_2O$ or $K_2HPO_4$ | 260 | 260 | 30.5 | 39 | - | 260 | - | - |
| $CaCl_2 \cdot 2H_2O$ | 13 | 13 | 36 | 36 | - | - | - | 55 |
| Fe-EDTA | 10 | 10 | - | - | - | 10 | - | - |
| $FeSO_4 \cdot 7H_2O$ | - | 130 | - | - | 2 | - | 20 | - |
| $MgSO_4 \cdot 7H_2O$ | 50 | 400 | 75 | 75 | 1250 | 50 | 550 | 109 |
| $Na_2CO_3$ | - | - | 20 | 20 | - | - | - | - |
| Citric acid | - | - | 6 | 6 | - | - | - | - |
| Na-EDTA | - | - | 1 | 1 | - | - | 200 | - |
| Ferric ammonium citrate | - | - | 6 | 6 | - | - | - | - |
| $NaSiO_3 \cdot 9H_2O$ | - | - | - | 25 | - | - | - | - |
| $Ca(NO_3)_2 \cdot 4H_2O$ | - | - | - | - | - | - | 1000 | - |
| KCl | - | - | - | - | - | - | 250 | - |
| $CO(NH_2)_2$ | - | - | - | - | - | - | - | 550 |
| Reference | 1 | 1 | 2 | 3 | 3 | 4 | 5 | 6 |
| Micronutrients (mg/L) | | | | | | | | |
| $Al_2(SO_4)_3 \cdot 18H_2O$ | 3.58 | 3.58 | - | - | - | | - | - |
| $MnCl_2 \cdot 4H_2O$ or $MnSO_4 \cdot 4H_2O$ | 12.98 | 12.98 | 181 | 2.23 | 2.5 | | 1.81 | - |
| $CuSO_4 \cdot 5H_2O$ | 1.83 | 1.83 | 7.9 | - | 0.079 | | 0.08 | - |
| $ZnSO_4 \cdot 7H_2O$ or $ZnCl_2$ | 3.2 | 3.2 | 22 | 0.287 | 0.22 | | 0.11 | - |
| $H_3BO_3$ | - | - | 286 | 3.1 | 2.86 | | 2.9 | - |
| $Na_2MoO_4 \cdot 2H_2O$ | - | - | 39 | - | 0.02 | | - | - |
| $Co(NO_3)_2 \cdot 2H_2O$ | - | - | 4.9 | 0.146 | - | | - | - |
| $(NH_4)_6MoO_{24} \cdot 4H_2O$ | - | - | - | 0.088 | - | | 0.018 | - |
| $Na_2WO_4 \cdot 2H_2O$ | | | | 0.033 | | | | |
| KBr | | | | 0.119 | | | | |
| KI | | | | 0.083 | | | | |
| $NiSO_4(NH_4)_2SO_4 \cdot 6H_2O$ | | | | 0.198 | | | | |
| $VOSO_4 \cdot 2H_2O$ | | | | 0.02 | | | | |
| $Al_2(SO_4)_3K_2SO_4 \cdot 24H_2O$ | | | | 0.474 | | | | |
| Reference | 1 | 1 | 2 | 3 | 3 | 4 | 5 | 6 |

* Micronutrients of ASM-1 are not provided. ** Urea formula is the only one using tap water as the water source.
1. Mandalam and Palsson 1998; 2. Tang et al. 2011; 3. Sakai et al. 1995; 4. Jeong and et al. 2003; 5. Ho et al. 2010; 6 This paper

## 3.3. Materials and Methods

### 3.3.1. Algae culture maintenance

*Scenedesmus acutus* (UTEX 72) was purchased from the algae culture collection at the University of Texas at Austin. *Chlorella vulgaris* (#152075) was obtained from the algae collection at Carolina Biological Supply Company (Burlington, NC). Seed was grown and maintained in M-8 or Urea media (Table 3.1). Media were prepared with city water (dechlorinated with 0.06 g/L sodium thiosulfate) and filtered through a 0.2 µm Nalgene nylon membrane filter (47 mm diameter). Once a week, microalgal cells were transferred into new media for subculture or used as inoculums for an experiment. Working in a laminar flow hood, 2

mL liquid stock culture was transferred to 400 mL fresh media in a 500 mL Erlenmeyer flask. Newly transferred flasks were incubated under warm (Philips F32T8/TL741 Alto, 32 Watts) and cool white (Philips F32T8/TL735 Alto, 32 Watts) fluorescent lights (70 µmol/$m^2$ per second) in a 16:8 hours light:dark illumination period. Flasks were bubbled with 3% anaerobic grade cylinder $CO_2$ (approximately 0.14 L/min $CO_2$ and 4.4 L/min air for the total system of flasks), this percentage of $CO_2$ being sufficient to ensure the saturation of the flask with $CO_2$ (Figure 3.1). Flasks were placed on a shaking table (100 rpm) and kept at room temperature (22°C).

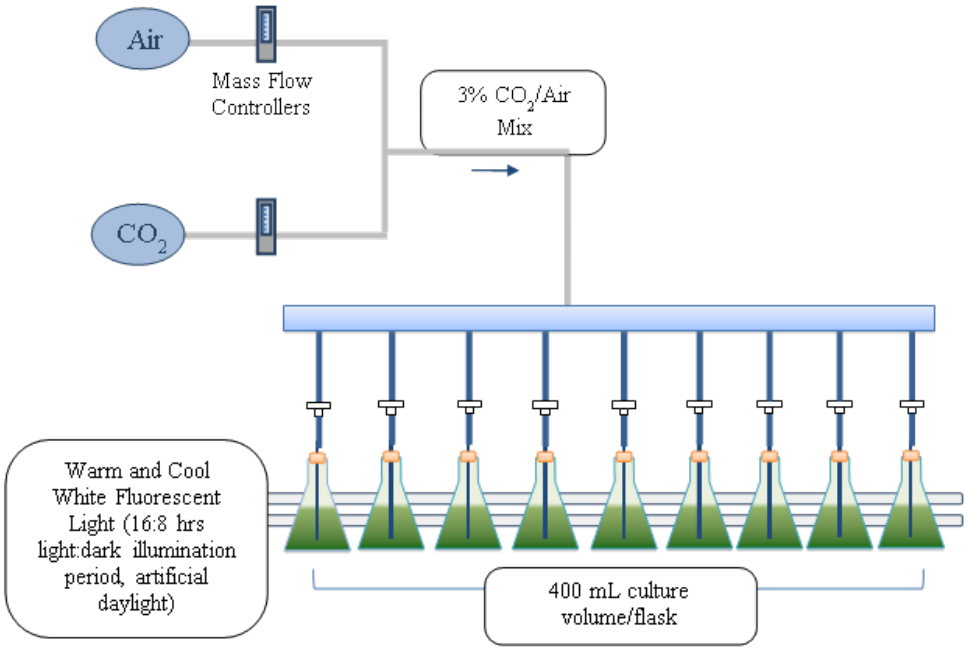


**Figure 3.1 The algae cultivation setup.**

### 3.3.2. Dry weight and growth rate

*Chlorella* and *Scenedesmus* biomass samples in suspension were filtered using Whatman binder-free glass microfiber filters (type 934-AH, 24 mm diameter). The dry weights (DW) of samples were measured by drying at 105°C for 24 hours. Biomass content was calculated from microalgal dry weight produced per liter (g/L). The slope of the growth curve at the exponential phase was used as the growth rate (mg/L/hr; Shuler and Kargi 1992).

### 3.3.3. Media formula development

M-8 medium was considered the starting point for optimization of the media for the flask experiments. Initial studies utilized the M-8 media recipe containing 3.0 g/L $KNO_3$ (Mandalam and Palsson 1998), which was developed for a higher final biomass density than possible in our set-up (due to light and reactor limitations). Based on the final biomass density achieved in our set-up (typically 1 g/L) the $KNO_3$ level was scaled down to 0.75 g/L and the remaining ingredient

levels were scaled down by the same factor. By using the elemental composition of *Chlorella vulgaris* biomass (Table 3.3) and a linear programming procedure (LPP), M-8 was evaluated to see to what extent it was stoichiometrically balanced. The media recipes compared were listed in Table 3.3.

**Table 3.2 Elemental composition of *Chlorella sp.* (by weight)[a].**

| Element % by weight | % by weight | Elemental composition % by weight | |
|---|---|---|---|
| | | Min | Max |
| Macro-elements | | | |
| N | 7.7 | 6.2 | 7.7 |
| P | 2.0 | 1.0 | 2.0 |
| K | 1.62 | 0.85 | 1.62 |
| Mg | 0.8 | 0.36 | 0.8 |
| S | 0.39 | 0.28 | 0.39 |
| Fe | 0.55 | 0.04 | 0.55 |
| Micro-elements | | | |
| Ca | 0.080 | 0.005 | 0.080 |
| Zn | 0.005 | 0.0006 | 0.005 |
| Cu | 0.004 | 0.001 | 0.004 |
| Mn | 0.01 | 0.002 | 0.01 |
| B | 0.0026 | - | 0.0026 |
| Mo | 0.001 | - | 0.001 |
| Co | 0.001 | - | 0.001 |

a. Adapted from Mandalam and Palsson (1998).

**Table 3.3 Summary of media recipes based on M-8 and urea used to culture *Scenedesmus* and *Chlorella.***

| Chemical compound (g/L) | M-8 (modified) | Urea - EDTA |
|---|---|---|
| $KNO_3$ | 0.75 | - |
| $CO(NH_2)_2$ | - | 0.55 |
| $KH_2PO_4$ | 0.185 | 0.1185 |
| $NaHPO_4$ | 0.065 | - |
| $CaCl_2.2H_2O$ | 0.00325 | 0.055 |
| $FeSO_4.7H_2O$ | 0.0325 | 0.015 |
| $MgSO_4.7H_2O$ | 0.1 | 0.109 |
| Na.EDTA.Fe | 0.0025 | - |

### 3.3.4. Experiment procedure

Prior to experiment, algae stock culture (cultivated in 400 mL Urea media with 3% $CO_2$ for 96 hours) was centrifuged at 1800 rpm for 30 min and the supernatant (media) was discarded.

The dewatered algae were re-suspended in deionized water to ensure a uniform algal concentration and no left over nutrients from the stock culture. *Chlorella* and *Scenedesmus* were cultured on a shaking table (100 rpm) in triplicate with M-8 or Urea media (Table 3.4) at 22°C for 96 hours. Media were prepared with city water (dechlorinated with 0.06 g/L sodium thiosulfate) and filtered through a 0.2 µm Nalgene nylon membrane filter (47 mm diameter). 15 mL of re-suspended algae was added to 400 mL media in a 500 mL flasks bubbled with 3% $CO_2$. The gas flow rates of 0.14 L/min $CO_2$ and 4.40 L/min air were regulated with a mass-flow controller (model 5850E, Brooks Instrument, Hatfield, PA) and a flow meter (model VA20439, Dwyer Instrument, Inc., Michigan City, IN), respectively. The culture was illuminated with warm and cool white fluorescent light in 16:8 hours light:dark period (70 µmol/$m^2$ per second). Dry weight (30 mL samples) and pH measurements were taken at time 0, 24, 48, 72 and 96 hours (±1 h). A schematic drawing of the experimental set up was shown in Figure2.1. The urea dosages were varied at four levels (0.07, 0.14, 0.275 and 0.55 g/L) and the other components were held constant. Triplicate treatments of growth media containing different levels of iron chelating agent (EDTA) were tested. Urea media were prepared by adding various amounts of EDTA (no EDTA, 0.02 g/L Na.EDTA.Fe, and 0.2 g/L Sprint 330 Na.EDTA.Fe(III)) and keeping other components constant ($FeSO_4$ was added to make up for the missing Fe in the no ETDA treatment). The effect of vitamins on algae growth was investigated by adding two levels of vitamin B complex (25 and 50 µg/L) to the Urea media (control). Micronutrients of boron (0.415 mg/L $H_3BO_3$), vanadium (0.07 mg/L $NH_4VO_3$), and molybdenum (0.06 mg/L $Na_2MoO_4.2H_2O$) were added to 400 mL Urea media individually or together into triplicate 500 mL flasks (a control was also included).

## 3.4. Results and discussion

### 3.4.1. Effects of nitrogen sources on growth rate

*Scenedesmus* and *Chlorella* grew in M-8 or Urea media respectively, and the growth curves were shown in Figure 3.2 and Figure 3.3. Two media contained similar amounts of N, K, P, Mg and Fe, but *Scenedesmus* cultivating in the Urea media grew faster than those in the M-8 media (3.90 vs. 2.13 mg/L/hr). *Chlorella* had the equivalent growth rate with both media. The major variance between M-8 and Urea media is the nitrogen form ($KNO_3$ vs. $CO(NH_2)_2$). The up-taking pathways for nitrate and urea by algae cells are different, even though both of them have to be reduced to ammonium before entering the same nitrogen assimilation pathway (Glass et al. 2009). The reduction of nitrate to ammonium is catalyzed by two enzymes in two steps:

$$NO_3^- + 2e^- + 2H^+ \xrightarrow{\text{nitrate reductase}} NO_2^- + H_2O \quad (3.1)$$

$$NO_2^- + 6e^- + 8H^+ \xrightarrow{\text{nitrite reductase}} NH_4^+ + 2H_2O \quad (3.2)$$

Urea is reduced by urease in one step:

$$CO(NH_2)_2 + HCO_3^- + 3H^+ + ATP \xrightarrow{\text{ureaamidohydrolase}} 2NH_4^+ + 2CO_2 + ADP + P_i \qquad (3.3)$$

$KNO_3$ was widely used as nitrogen source for microalgae cultivation (Table 3.1), and the results confirmed urea can be another choice of nitrogen source. Especially in the large-scale operation, urea is more cost effective than $KNO_3$.

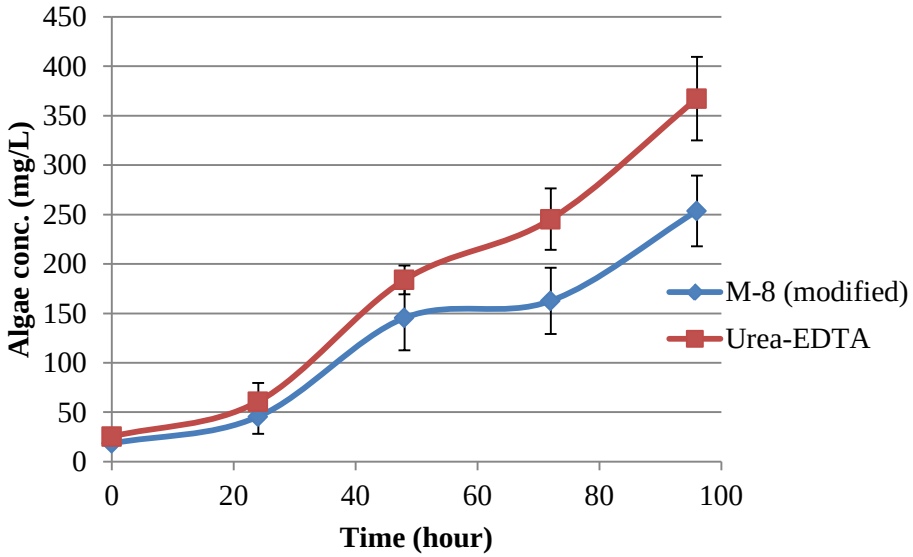


**Figure 3.2 *Scenedesmus* growth curves under two media: M-8 and Urea (0.55 g/L urea). Error bars are standard deviations (n=3).**

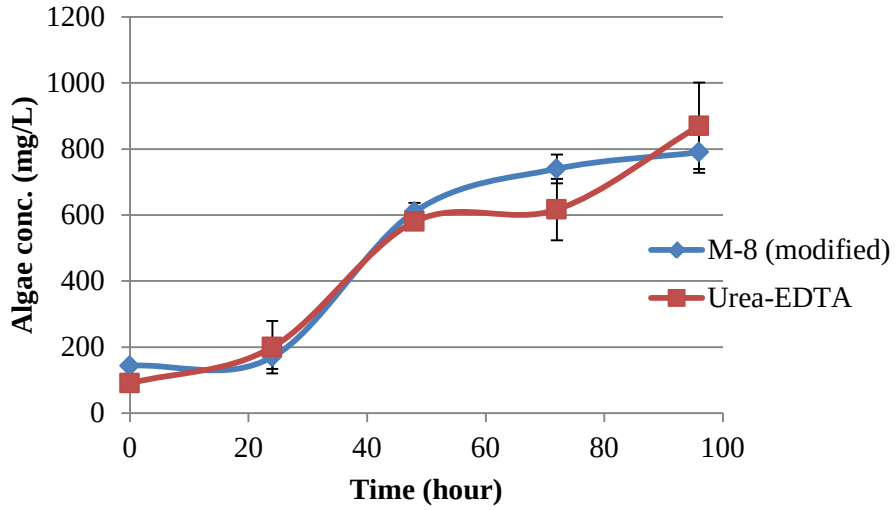


**Figure 3.3 *Chlorella* growth curves under two media: M-8 and Urea (0.55 g/L urea). Error bars are standard deviations (n=3).**

### 3.4.2. Effects of urea dosage on the *Scenedesmus* growth rate

To potentially reduce media cost, experiments were done to test three different levels of urea (Table 3.5). The resulting growth rates were not statistically different ($\alpha = 0.05$). It even appeared that the lower urea level supported better growth rates overall. This indicates the growth rate was partially determined by the urea assimilation rate, but not limited by the urea concentration under these four dosages.

**Table 3.4 Growth rate ± standard error for *Scenedesmus* grown with four urea levels (n=3).**

| Urea Level | Growth rate (mg/L/hr) |
|---|---|
| 0.55 g/L | 6.84 ± 0.36 |
| 0.275 g/L | 7.03 ± 0.14 |
| 0.14 g/L | 6.96 ± 0.074 |
| 0.07 g/L | 7.21 ± 0.18 |

### 3.4.3. Effects of addition of EDTA, vitamin B, B, V and Mo on *Scenedesmus* growth

EDTA, as a chelating agent, improves the solubility of iron. Experiments were done to test whether the presence of EDTA would enhance the growth rate (Table 3.6). Na.EDTA.Fe is the laboratory grade EDTA reagent (purchased in small quantities from Sigma-Aldrich), while Sprint 330 EDTA is an industrial grade of EDTA (purchased in bulk from a fertilizer supplier), being more cost effective for large-scale applications. There was no significant difference between Sprint 330 EDTA and the absence of EDTA. There was a significant difference between the lab and commercial grade ($p = 0.031$). While these results show that the presence of EDTA doesn't necessarily improve the growth rate, it was kept in the final version of the urea media because it did help keep iron in solution and made various laboratory tasks easier.

**Table 3.5 Growth rate ± standard error of *Scenedesmus* in the absence of EDTA and with a lab grade (Na.EDTA.Fe) and commercial grade EDTA (Sprint 330 EDTA) (n=3).**

| EDTA Type | Growth rate (mg/L/h) |
|---|---|
| Na.EDTA.Fe | 5.64 ± 0.28 |
| Sprint 330 EDTA | 6.83 ± 0.23 |
| No EDTA | 6.84 ± 0.36 |

The inclusion of B vitamins was tested at two levels (25 and 50 μg/L) and compared to a control (Table 3.7). The addition of vitamins did not have an effect on the growth rate of *Scenedesmus* in this system. This does not totally agree with Krichnavaruk's results on *Chaetoceros calcitrans* (Krichnavaruk et al. 2005). In that study, it was proved that 1 μg/L of Vitamin $B_{12}$ gave a significant boost on growth rate. It is possible with this small volume (batch cultivation) vitamins are not necessary or advantageous for growth. However, the addition of vitamins in an industrial, continuous system could prove to be advantageous.

**Table 3.6 Growth rates ± standard error of *Scenedesmus* with the addition of vitamin B (n=3).**

| Vitamin B Level | Growth rate (mg/L/hr) |
|---|---|
| 0 μg/L | 5.19 ± 0.14 |
| 25 μg/L | 5.10 ± 0.17 |
| 50 μg/L | 5.06 ± 0.09 |

There are several micronutrients that could be available from the water source, but there are some trace micronutrients suggested by various media compositions that may not be. Specifically, B, V and Mo were selected as possible micronutrients that might help increase the algae growth rate. However, there was no significant difference between the control and the medium with each of the elements or when all three were added (Table 3.8).

**Table 3.7 Growth rates ± standard error of *Scenedesmus* with the addition of B, V and Mo (n=3).**

| Additions | Growth rate (mg/L/hr) |
|---|---|
| none | 4.18 ± 0.23 |
| + B | 4.11 ± 0.06 |
| + V | 3.39 ± 0.28 |
| + Mo | 4.26 ± 0.12 |
| + B, V, Mo | 4.14 ± 0.16 |

## 3.5. Conclusions

The influence of media composition on the *Scenedesmus* growth rate was investigated. A linear programming procedure (LLP) was used in conjunction with the elemental composition of *Chlorella* to estimate the optimum formulation of the growth medium. The LLP was used to revise the M-8 recipe and to develop a new medium using urea as nitrogen source. For *Scenedesmus*, the addition of EDTA, vitamin B, or B, V and Mo did not increase the growth rate.

When the urea level was reduced, the growth appeared to increase slightly, showing that the urea level was not the limiting growth factor.

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# Chapter 4: Pretreatment of *Scenedesmus* biomass as the potential anaerobic digestion substrate[2]

## 4.1. Summary

*Scenedesmus* biomass can be a potential substrate for anaerobic digestion. However raw algae cells show a resistance to biological degradation, and result in a slower methane production rate. Therefore thermal chemical pretreatment was applied to the algae biomass to enhance the methane production. For algae cells with no flocculant addition, the highest soluble organic matter (SOM) increase was 15% when heating at 50 °C with addition of 3% of NaOH or heating at 90 °C with addition of 12% of NaOH both heating for 60 mins. Flocculation inhibits the cell wall disruption, but thermal chemical treatment can hydrolyze some flocculant polymers, which ease the flocculation and facilitate cell destruction. Pretreated algae showed a higher methane production rate for the first 7 days compared to untreated algae. Highest specific gas production was 594 L/kgVS/d at organic loading rate of 0.75 kgVS/$m^3$/d.

***Keywords:*** *Scenedesmus*, anaerobic digestion, pretreatment.

## 4.2. Introduction

Anaerobic digestion has been studied for decades as a practical approach to decompose microalgae biomass and produce methane as fuel gas (Golueke et al. 1957, Foree and McCarty 1970, Chen 1987, Ras et al. 2011) In fact, anaerobic decomposition of microalgae takes place in natural aquatic systems. Because of gravitational sedimentation, microalgae eventually sink to the anoxic and aphotic zones, and are broken down by anaerobic bacteria (Foree and McCarty 1970). If this anaerobic digestion takes place in a bioreactor, microalgae can be degraded into inorganic elements, which can be transferred back to the photo bioreactor and support microalgae growth. Thus there is a strong motivation to investigate the potential of algae biomass as the anaerobic digestion substrate, and explore the possibility to improve the digestion efficiency.

Anaerobic digestion is potentially suitable for *Scenedesmus* degradation, however living algal cells are reported to resist bacterial attack, due to their rigid cell wall structure (Chen 1987). Golueke et al. (1957) compared the degradation rate between raw sewage and microalgae, and it showed that after 35°C mesophilic digestion, 60% of raw sewage was degraded, but only 40% of microalgae was destructed. Chen (1987) studied anaerobic digestion of microalgae collected from the effluent of high-rate sewage stabilization ponds. Prior to thermal pretreatment, the methane

[2] This chapter has been submitted to Biological Engineering Transactions.

production was 0.14 L/gVS; by contrast, the methane production increased to 0.20 L/gVS, when the substrate microalgae were heated to 50°C for 30 min. Apparently heating improved the microalgae biodegradability.

In order to explore more ways to improve the microalgae biodegradability, algal cell structure needs to be understood. Cell wall structures of *Scenedesmus* and *Chlamydomonas* are significantly different from each other. Based on the observations under light and electron microscopy, Bisalputra et al. (1963) described the cell wall of *Scenedesmus quadricauda* as a sandwich-like structure consisting of cellulosic layer, middle layer with unknown composition and pectic layer. Given the sugar composition of cell walls isolated from array of *Scenedesmus*, Takeda (1996) concluded that glucose was the major sugar component in the rigid wall, along with galactose and mannose. The isolated cell wall from *Scenedesmus communis* was examined by $^{13}$C-NMR (solid state $^{13}$C-nuclear magnetic resonance spectroscopy) and FTIR (Fourier transform infrared spectroscopy), which was revealed to compose of a highly aliphatic material; meanwhile pyrolysis-GC/MS's measurement confirmed the dominance of *n*-alkene/*n*-alkane doublets in the aliphatic molecules, which were cross-linked via ether-linkages (Blokker et al. 1998).

On the other hand, *Chlamydomonas* presents a distinct type of cell wall structure. *Chlamydomonas* was firstly considered to contain mainly hydroxyproline heterooligosaccharides in its cell wall, which was linked to the fact that *Chlamydomonas* cell walls are more susceptible to sonication rupture than *Chlorella* walls (Miller et al. 1972). After short sonication (less than 1 minute), 80% of *Chlamydomonas* cell walls were disrupted and quickly disintegrated into small fragments with further sonication. By contrast, the destruction of *Chlorella* walls took longer time, and fragments were hard to be further disintegrated. Shortly after this discovery, the chemical composition of cell walls of *C. gymnogama* was determined, and arabinose (24.8% w/w) and galactose (19.6% w/w) were proved to be the predominant sugars, and amino acids accounted for about 10.2% w/w of the cell wall (Miller et al. 1974). Sugars were bound to amino acids and formed glycoprotein structure, which was estimated to be at least 32% of the cell wall weight (Miller et al. 1974, Catt et al. 1976). The cell wall of *Chlamydomonas reinhardtii* was a multilayered membrane composed of carbohydrates and polypeptides, meanwhile it lacked cellulose in the structure (Imam et al. 1985). Mussgnug et al. (2010) carried out anaerobic digestion on six common microalgae species including *Scenedesmus obliquus* and *Chlamydomonas reinhardtii* without any pretreatment. The methane production rates for *S. obliquus* and *C. reinhardtii* were 0.287 and 0.587 L/gVS respectively. The light microscopic

images of microalgal cells indicated after 28 days mesophilic (38°C) anaerobic digestion, intact cells of *Scenedesmus* or *Chlamydomonas* still existed in the sludge.

To improve the cell degradation and methane production, pretreatment should be carried out prior to the anaerobic digestion. Cell wall disruption can be executed by thermal, chemical, enzymatic or mechanical pretreatment (Gonzalez-Fernandez et al. 2011). Thermal pretreatment is one of the most discussed methods. Chen (1987) studied the thermal effects on the methane production efficiency from the microalgae collected from the effluent of high-rate sewage stabilization ponds. When heating duration was 30 min, high temperature pretreatment appeared to significantly improve the methane production, and especially the highest yield (0.25 L/gVS) was carried out by the microalgae subjected to 100 or 120°C treatment. Meanwhile moderate temperature (40°C) also benefited the methane yield, but showed less increase (0.17 vs. control 0.14 L/gVS). Heating duration was also studied. Comparing 15 min to 4 h, the latter had 0.04 L/gVS more methane being produced. Besides temperature and duration, he also tested the effects of substrate concentrations. Interestingly increasing the concentration from 3.7% to 22.5% made the methane yield plunge by 18%. Another study carried out by Gonzalez-Fernandez et al. (2012) revealed the effects of heating temperature (70°C vs. 90°C) on the soluble COD of substrate and methane yield. After 180 min of heating, soluble COD of *Scenedesmus* sludge was increased 4 or 5 folds with 70°C or 90°C respectively. The methane production over 33 days showed that raw microalgae and 70°C treated microalgae resulted in a similar amount of methane at the end, but 90°C treated microalgae produced two times more biogas. For the first 10 days, pretreated microalgae showed significantly higher methane yield rate than did raw microalgae, especially for the one heated at 90°C. Appels et al. (2010) and co-workers studied the pretreatment of wastewater sludge and demonstrated the similar trend, but with more significant difference. Pretreatments at 80°C and 70°C rendered same amount of methane when treatment duration was 15 min. Pretreatment at 80°C showed slightly higher biogas yield when duration prolonged to 60 min. By contrast, pretreatment at 90°C gave superior results at all three heating duration (15, 30 and 60 min). Thermal pretreatment was often companioned by high pressure if the process was implemented in an autoclave (Mendes-Pinto et al. 2001, Barjenbruch and Kopplow 2003, Miranda et al. 2012). Barjenbruch and Kopplow (2003) investigated the thermal pretreatment of sewage sludge by autoclave under three temperatures (80, 90 and 121°C), and it turned out 90°C carried out the highest improvement.

Alkaline or acid was also incorporated in the thermal pretreatment of microalgae to disrupt cell walls (Chen 1987, Mendes-Pinto et al. 2001, Halim et al. 2012, Miranda et al. 2012). Chen (1987) chose to heat the microalgae sludge at 100°C, for 8 h with various NaOH dosages

(w NaOH/w dry microalgae %). Interestingly it showed that no addition of NaOH gave the highest methane yield, and increasing NaOH dosage from 2% to 20%, the methane yield plunged to almost zero, even though samples were neutralized to pH 7 after all pretreatments. Sulfuric acid solution was also studied to aid the cell wall disruption (Halim et al. 2012). The most severe cell wall disruption took place when *Chlorococcum* sp. was subjected to 8 vol% sulfuric acid and heated to 160°C for 45 min. One thing need to be noted is that chemical treatment can potentially impair the molecular structure, which becomes an issue if the molecules were the product to recover. *Haematococcus pluvialis,* which was considered as an important natural source of carotenoid astaxanthin for the aquaculture industry, was subjected to several pretreatments to improve the carotenoid recovery efficiency (Mendes-Pinto et al. 2001). Both acidic and alkaline treatments resulted a significant loss of carotenoid compared to the control. By contrast, mechanical disruption and autoclave pretreatment led to minimum loss of carotenoid and higher product recovery.

As aforementioned, mechanical disruption and ultrasonication have also been proposed to facilitate the cell wall disruption (Barjenbruch and Kopplow 2003, Mendes-Pinto et al. 2001, Halim et al. 2012). Scanning electron micrographs of *Haematococcus pluvialis* (Mendes-Pinto et al. 2001) illustrated that both homogenization and autoclaving can effectively disrupt cell walls into fragments. Spray-drying only slightly decrease the size of the cells without impairing the cell wall, which supported the results from another study comparing the biogas yield by fresh vs. dried microalgae (Mussgnug et al. 2010). In this study, drying the algae at 105°C for 24 h prior to anaerobic digestion significantly decreased the biogas production compared to the fresh biomass. In another study, researchers (Miranda et al. 2012) compared the effects of thermal, chemical-thermal, sonication, bead-beating and homogenization pretreatments on sugar extraction from *Scenedesmus obliquus* cells, and results showed acidic-thermal pretreatment had the superior effects on sugar extraction, and alkaline-thermal along with all three mechanical approaches were less effective. Acid pretreatment can be beneficial for microalga bioethanol production, however in the case of biogas production, alkaline pretreatment should be more appropriate since the fermentation pH is more basic (Chen 1987).

In this study, the ideal algal pretreatment was chosen based on two criteria: 1) significant cell wall disruption determined by microscopic observation, soluble organic matter (SOM) test, and methane production in the digester; 2) economic energy consumption which requires a simple pretreatment procedure and minimum feedstock adjustment (pH) prior to anaerobic digestion. Therefore thermal treatment aided by NaOH instead of acid was studied.

## 4.3. Materials and Methods

### 4.3.1. Algae pretreatment

*Scenedesmus* was cultivated in both tubular photobioreactors (PBRs) and 500 mL glass flasks. Two cultivation set-ups adopt the similar media and same $CO_2$ level (3%), but different light sources: sun light for PBRs and artificial fluorescent light for flasks. The medium for flasks cultivation was composed of $CO(NH_2)_2$ 0.137, $KH_2PO_4$ 0.118, $MgSO_4.7H_2O$ 0.109, $CaCl_2.2H_2O$ 0.055 and Na.EDTA.Fe 0.02 in g/L. Considering the significant size (3800 L) of PBRs, commercial fertilizers (triple super phosphate and pot ash) replaced $KH_2PO_4$, and $Mg^{2-}$ was supplied by tap water. Nevertheless the nutrients' elemental levels were kept consistent between the media of the flasks and PBRs. Both media were prepared with tap water. *Scenedesmus* harvested from PBRs underwent two dewatering procedures prior to storage: 1) flocculating, or 2) flocculating, filtering and oven-drying. Cationic polyacrylamide (5~10 ppm) was applied to aid the flocculation of algae, and the algal biomass was concentrated from 1 g/L to 25 g/L. In oven-dry procedure, the filtrated algal cakes were dried at 60 $^{\circ}$C until the moisture content was reduced to less than 5%. *Scenedesmus* harvested from flasks was dewatered by moderate centrifugation (3000 rpm for 20 min), and reconstituted into 10 g/L algal stock by adding room temperature boiled tap water. The other two types of *Scenedesmus* biomass were also reconstituted into 10 g/L algal stock. For convenience, three types of biomass were denoted as Flocculated Algae, Dried Algae and Fresh Algae. Fresh Algae were strictly from flask cultivation.

The complete 2×3×4 factorial design was applied for the algal biomass pretreatment study, including two heating temperatures (50$^{\circ}$C or 90$^{\circ}$C), three heating durations (10, 30 or 60 min) and four NaOH concentrations (0, 3, 6 or 12% g NaOH/g DW of algae) in 10 mL of the 10 g DW algae/L algae stock solutions. Temperatures and duration were based on the study by Gonzalez-Fernandez et al. (2012), and NaOH dosage was based on the study by Chen (1987).

Chemical oxygen demand (COD) was used to determine the release of soluble organic matter (SOM) after the cell wall destruction due to pretreatment (Gonzalez-Fernandez et al. 2012). Soluble COD (SCOD) and total COD (TCOD) were measured for each sample, and the ratio of SCOD to TCOD was compared at the end. The pretreated samples (0.5 mL) were diluted with DI water (2 mL), and centrifuged at 4000 rpm for 8 min. Supernatants were pipetted into 5 mL centrifuge vials, and 2.5 mL DI water was mixed with the pellet to undergo another centrifugation. Supernatants from the second centrifugation were pipetted and combined with the supernatants from the first centrifugation. The combined supernatants were centrifuged again to remove any residual debris. The dilution factor for SCOD was 10. TCOD samples were diluted 20 times with DI water before the test. COD tests were conducted in HACH® (Loveland,

Colorado) pre-packed tubes. Each tube contained a 2 mL sample that was digested at 150°C for 2 h. After cooling to room temperature, the absorbance of the tube was read at 620 nm using the spectrophotometer. The standards were serial dilutions of potassium dichromate solutions (25, 50, 100, 200, 400, 600, 800, 1000, 1289 and 1475 ppm). The SCOD and TCOD of each sample were calculated from the calibration curve. The increase of soluble organic matter (SOM) in the algae slurry was determined as the difference between the ratios of SCOD/TCOD of raw and treated samples.

### 4.3.2. Dual-fluorescence microscopy

The raw or pretreated *Scenedesmus* was centrifuged and pellets were kept frozen at -20°C for less than one month before the fluorescent labeling. Sytox® Green nucleic acid stain (Molecular Probes Inc., Eugene, OR) was used to label cells with compromised cell walls and cell membranes (Sato et al. 2004). The stain was diluted 100 times with DI water prior to use, and the final concentration was 50 μM. The algae pellets were suspended in the media, and 1 μL of the algae culture was mixed with 1 μL of the diluted stain on the microscope slides. The samples were incubated in the dark for 5 min. The fluorescence microscope (Eclipse E600, Nikon, Japan) was used, and images were taken by a digital camera (Coolpix 5000, Nikon, Japan). The chlorophylls were autofluorescent pigments, and were excited by 540-580 nm light emitting bright red. The Sytox® Green was excited by 465-495 nm light, and emitted bright green. The light source was a mercury lamp (C-SHG1, Nikon, Japan).

### 4.3.3. Anaerobic digestion

The bio-methane potential (BMP) of raw algae was studied to obtain a full profile of the anaerobic digestion process including lag phase, exponential phase, plateau phase and death phase. BMP tests were performed in a Bioprocess Control Automatic Methane Potential Test System (AMPTS) (Bioprocess Control, Sweden). This system consists of 500 ml glass bottles with stoppers, motors and stirring shafts, water bath, sodium hydroxide bottles for carbon dioxide fixation, a flow cell with backflow prevention valves, inline gas sampling port, and a real-time data logging system. Inoculum was obtained from an operational full scale anaerobic digester running at a wastewater treatment plant and allowed to degas for three days, then the total and volatile solids (TS/VS) were measured. Batch scale reactors were prepared in triplicate for both the blank and algae substrate. Glass bottles were filled to a total of 400 g in a 2:1 VS ratio of inoculum to substrate. The bottles were kept at 37°C with continuous stirring. The system was flushed with nitrogen, and each flow cell was emptied to ensure the correct initial gas count. The experiment ran for 40 days.

Continuous stirred tank reactors (CSTR) were used to test the anaerobic degradability of raw Flocculated Algae. The CSTR system used in this experiment consisted of a 5 L glass tank, feeding tube, stirring shaft and motor, heating mantel and temperature probe to keep the temperature at 37°C, gas outlet three-way valve, condensate trap, and a Ritter Milli Gas Counter (Ritter, German). The reactor was fed with primary sludge until stabilized, and then switched to raw algae to acclimate for 3 months prior to all tests. The status of the CSTR was recorded daily and when complications occurred (e.g. foaming, seized motor) the data from those days was removed, such that the data represented the proper operation of the CSTR. Once biogas production was stabilized, the organic loading rate (OLR) was incrementally increased from 0.45 to 2.5 kgVS/$m^3$/$day^1$. Hydraulic retention time (HRT) was 30 days, and reactor was fed daily and the amount of biogas produced was tallied. The specific gas production (SGP) was calculated as: $\mathrm{SGP} = \frac{\text{Daily Gas Production}}{\text{Daily VS Input}}$.

### 4.3.4. Total solid and volatile solid of algae biomass

One aliquot algae solution was withdrew, and filtered with Whatman® binder-free glass microfiber fitlers, and washed with DI water three times. The filtered wet algae were dried at 105 °C for 24 hours, and the dry weight was used to calculate the total solid content of the algae solution. After drying at 105°C, the dried samples were transferred to muffle furnace and heated to 550°C for 2 hours. The volatile solid content was calculated as: $\mathrm{VS\%} = \frac{\mathrm{TS} - \mathrm{Ash}}{\mathrm{TS}} \times 100\%$.

### 4.3.5. Statistical analysis

All pairwise comparison was conducted according to Tukey's honest significant differences method with $\alpha$=0.05. SAS® 9.3 (SAS Institute Inc., USA) was used to perform the statistical tests.

## 4.4. Results and Discussion

### 4.4.1. Pretreatment of *Scenedesmus*

Three types of algae biomass (Fresh Algae with no flocculation and without drying, Flocculated Algae with flocculation and Dried Algae with both flocculation and drying) were collected and pretreated using the 24 scenarios. Soluble organic matter (SOM) of the sample was expressed as the net ratio of SCOD to TCOD. The ratios of SCOD/TCOD of blanks were listed in Table 4.1.

**Table 4.1 The ratio of SCOD to TCOD in untreated samples (n=3)[a].**

| | SCOD/TCOD | SD |
|---|---|---|
| Dried Algae-1 | 7.5% | 0.6% |
| Dried Algae-2 | 7.4% | 0.4% |
| Flocculated Algae-1 | 10.3% | 0.6% |
| Flocculated Algae-2 | 2.7% | 0.1% |
| Fresh Algae-1 | 3.4% | 0.5% |
| Fresh Algae-2 | 1.7% | 0.2% |

a. First batches of algae samples were collected for testing NaOH dosages of 0 and 3%. Second batches of algae samples were collected for test NaOH dosages of 6 and 12%.

The SOM contents of Fresh Algae increased 6% to 15% after thermal chemical treatments (Table 4.2). High temperature (90°C) did not give a superior effect on SOM increase compared to the mesophilic temperature (50°C). The maximum SOM increase after 50°C or 90°C heating was 14.8% or 15.5%. A study by Gonzalez-Fernandez et al. (2012) showed that two heating temperatures (70°C or 90°C) had the similar effects on the soluble COD yield when heating duration was less than 50 min. Current result agreed with this. The effect of alkali (NaOH) addition was significant (Table 4.2, $P<0.0001$). The average pH of algae slurries with various amount of NaOH (0, 3, 6 or 12% g NaOH of g DW algae) was 6.5, 7.0, 9.5 and 11.0 respectively. When heating at 50°C for the same period of time, increasing the alkali dosage inhibited the release of SOM from the algal cells. When heating at 90°C for the same amount of time, increasing NaOH concentrations apparently accelerated the release of SOM from the cells. Chen (1987) studied the alkali thermal treatment on algae collected from the effluent of high-rate sewage stabilization pond. He found as the dosage of NaOH increased from 3% to 12% of DW algae the methane specific gas production (SGP) of the algae decreased from 0.24 L/gVS to nearly zero, when the heating temperature was 100°C. He also found no addition of NaOH rendered the highest gas production rate. In the current study, the pretreatment index is SOM (expressed as SCOD/TCOD) instead of SGP. But the soluble COD of algae biomass showed a positive relation to SGP (Ras et al., 2011; Gonzalez-Fernandez et al., 2012). Current results showed higher NaOH dosage aided the release of organic matter from algal cells at 90°C but not at 50°C. The heating duration did not play a significant role in terms of cell wall destruction. In

fact, Chen (1987) demonstrated that when heating duration was over 20 hours it drastically inhibited the gas production.

**Table 4.2 The increase of soluble organic matters (SOM) in fresh, flocculated and oven-dried algae samples after pretreatments[a]. The increase of SOM is presented as the increase of SCOD/TCOD between raw and treated samples (n=3).**

| Temp (°C) | Alkaline (%) | Duration (min) | Fresh Algae (%) | Flocculated Algae (%) | Dried Algae (%) |
|---|---|---|---|---|---|
| 50 | 0 | 10 | 12.0$^{b}$ | 7.63$^{a}$ | -0.2$^{a}$ |
| | | 30 | 12.7$^{b}$ | 8.70$^{ab}$ | -0.4$^{a}$ |
| | | 60 | 11.8$^{b}$ | 7.02$^{a}$ | 0.0$^{a}$ |
| | 3 | 10 | 13.0$^{c}$ | 10.56$^{b}$ | -0.4$^{a}$ |
| | | 30 | 14.3$^{cd}$ | 9.51$^{b}$ | 1.1$^{ab}$ |
| | | 60 | 14.8$^{cd}$ | 9.71$^{b}$ | 1.1$^{ab}$ |
| | 6 | 10 | 11.5$^{b}$ | 9.15$^{b}$ | 3.0$^{b}$ |
| | | 30 | 11.5$^{b}$ | 7.80$^{a}$ | 2.5$^{ab}$ |
| | | 60 | 8.7$^{ab}$ | 7.11$^{a}$ | 1.8$^{ab}$ |
| | 12 | 10 | 7.4$^{ab}$ | 8.32$^{ab}$ | 4.3$^{c}$ |
| | | 30 | 7.9$^{ab}$ | 8.67$^{ab}$ | 3.0$^{b}$ |
| | | 60 | 8.1$^{ab}$ | 7.92$^{a}$ | 3.8$^{c}$ |
| 90 | 0 | 10 | 6.2$^{a}$ | 6.91$^{a}$ | -1.0$^{a}$ |
| | | 30 | 6.8$^{a}$ | 7.37$^{a}$ | -0.7$^{a}$ |
| | | 60 | 6.9$^{a}$ | 7.15$^{a}$ | -1.1$^{a}$ |
| | 3 | 10 | 8.1$^{ab}$ | 9.05$^{ab}$ | 0.0$^{a}$ |
| | | 30 | 6.8$^{ab}$ | 9.84$^{b}$ | 0.1$^{a}$ |
| | | 60 | 7.6$^{ab}$ | 10.84$^{b}$ | 2.3$^{ab}$ |
| | 6 | 10 | 8.9$^{ab}$ | 6.22$^{a}$ | 3.3$^{b}$ |
| | | 30 | 9.0$^{ab}$ | 7.33$^{a}$ | 6.0$^{cd}$ |
| | | 60 | 10.6$^{b}$ | 8.57$^{ab}$ | 5.6$^{cd}$ |
| | 12 | 10 | 13.1$^{c}$ | 12.34$^{c}$ | 8.9$^{d}$ |
| | | 30 | 13.8$^{c}$ | 13.98$^{d}$ | 10.4$^{d}$ |
| | | 60 | 15.5$^{cd}$ | 17.24$^{e}$ | 11.5$^{de}$ |

a. Mean values followed by the same letter are not significantly different at $\alpha$=0.05. There is no comparison between columns.

Under fluorescent microscope, algal cells with de-activated chlorophyll pigments emitted very dim red, and cells containing intact chlorophyll pigments emitted intense red. The intensity of green related to the amount of dye penetrating the cells, so the intensity of green indicated the severity of cell wall destruction. The alkali solution provided protection to chlorophyll pigments after heating at 90°C, since the red fluorescence was observed (Figure 4.1n). However, alkali did not provide any protection to the cell wall, but facilitated its disruption (Figure 4.1l and o). Visually it appeared that heating at 90°C caused more severe cell wall destruction compared to

heating at 50°C, even though the former treatment failed to bring out a high SOM increase. The cell matrix is made of proteins, carbohydrates and lipids, which dynamically interact and bond with each other. When cell walls and membranes are disrupted, this organic matter does not completely dissolve and disperse in the extracellular space. Therefore partially destroying the cell wall causes some organic matter to leach from the cells, but does not guarantee they are in a soluble form (SOM). Therefore when maximum cell wall destruction was observed under the fluorescent microscope, a corresponding increase of SOM was not always observed.

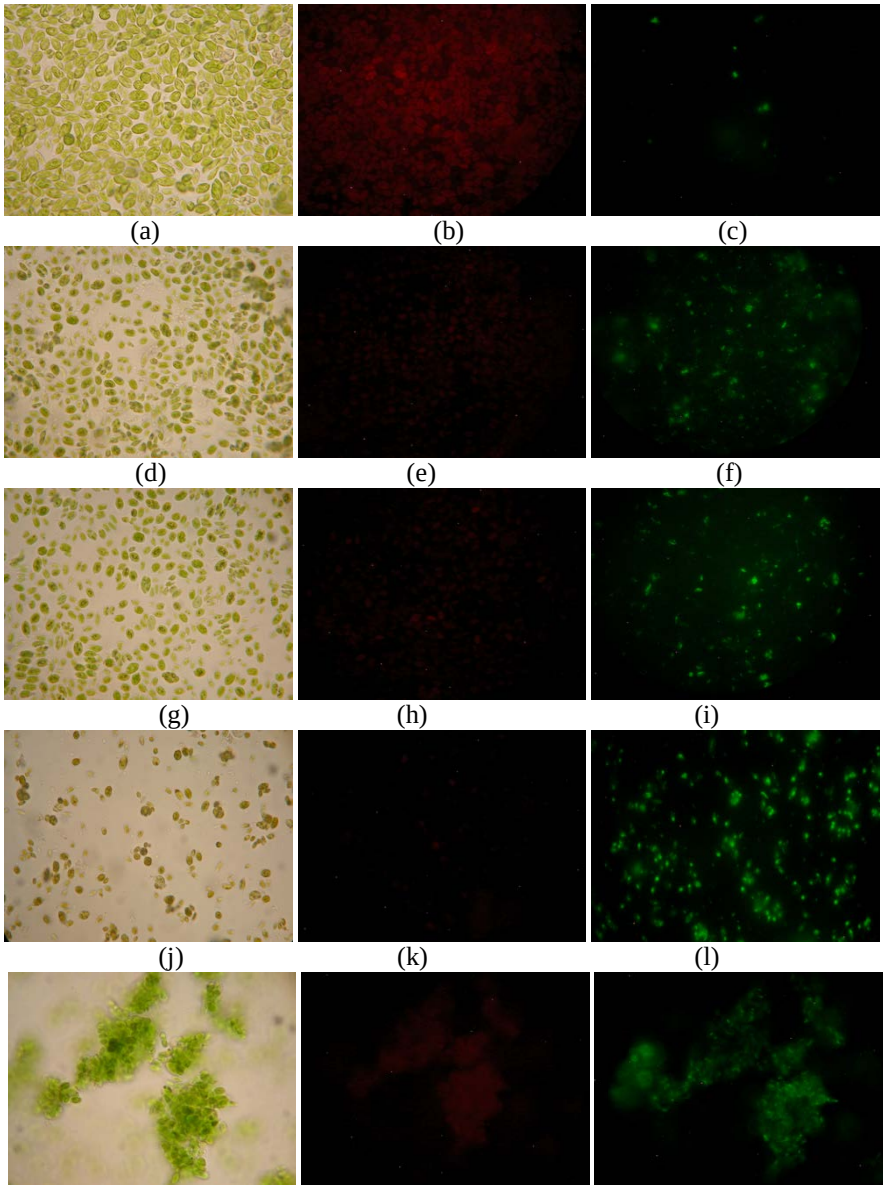


**Figure 4.1 The microscopic images of Fresh Algae. From left to right, there are light microscopy, red fluorescent microscopy representing live cells, and green fluorescent microscopy representing dead cells. (a-c) raw Fresh Algae; (d-f) Fresh Algae treated with 0% NaOH at 50 °C for 10 min; (g-i) Fresh Algae treated with 12% NaOH at 50 °C for 10 min; (j-l) Fresh Algae treated with 0% NaOH at 90 °C for 10 min; (m-o) Fresh Algae treated with 12% NaOH at 90 °C for 10 min.**

In the case of cation polyacrylamide (CPAM) flocculated fresh algae (Flocculated Algae), the combination of high temperature (90°C) and a high dose of NaOH (12% of DW algae) resulted in a significant increase in SOM ranging from 12.3% to 17.2%. Interestingly, heating at 90 °C with 6% NaOH produced the smallest of increase in SOM (6%) among all

treatments. The dosage of NaOH significantly impacted the SOM extraction when the heating temperature was 90°C, but not the case when heating was at 50°C (Table 4.2). The pH values had no significant difference between samples treated at the two temperature levels. Microscope images showed algae cells start aggregating as treatment conditions intensified (Figure 4.2).

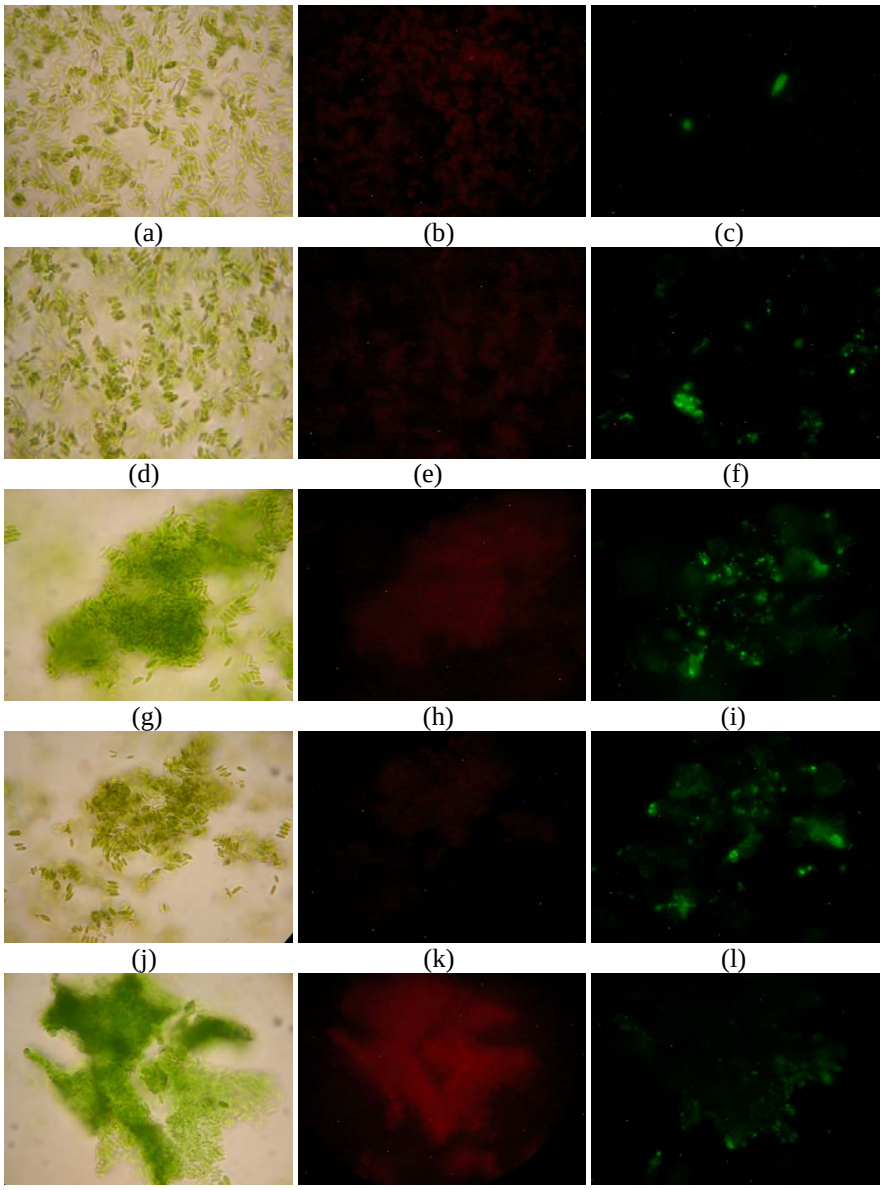


**Figure 4.2 The microscopic images of Flocculated Algae. From left to right, there are light microscopy, red fluorescent microscopy representing live cells, and green fluorescent microscopy representing dead cells. (a-c) raw Flocculated Algae; (d-f) Flocculated Algae treated with 0% NaOH at 50 °C for 10 min; (g-i) Flocculated Algae treated with 12% NaOH at 50 °C for 10 min; (j-l) Flocculated Algae treated with 0% NaOH at 90 °C for 10 min; (m-o) Flocculated Algae treated with 12% NaOH at 90 °C for 10 min.**

After heating at 50°C without addition of alkali, cells appeared as individuals and slight cell wall destruction occurred (Figure 4.2). As 12% NaOH was added in the slurry, cells clumped and formed flakes. Meanwhile more cell walls were disrupted (comparing Figure 4.2 f to i). At 90°C, even without addition of alkali, cells coagulated, along with considerable cell wall destruction (Figure 4.2l). Based on fluorescent images, no significant difference in term of cell

wall disruption was observed between alkali treated algae and non-alkali treated algae (comparing Figure 4.2l to o).

The way SOM reacted to alkali and heating in the Flocculated Algae samples showed a different pattern compared to the Fresh Algae samples (Table 4.2). At 50°C, the SOM increases in Flocculated Algae samples was slightly less than those for Fresh Algae; at 90°C, as the dosage of NaOH increased, both algae samples had a similar amount of SOM increase. Flocculants should play a role in how algal cells react to the altered extracellular pH and temperature. The flocculant polymers are water soluble, and can adsorb onto the algae cell surface by electrostatic interaction, hydrogen bonds or ionic bonds (Bolto and Gregory 2007). Once the equilibrium of the adsorption is attained, three distinct segments are formed: trains where portions of the polymer are attached to the surface, tails where portions of polymer are projected into the solution and loops where a portion of polymer is between two trains (Bolto and Gregory 2007). When the pH is above 8.5, degradation can occur to CPAM and as a result, flocculation is cancelled (Bolto and Gregory 2007). Heating at 50°C without alkali increased the SOM of Fresh Algae and Flocculated Algae by 12% and 8% (Table 4.2). It seemed the flocculation of algae cells inhibited the cell wall disruption. When heating at 90°C with alkali addition, CPAM degradation took place and flocculation was inhibited. Under this condition, cell wall disruption happened at a similar intensity for both algae samples.

The pH of Dried Algae samples with NaOH addition fell into a similar range (9~12) of those for Fresh and Flocculated Algae. But the pretreatment appeared to have less effect on increasing extracellular SOM in the Dried Algae samples (Table 4.2). It was worth noting that the SOM of the Dried Algae sample prior to the pretreatment was the highest among the three algae sources (Dried Algae 7.5, Flocculated Algae 2.7 and Fresh Algae 1.7% SCOD/TCOD). This was because drying altered cell structure and disrupted cell wall continuity (Mendes-Pinto et al. 2001). Heating (50°C or 90°C) without NaOH had no effect on improving the release of SOM from the cell matrix (Table 4.2). The addition of NaOH rendered the release of additional SOM, however increasing the dosage at 50°C had no significant effect ($P=0.2184$) on releasing additional SOM. When heating at 90°C, high dosage of NaOH had increased the impact on the destruction of cell walls and the release of SOM (Table 4.2). The untreated Dried Algae did not easily dissolve in water, and under microscope the coagulated clumps were observed (Figure 4.3).

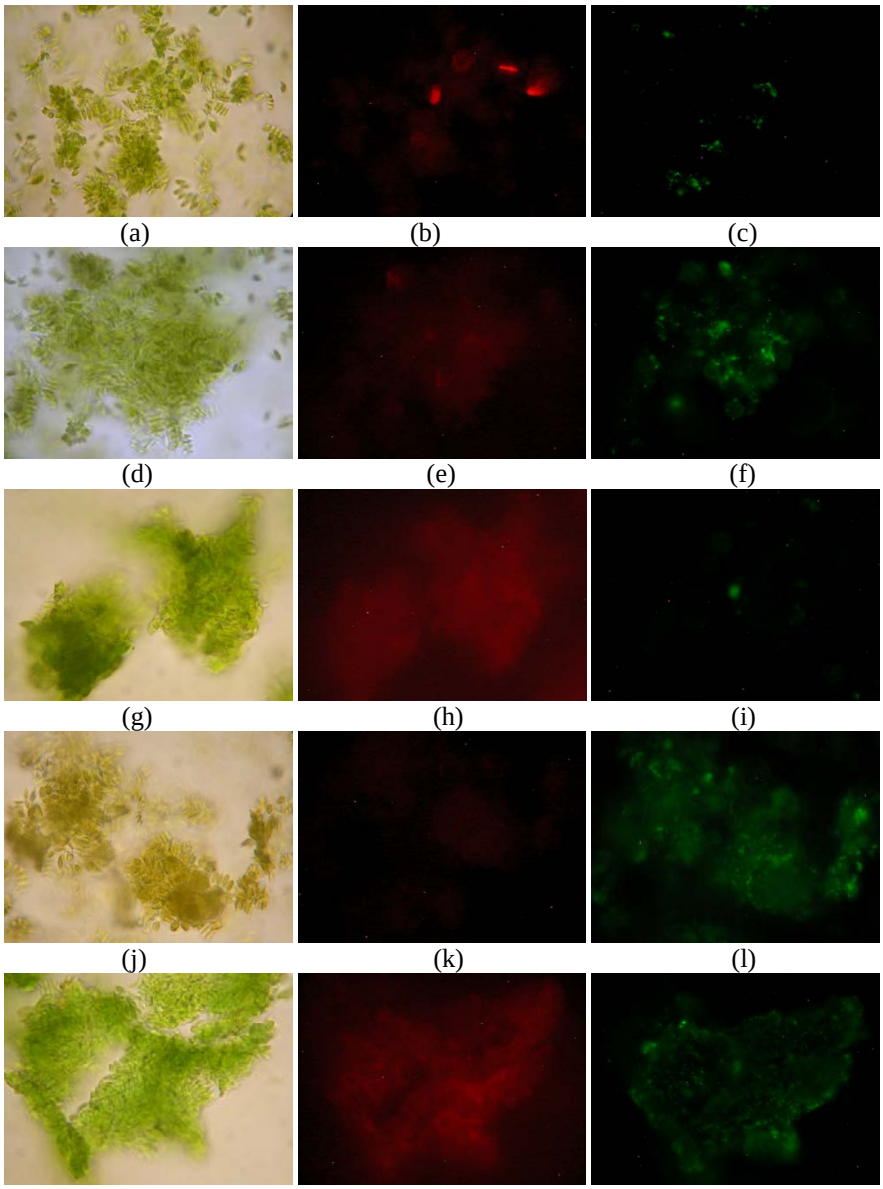


**Figure 4.3 The microscopic images of Dried Algae. From left to right, there are light microscopy, red fluorescent microscopy representing live cells, and green fluorescent microscopy representing dead cells. (a-c) raw Dried Algae; (d-f) Dried Algae treated with 0% NaOH at 50 °C for 10 min; (g-i) Dried Algae treated with 12% NaOH at 50 °C for 10 min; (j-l) Dried Algae treated with 0% NaOH at 90 °C for 10 min; (m-o) Dried Algae treated with 12% NaOH at 90 °C for 10 min.**

The shining green clouds seen in the untreated samples indicated significant cell destruction. Pretreated samples also had green patches, but the intensity was similar to the untreated one, or even less in one case (Figure 4.3c vs. i). Drying helped to alter cell structure and release SOM from cell matrix, but it can decrease the fermentative potential of algae biomass (Mussgnug et al. 2010). Drying changed both the biological structure of cells and the chemical

merits of the organic compounds, and the latter determined the fermentative potential. If the change was detrimental, drying can limit the accessibility of organic compounds to bacteria.

### 4.4.2. Anaerobic digestion of algae

Flocculated Algae was used as the primary biomass in the anaerobic digestion study. Based on microscopic images after anaerobic digestion the majority of algae cells were completely decomposed. However, even when the substrate was pretreated algae, some intact cells still existed in the effluent.

Flocculated Algae was pretreated at 50°C for 10 min without alkali. The pretreated algae had a steeper and shorter exponential phase than the untreated one (Figure 4.4). Due to the limited access to the BMP system, only two treatments (pre-treated vs. raw algae) were able to be tested. Focusing on the most economical approach, the least energy intensive pretreatment (50°C, 10 min, no alkali) was selected to determine if it would have a significant effect on the digestibility of the algae.

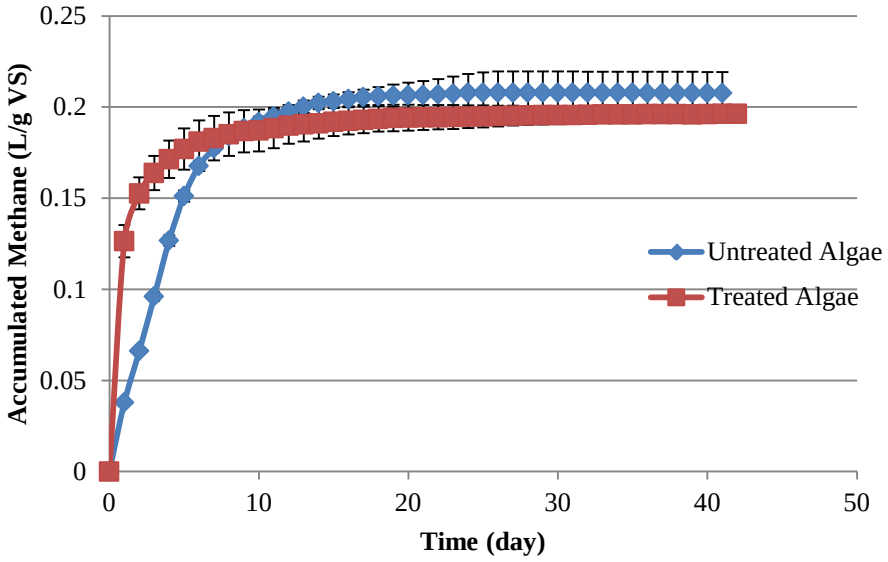


**Figure 4.4 Accumulated methane production of raw Flocculated Algae and pretreated Flocculated Algae (50 °C 10 min no alkali). The error bars are standard deviation of two replications.**

After one day's fermentation, pretreated algae (50°C for 10 min and no addition of NaOH) produced 0.135 L/gVS, and then methane production immediately slowed down, and reached the maximum of 0.2 L/gVS after 20 days. As to the untreated algae, methane production gradually increased within the first 7 days, and there was no significant difference between the

maximum methane production per gram of VS in the two algae samples. This result agreed with the one reported by Gonzalez-Fernandez et al. (2012). The treated algae gave a quick start of gas production mainly due to the significant amount of soluble organic matter leaching out from the cells after thermal treatment. However once all soluble organic matter was depleted after first 2 days, the digestion slowed down, and ended up with the same specific gas production from untreated counterpart. The cell wall destruction was not complete after minor treatment, and intact cells can be seen in the digestate of both algae samples (the image was not shown here). When running the anaerobic digestion for untreated Flocculated Algae in CSTR, the OLR had a significant effect on the gas production (Table 4.3).

**Table 4.3 The effect of loading rate on the specific gas production in CSTR (n=3).[a]**

| Loading rate kgVS/$m^3$/d | Specific gas production L/kgVS/d |
|---|---|
| 2.5 | $316^{c}$±37 |
| 1.78 | $414^{b}$±35 |
| 1 | $378^{bc}$±141 |
| 0.85 | $347^{bc}$±44 |
| 0.75 | $594^{a}$±52 |
| 0.64 | $482^{a}$±33 |
| 0.59 | $325^{c}$±13 |
| 0.45 | $431^{ab}$±173 |

(a) Mean values followed by the same letter are not significantly different at α=0.05.

The highest specific gas production was 594 L/kgVS/d at OLR of 0.75 kgVS/$m^3$/d. When raising OLR to 2.5 kgVS/$m^3$/d, specific gas product decreased to 316 L/kgVS/d. One low OLR (0.59 kgVS/$m^3$/d) also caused low gas production. However OLR was not proved to be linearly related to SGP. Overloading can induce digestion imbalance including volatile fatty acids accumulation, pH drop, $H_2$ overproduction, foam formation and eventually the inhibition of methane production (Charles et al. 2011, Gomez et al. 2006). The determination of the threshold of overloading is important to design an efficient reactor where maximum amount of substrate is digested in the minimum size of digester. Based on the current data, the lowest SGP occurred at two OLR (0.59 and 2.5 kgVS/$m^3$/d), which indicated 2.5 kgVS/$m^3$/d was the possible threshold for the high loading rate. A study focusing on the batch anaerobic digestion of municipal wastewater sludge concluded that organic loading of 2.5 kgVS/$m^3$ was the critical threshold for foam initiation (Ganidi et al. 2011).

## 4.5. Conclusions

Thermal pretreatment can effectively weaken the algae cell wall and lead to the release of soluble organic matter from the cell with algae at a 10 g/L concentration. Different temperatures (50°C and 90°C) showed more significant effect on the flocculated algae and the dried algae than on the fresh algae. NaOH addition showed deleterious impacts on organic matter release for the fresh algae at 50°C, but increased the amount of soluble organic matter released for the fresh algae at 90°C and the other two samples at both 50°C and 90°C. Duration (10, 30 or 60 min) failed to show a significant effect in most cases, however when heating at 90°C with 12% of NaOH, more soluble organic matter was leached out as the treatment duration was extended. Fluorescent microscope images confirmed that the thermal chemical treatments disrupt the cell walls, however they failed to show the magnitude of the disruption among various pretreatments. Both raw and pretreated algae were confirmed to be potential anaerobic digestion substrates. The pretreated algae appeared to have a stiffer exponential growth curve, but ended up with a similar accumulated methane production (L/gVS) with the raw algae sample. The highest SGP occurred at OLR of 0.75 kgVS/$m^3$/d, and increasing the loading rate to 2.5 kgVS/$m^3$/d induced the decline of gas production.

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# Chapter 5: Application of used media and algae biomass anaerobic digestate in *Scenedesmus* photobioreactor cultivation

## 5.1. Summary

Used media and algae biomass are products from algae harvesting. After harvesting, algae biomass was subjected to anaerobic digestion, which generated mineralized nutrients. Recycling used media and mineralized nutrients during *Scenedesmus* cultivation made the system more sustainable. *Scenedesmus* was cultivated in flasks with artificial lights and elevated $CO_2$ concentration (3%). To maintain the optimum growth rate, algal biomass was harvested continuously to keep a constant biomass concentration. The used media proved to be able to support the cell growth with nutrient replenishment. Algae biomass was subjected to the anaerobic digestion, and the digestate was tested as nutrient source for algae cultivation. The digestate was rich in ammonium, and proved to be a sufficient replacement for urea. When both urea and ammonium were available in the media, the assimilation of urea by algal cells slowed down compared to the case where urea was the only nutrient source.

***Keywords:*** Ammonium ion, nitrogen source, *Scenedesmus*, photobioreactor.

## 5.2. Introduction

Nutrient supplementation is critical for algae cultivation. Commercial fertilizers or lab grade chemicals can provide nutrients for algae growth (Chapter 3), and as the cultivation scale expands, the amount of nutrient supplied becomes significant (Chapter 6). When the cultivation is operated in a continuous mode, algae biomass has to be harvested intermittently, which generates used media containing residual nutrients and algae biomass. Algae biomass contains proteins, carbohydrates, oils, nucleic acids and inorganic compounds, which are potential nutrients for further algae cultivation. To alleviate the dependence on pre-formed commercial fertilizers, used media and algae biomass should be considered as a secondary nutrient source for the system.

The four most abundant elements in *Scenedesmus* biomass are carbon, oxygen, hydrogen and nitrogen (Table 5.1), where oxygen and hydrogen originate from water molecules, and carbon is assimilated from $CO_2$.

**Table 5.1 Elemental composition of *Scenedesmus* used in this study (by weight).[a]**

| Element | % |
|---|---|
| Carbon (C) | 50.02 |
| Oxygen (O) | 25.98 |
| Hydrogen (H) | 7.43 |
| Nitrogen (N) | 7.80 |
| Phosphorus (P) | 2.20 |
| Potassium (K) | 0.82 |
| Magnesium (Mg) | 0.39 |
| Sulfur (S) | 0.69 |
| Iron (Fe) | 0.43 |
| Calcium (Ca) | 1.3 |
| Manganese (Mn) | 0.0023 |
| Copper (Cu) | 0.0021 |
| Zinc (Zn) | 0.0053 |

a. Method was described in Materials and Methods.

In the photobioreactor (PBR), nitrogen is not readily available for algae cells, instead it is supplied through fertilizers including urea, ammonium salts, and nitrate salts. Only the most reduced form of nitrogen ($NH_4^+$) can directly join the assimilating pathways and become the building block of proteins and nucleotides (Horton et al. 2002, Glass et al. 2009). Therefore urea and nitrate have to be reduced to $NH_4^+$ by algae cells prior to assimilation. Urea has been used as nitrogen source for many algae, including dinoflagellates, *Chlorella* and *Chlamydomonas* (Healey 1973, Syrett 1988, Solomon and Glibert 2008). Urea is transported into the cells via either passive or active transport (Syrett 1988). Inside the cell, urea is further decomposed by urease, and converted to ammonium and carbon dioxide.

$$CO(NH_2)_2 + H_2O + 2H^+ \xrightarrow{\text{urease}} 2NH_4^+ + CO_2 \qquad (5.1)$$

However *Chlorella* and *Chlamydomonas* have been found lack of urease (Healey 1973, Syrett 1988). These algae use urea amidohydrolase coupling with one molecule of ATP to break down one urea molecule (Syrett 1988):

$$CO(NH_2)_2 + HCO_3^- + 3H^+ + ATP \xrightarrow{\text{ureaamidohydrolase}} 2NH_4^+ + 2CO_2 + ADP + P_i \qquad (5.2)$$

Another theory is that there are two enzymes involved in the hydrolysis: urea carboxylase coupled with ATP converts urea into allophanate, which is later hydrolyzed into ammonium and bicarbonate by allophanate hydrolase (Thompson and Muenster 1971).

When nitrate is fed as nitrogen source, nitrate reductase (NR) and nitrite reductase (NiR) are assimilated in algal cells to catalyze the reduction reaction (Glass et al. 2009).

$$NO_3^- + 2e^- + 2H^+ \xrightarrow{\text{nitrate reductase}} NO_2^- + H_2O \qquad (5.3)$$

$$NO_2^- + 6e^- + 8H^+ \xrightarrow{\text{nitrite reductase}} NH_4^- + 2H_2O \quad (5.4)$$

In algae, NAD(P)H is the electron donor, whereas in cyanobacteria, electrons are donated by ferredoxin (Glass et al. 2009). Besides the commercial nitrogen fertilizers, dissolved organic nitrogen like amino acids can also be taken up by algae (Healey 1973). *Chlorella pyrenoidosa, Platymonas subcordiformis, Nitzschia ovalis, Melosira nummuloids* and *Coscinodiscus asteromphalus* have been reported to perform energy dependent amino acid uptake which is not affected by the presence of ammonia and nitrate (Healey 1973). On the other hand, some red algae (including coccolithophorids and dinoflagellates) can deaminate amino acid outside the cell by a Cu-containing cell-surface amine oxidase (GuAO), and then take up $NH_3$ (Glass et al. 2009).

$$RCH_2NH_2 + O_2 \xrightarrow{\text{GuAO}} H_2O_2 + RCHO + NH_3 \quad (5.5)$$

Once N compounds are converted into $NH_4^+$, $NH_4^+$ enters the glutamine synthetase-glutamate synthase (GS-GltS) pathway, and then constitutes protein, chlorophyll, nucleotide and phospholipid (Glass et al. 2009). The initial step of this pathway is to generate glutamine:

$$\text{Glutamate} + \text{ATP} + NH_4^+ \xrightarrow{\text{Glutamine synthetase}} \text{Glutamine} + \text{ADP} + P_i \quad (5.6)$$

GS catalyzes the formation of glutamine, and requires Mg or Mn ions for activation (Glass et al. 2009).

Another important element is phosphorus, the building block of nucleic acids and ATP. In a photobioreactor, phosphorus is added as phosphate, which also functions as buffer to maintain a neutral pH. When phosphorus is not limited in the environment, algae cells utilize it for synthesizing macromolecules (nucleic acids) and energy storage unit (ATP). When external phosphorus becomes scarce, the polyphosphate is degraded to provide the phosphate for metabolic assimilation (Healey 1973).

To make the system sustainable, commercial fertilizers input should be minimized, and mineralized nutrients from AD digestate should be reused. Organic macromolecules are mineralized through anaerobic decomposition (Moller and Muller 2012) (Figure 5.1). Anaerobic consortia break down hydrocarbons (carbohydrate and fatty acids) and generate biogas (a mixture of $CH_4$ and $CO_2$). Organic nitrogen is mineralized to $NH_3$ venting out as biogas, or $NH_4^+$ dissolving in liquid. Phosphate as essential building blocks for nucleic acid is released from the macromolecules, and available for anaerobic bacteria to assimilate. Sulfur existing in cysteine or methionine to form disulfide bonds is mineralized to $H_2S$.

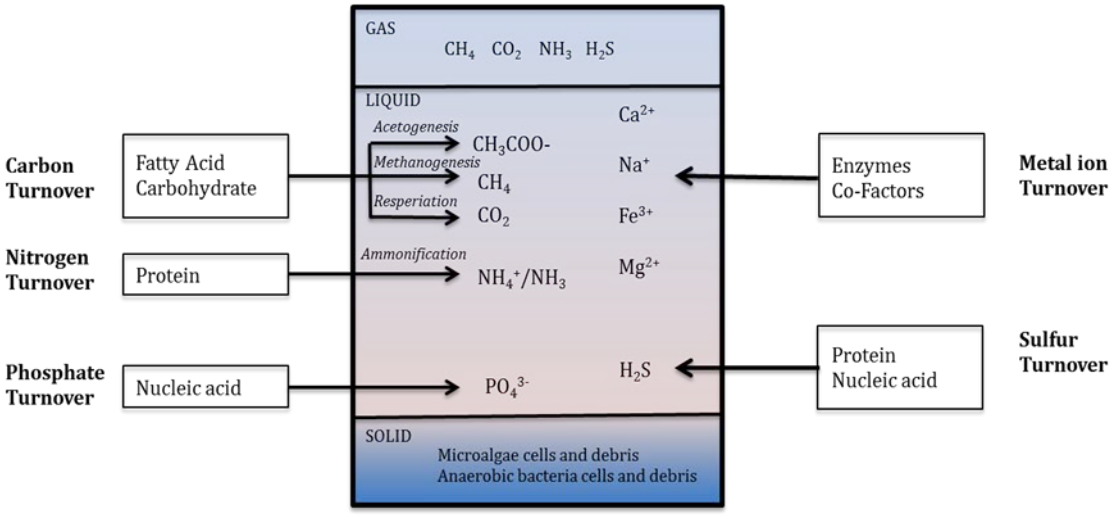


**Figure 5.1 Mineralization of macromolecules by anaerobic digestion.**

Using digestates as fertilizer is not a new concept. Researchers have been studying the impacts of digestates to soil fertility and crop phytotoxicity. Farm manures are common substrates for anaerobic digesters, so digested manure slurry or co-digested crop residue and manure slurry are studied the most. Digestate is best known as rich in mineralized nutrients (N and P), which also plays a vital role in determining the suitability of a digestate as fertilizer (Alburquerque et al. 2012). Alburquerque et al. (2012) investigated the phytotoxicity of anaerobically digested manure slurries in lettuce and cress hydroponics. Directly using the digestate without any dilution caused no germination or biomass accumulation, which was explained by the high salinity of the digestate. Diluting to 20% of concentration, seeds started to germinate, and 1% of digestate concentration gave the best germination and highest biomass accumulation. Researchers linked the fertilizing performance of various dilutions of digestate to organic matter content, and concluded that when DOC (dissolved organic carbon) and  (5 d biochemical oxygen demand) were less than 1.5 or 2.5 g/L respectively, the digestate was suitable for lettuce and cress hydroponics. Nishikawa et al. (2012) conducted a six years experiment on the effects of digested cattle manure in the rice paddy in the warmer region of Japan. The digestates were compared to commercial fertilizers in terms of grain yield and nitrogen efficiency, which was normalized nitrogen uptake by control and varying nitrogen dosages. Regardless of the dosage level and application method, first three years' results showed commercial fertilizer yielded higher N efficiency, but the second three years' result indicated a superior effect of digestates on N efficiency. When it came to grain yield, two kinds of fertilizers rendered similar rice production rates. They considered manure digestate as an appropriate alternative to commercial fertilizers in rice cropping since rice plants preferred ammonia N rather

than nitrate N. Bachmann et al. (2011) tested the fertilizing effects of codigested slurry of dairy slurry, maize silage and wheat grain on two crops species: *Zea mays* L. and *Amaranthus cruentus* L.. They concluded that dairy slurry and codigested dairy slurry were as effective as the highly soluble mineral P source with respect to the plant P uptake rate. In particular for amaranth growing on the sandy soil, digestates offered even higher plant P uptake. However an opposite pattern occurred for N uptake, organic fertilizers caused more than 50% less plant N uptake than commercial counterpart did. Interestingly the dry matter yields did not drop dramatically with the lowing N uptake for both *Zea mays* L. and *Amaranthus cruentus* L.. They also studied the microbial activity in the soils fertilized by digestates and commercial fertilizers. It turns out significant high microbial activity has been maintained in the soils fertilized by digestates, which can benefit the organic-matter turnover and the plant growth. Studies has shown anaerobic digestates can potentially benefit crop growth as well as nitrogen uptake, however this application should be carefully monitored to ensure a constant soil quality at the early stages (Arthurson 2009). Due to the high content of organic matter (especially organic C), the soil can undertake a different route to adjust to the addition of digestates compared to commercial fertilizers. Overlooking the short-term alternation of soil quality can be risky to maintain the sustainability of soil quality in the long run.

In this study, a nutrient recycling strategy was tested with *Scenedesmus* cultivation. The used media generated through harvesting and dewatering of biomass was added back to the algae cultivation system. Meanwhile anaerobic digestate was considered as nutrient source to replace the commercial fertilizers. The effects of nutrient recycling on algae growth were investigated.

## 5.3. Materials and Methods

### 5.3.1. Used media recycling

*Scenedesmus actus* were cultivated in Urea medium consisting of $CO(NH_2)_2$ 0.137, $KH_2PO_4$ 0.118, $MgSO_4.7H_2O$ 0.109, $CaCl_2.2H_2O$ 0.055 and Na.EDTA.Fe 0.02 in g/L. Tap water was autoclaved to remove chlorine and kill live cells. All tests were conducted in 500 mL flasks and each treatment had three replications. The inoculation level was approximately 0.05~0.10 g dw/L. All test flasks were placed on a shaking table with 140 rpm rotation. Filtered ambient air mixed with 3% $CO_2$ was bubbled into each flask with flow rate of 0.15 L/min. Warm and cool white fluorescent lights (40 Watts, 60.5 μmol/$m^2$ per second) were provided as light source for a 16h : 8h light: dark illumination period. The chamber temperature was controlled at 25 °C. Each 24 hours, 8 mL of liquid samples were withdrawn, filtered and oven-dried to obtain the algae dry mass.

Due to the limited cultivation volume (400 mL per flask), stocks were prepared in advance (Figure 5.2). The stock flasks were kept at the exactly same condition as aforementioned, and were replenished with fresh nutrients (50% of the original formula) weekly. Prior to being used as used media, stocks were centrifuged (3000 rpm for 20 min) to remove algae cells. Supernatants were collected and replenished for the next generation of algae. In the first trial, 50% of the original formula was mixed into the spent medium, and the reconstituted spent medium was used to cultivate the next generation of algae for 7 days. In the second trial, no nutrient replenishment was applied prior to use.

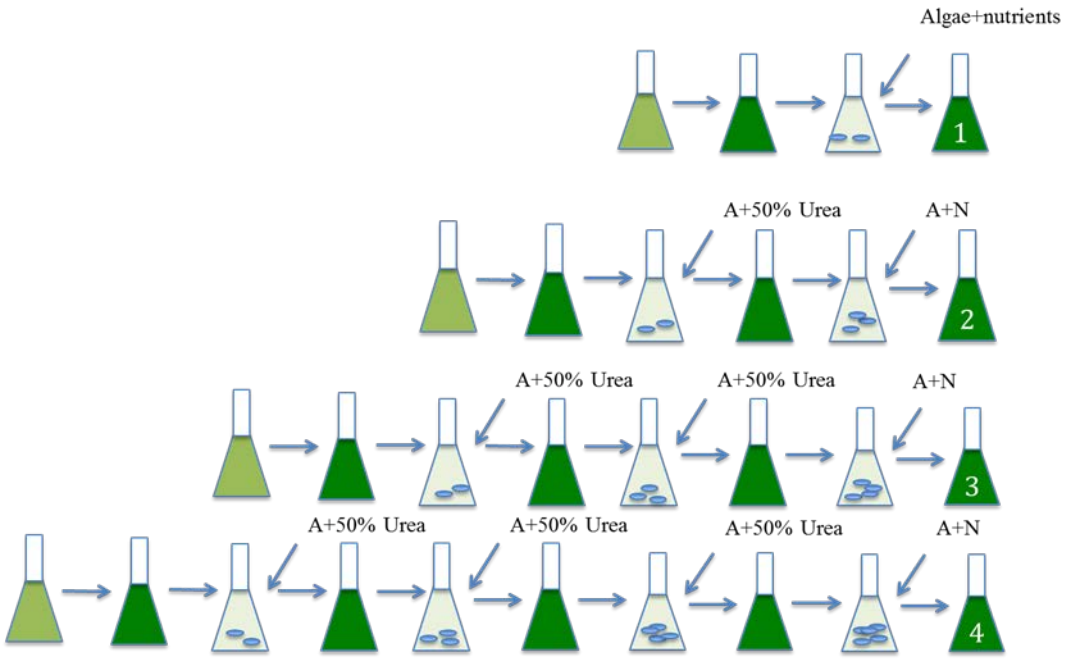


**Figure 5.2 Stock media preparation. Dots in flasks represent potential growth inhibitors. Before inoculating algae into the last set of used media, nutrients were added except the test without nutrient supplement. In the test of anaerobic digestate, nutrient was replaced by liquid or total digestate.**

The urea concentration in the medium was analyzed by HPLC. Samples were centrifuged to remove algal cells, and filtered with a 0.45 micron membrane syringe filter (Corning®) before being injected into a HILIC-1 column (Acclaim® Mixed-Mode, 4.6 × 150mm) with a UV detector (Dionex® Ultimate 3000). The eluent was 30/70 v/v $CH_3CN$/ de-ionized water, and the flow rate was 0.7 mL/min. The operation temperature was 25 °C. The injection volume was 50 µL.

### 5.3.2. Digestate application

Anaerobic digestate of raw algae was obtained from a CSTR reactor with a solid content of 7%. The digestate was added in the media in two forms: liquid only (ADL) or total digestate (the mixture of liquid and solid slurry) (ADT). The phase separation was performed by

centrifugation (3000 rpm for 10 min). Prior to algae inoculation, liquid and slurry were diluted to match the nutrient level in Urea medium. The algae cultivation condition was as aforementioned. Growth curves and $NH_4^+$ consumption curves were recorded to evaluate differences between the digestates and commercial mineralized chemicals. Samples were named according to the media composition.

C: Urea media (control)

FADL: Urea media + liquid digestate

FADT: Urea media + total digestate

R1ADL: 1 week used media + liquid digestate

R2ADL: 2 week used media + liquid digestate

R3ADL: 3 week used media + liquid digestate

R4ADL: 4 week used media + liquid digestate

R1ADT: 1 week used media + total digestate

R2ADT: 2 week used media + total digestate

R3ADT: 3 week used media + total digestate

R4ADT: 4 week used media + total digestate

ADLW: liquid digestate + tap water

ADLU: liquid digestate + urea media

ADTW: total digestate + tap water

ADTU: total digestate + urea media

### 5.3.3. Nutrient analysis

The soluble nutrients (ammonia, phosphate, potassium and magnesium) in the anaerobic digestate and used media were determined by ion chromatography (Dionex® ICS-1100). Filtered samples were sealed in Dionex® Polyvials and loaded into Dionex® ICS AS-DV autosampler. The eluent was 4.5 mM sodium carbonate and 1.4 mM sodium bicarbonate, and the flow rate was 1.0 ml/min. Samples were injected into the cation Column CS16 at 40°C, and signals were detected by conductivity detector. Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) was used to identify and quantify the elemental composition of algae and the solid digestate. The instrument used was a Varian Vista Pro ICP-OES under the following operating conditions: 1.2 kW power, 15 L/min plasma flow, 0.9 L/min nebulizer flow, 8 second replicate read time and 1 ppm Yttrium internal standard. Algae cells or solid digestates were washed with DI water, and centrifuged to remove excess water, and oven-dried overnight. The solid samples were acid digested, and diluted with DI water before injected into the nebulizer.

### 5.3.4. Algae dry weight and growth rate

*Scenedesmus* biomass samples in suspension were filtered using Whatman binder-free glass microfiber filters (type 934-AH, 24 mm diameter). The dry weights (DW) of samples were measured by drying at 105°C for 24 hours. Biomass content was calculated from microalgal dry weight produced per liter (g/L). The slope of the growth curve at the exponential phase was used as the growth rate (g/L/d). For algae cultivated in the media with total digestate, a blank was taken before inoculation, and the algae dry weight was calculated as the difference between the sample dry weight and dry weight of the blank.

## 5.4. Results and Discussions

### 5.4.1. Composition of anaerobic digestate

Anaerobic digestate was rich in soluble nutrients and its solid fraction also contained significant amount of N, P and K (Table 5.2). Ammonium is an ideal replacement of urea, and the presence of soluble $K^+$ and $PO_4^{3-}$ made the digestate a viable nutrient source. The composition of solid fraction was similar to the composition of *Scenedesmus* (Table 5.1), especially for N, P and Ca. The similarity between these two composition profiles indicated the major component in the solid digestate were likely undigested algal cells.

**Table 5.2 Nutrients profile of soluble and solid anaerobic digestate (n=3).**

| | Nutrient | Concentration |
|---|---|---|
| Soluble fraction (ppm) | $NO_3^-$ | 72 ± 3 |
| | $NH_4^+$ | 967 ± 9 |
| | $K^+$ | 238 ± 6 |
| | $PO_4^{3-}$ | 448 ± 15 |
| Solid fraction (% w/dw) | N | 8.68 ± 0.21 |
| | P | 2.13 ± 0.06 |
| | K | 0.39 ± 0.01 |
| | Mg | 0.62 ± 0.04 |
| | Fe | 0.96 ± 0.01 |
| | Ca | 1.90 ± 0.03 |

### 5.4.2. Effects of used media on growth

The current cultivation of *Scenedesmus* is in batch mode, with retention time of 7 days. The slopes of the growth curves in fresh or used media varied from week to week, but the

patterns were similar, with 50% nutrient replenishment in the used media except the first week (Figure 5.3). In the first week, two curves were almost overlapping; since both media were freshly prepared, and specific growth rates were 0.12 or 0.11 g/L/day. In the second week, the two curves still overlapped until towards the end of 7 days, when used media samples had slightly less robust growth. In the third week, fresh media samples encountered an environmental shock ($CO_2$ supply was interrupted for about 12 hours) inducing a prolonged lag phase. Even though the two sets of samples were placed in the exact same cultivation condition, used media samples seem to be not affected by the environmental shock, but produced less biomass at the end (0.46 vs. 0.54 g/L). On the other hand, fresh media samples had a 4-day lag phase, but had a higher biomass accumulation (0.54 g/L). After media had been recycled two times (the third week), it still showed great potential to support algae growth: no significant lag phase was observed, and the biomass accumulation was comparable to previous weeks. Fresh media presented a superior specific growth rate in the third week: 0.97 vs. 0.7 g/L/day. However considering used media were only dosed with 50% of urea, it indeed carried out a comparable growth rate. After media had been recycled three times, the two growth curves were still very similar to each other. Apparently media can be reused with nutrient replenishment in the dilute cultivation system (biomass density less than 1 g/L). In the ultrahigh cell density cultures (UCDC), defined as cultures having more than 10 g/L cell mass, frequent replacement of the growth media instead of nutrient replenishments is need to ensure the much higher areal productivity (Richmond 2004). Several microalgae have been identified to excrete certain growth inhibitors to control the total cell population, like *Chlorella vulgaris*, *Skeletonema costatum*, *Cladosiphon okamuranus* and *Haematococcus pluvialis* were inhibited by *Chlamydomonas reinhardtii* (Richmond 2004). Growth inhibition was most prominent in UCDC compared to low density cell cultures, therefore inhibitors need to be frequently removed by replacing the media to ensure a high productivity (Richmond 2004).

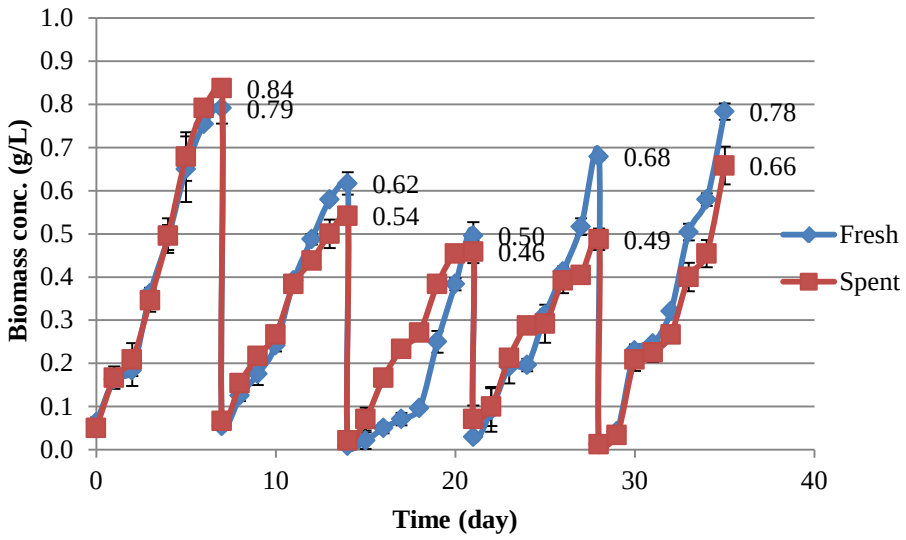


**Figure 5.3 Growth curves of *Scenedesmus* in fresh and used media, where the used media was replenished with 50% Urea formula at the beginning of each week except the first week. Error bars represent standard errors (n=3). Numbers are accumulated algae yields (g/L).**

Urea consumption curves were almost the reverse of growth curves (Figure 5.4). In fresh media samples, urea started with 140 ppm and was gradually consumed as algae cells proliferated, and was exhausted at the end of cultivation. This pattern was repeated for most batches, except for the third week. When algae cells underwent a prolonged lag phase, urea was not rapidly decomposed for the first four days, and the prompt consumption occurred only after the exponential growth took off. In the case of used media, only 50% of urea was added in the first day of each week, and was not exhausted until the last day.

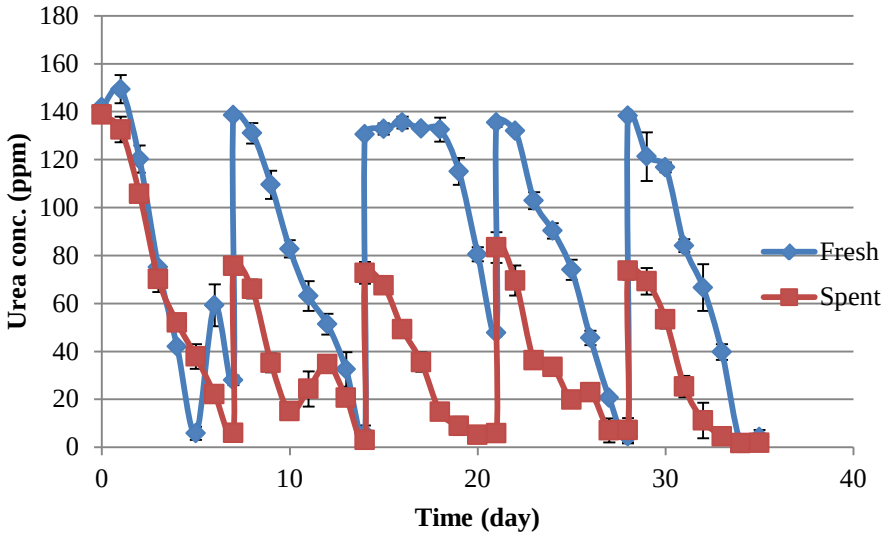


**Figure 5.4 Urea consumption curves in fresh and used media, where the used media was replenished with 50% Urea formula at the beginning of each week. Error bars represent standard errors (n=3).**

Without nutrient replenishment in the used media, algae growth showed a rather different pattern (Figure 5.6). As starvation occurred, the growth gradually ceased and algae become yellowish (Figure 5.5). Compared to the control, algae growing in the nitrogen limited used media showed only 30% of growth. Nitrogen is the building block for proteins including various enzymes. Photosynthesis and carbon assimilation rely on the activity of enzymes and chlorophylls. The photosynthesis rate is positively related to the concentration of N in the cell regardless the intensity of irradiation (McGlathery and Pedersen 1999, Young and Beardall 2003). When *Chaetomorpha Linum* encountered nitrogen depletion, both protein and chlorophyll contents declined within days (McGlathery and Pedersen 1999, Young and Beardall 2003). Interestingly during nitrogen starvation, both starch and sugar contents increased compared to nitrogen saturation condition (McGlathery and Pedersen 1999). In this study, when nitrogen was limited, the growth of *Scenedesmus* was not completely zero, (Figure 5.6 and Figure 5.7), despite the obvious chlorosis. Clearly when *Scenedesmus* had limited nitrogen supply and internal N was continuously diluted as cell division occurred (Young and Beardall 2003), photosynthesis slowed down due to the impaired chlorophyll synthesis, but carbon assimilation still occurred although at 30% of the maximum growth rate. Among the four used media, the one recycled twice showed the highest specific growth rate (0.04 g/L/d, P=0.0074).

**Figure 5.5 The effect of nitrogen deprivation on algae color. Flasks from left to right contain fresh media, used media recycled once, used media recycled twice, used media recycled three times and used media recycled four times respectively.**

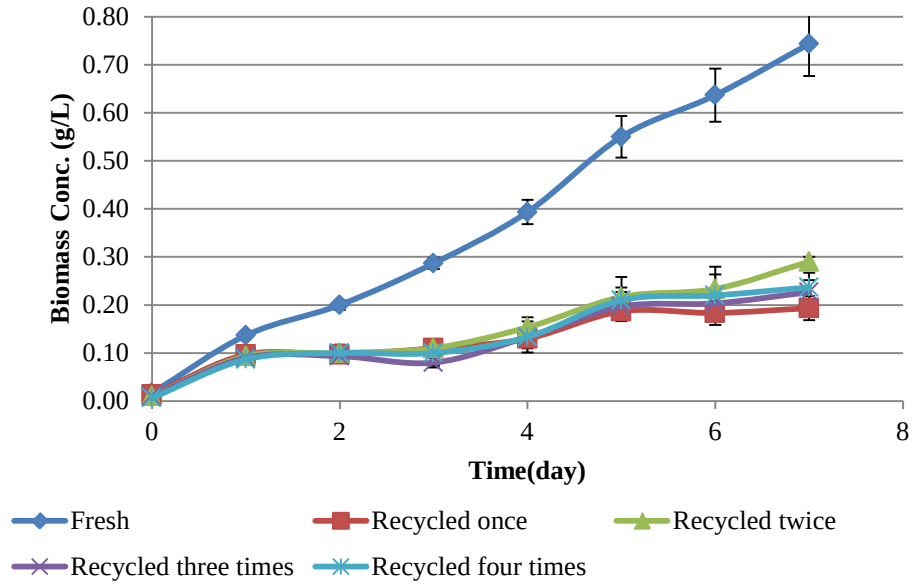


**Figure 5.6 Growth curves of *Scenedesmus* growing in fresh or used media without nutrient replenishment. Error bars represent standard errors (n=3).**

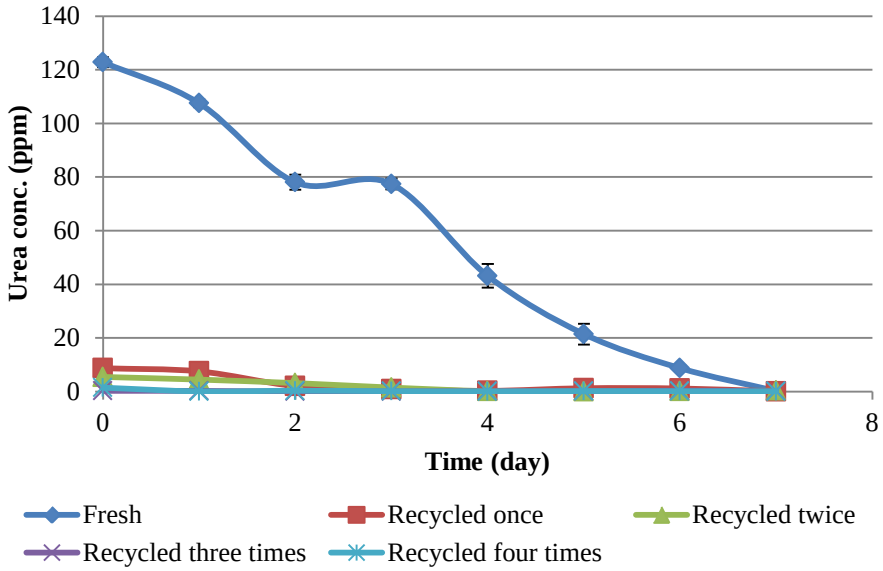


**Figure 5.7 Urea consumption of *Scenedesmus* growing in fresh or used media without nutrient replenishment. Error bars represent standard errors (n=3).**

### 5.4.3. Effects of using anaerobic digestate as nutrient resource

Anaerobic digestate is rich in mineralized nutrients, and contains a substantial amount of insoluble particles mainly algae cell debris. The insoluble particles contribute to the high turbidity in media (Figure 5.8).

**Figure 5.8 The effect of liquid and solid digestate on culture color and turbidity. From left to right, flasks contain fresh urea media, tap water mixed with liquid digestate, fresh urea media mixed with liquid digestate, tap water mixed with total digestate and fresh urea media mixed with total digestate.**

Firstly anaerobic digestate was tested in tap water as the sole nutrient source. The amount of digestate added in media was adjusted to be similar to the control, however due to the different

nutrient composition between digestate and the Urea formula it was not possible to make all chemicals have the exact amount as they were in the control (Table 5.2 and Table 5.3).

**Table 5.3 The elemental composition of Urea (Control) and digestate (ADLW and ADTW) formula (n=3).**

| | Control | ADLW | ADTW |
|---|---|---|---|
| | ppm | ppm | ppm |
| N | 54.2±2.7 | 76.6±2.5 | 40.6±1.1 |
| P | 24.7±0.3 | 14.0±0.5 | 9.4±0.3 |
| K | 31.6±0.1 | 13.9±0.6 | 4.3±0.1 |
| Mg | 20.7±0.1 | 6.3±0.3 | 10.4±0.1 |

The addition of liquid digestate or total digestate showed a positive impact on algae growth (Figure 5.9). Algae in dark turbid media containing insoluble particles (ADTW or ADTU) did not appear to have a prolonged lag phase compared to the control or liquid digestate samples (ADLW or ADLU). For all samples, the exponential growth took off after the first day. Over 9 days of cultivation, the control had the lowest specific growth rate of 0.086 g/L/day ($P<0.0001$) and lowest biomass accumulation of 0.86 g/L. When mixing liquid digestate with tap water or Urea media to constitute the growth media, specific growth rates were 0.095 or 0.11 g/L/day ($P<0.001$), and final biomass accumulations were 0.95 or 1.13 g/L respectively. When mixing the total digestate including both liquid and solid fractions with tap water or Urea media to constitute the growth media, the specific growth rates were 0.11 or 0.12 g/L/day ($P=0.1742$), and final biomass accumulations were 1.10 or 1.19 g/L. Interestingly using total digestate in both tap water or Urea formula seemed to give a higher specific growth rate than using liquid digestate (0.11 vs. 0.095 g/L/d, $P=0.0918$; 0.12 vs. 0.11 g/L/d, $P=0.0993$), but the results were not significant.

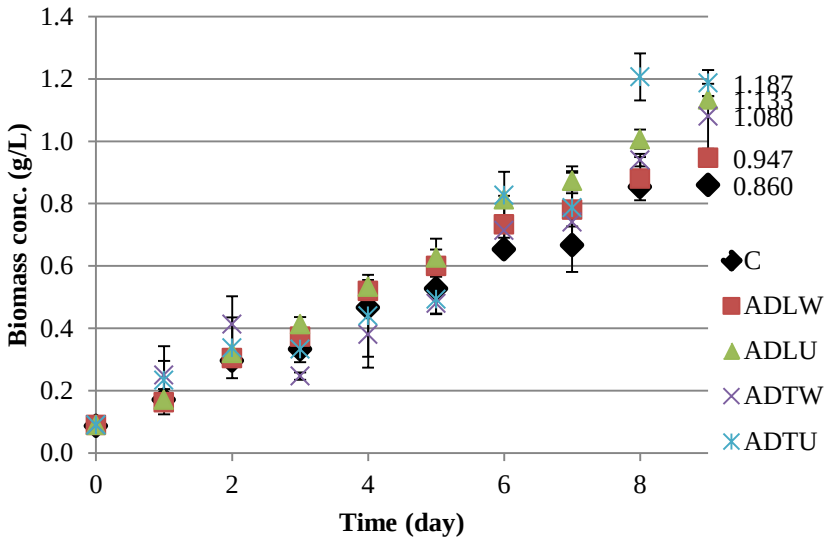


**Figure 5.9 Growth curve in the media with addition of liquid digestate or total digestate. Error bars represent standard errors (n=3).**

Nitrogen as one of major building blocks for protein and chlorophyll was significantly consumed during algae growth (Figure 5.10). For the control, where urea was the only nitrogen source, there was a linear decrease in urea concentration over 7 days and only 15 ppm of urea was left. For ADLW and ADTW where ammonium was the only nitrogen source, ammonium was quickly depleted, especially for ADTW where ammonium was exhausted at day 5. Since ADLW had more ammonium (98 vs. 52 ppm) to start with compared to ADTW, it had 22 ppm of ammonium left out at day 7. Nevertheless both samples had similar ammonium consumption rates. When both urea and ammonium were provided as nitrogen for algae growth (ADLU and ADTU), consumption curves were skewed from the sole nitrogen samples, especially for ammonium (Figure 5.10). ADLU contained more ammonium than ADTU (97 vs. 58 ppm) and showed a modest ammonium consumption that remained at 71 ppm of ammonium on day 7. By contrast, ADTU had a bell shape consumption curve, where the ammonium concentration increased from 58 to 88 ppm after 5 days, and ended with 70 ppm at day 7. Less urea had been consumed in both ADLU and ADTU compared to the control. It seemed urea uptake was affected by the presence of ammonium. The competitive uptake of urea and ammonium can be the consequence of the *Scenedesmus* urea uptake pathway and cell feedback regulation. Prior to joining the nitrogen assimilation pathway, urea was passively transported into *Scenedesmus* cell and reduced to $NH_4^+$ by ATP: urea amidolyase (Thompson and Muenster 1971, Thomas and Syrett 1976). The accumulation of intracellular $NH_4^+$ can result in the feedback inhibition of

extracellular $NH_4^+$ and urea uptake by repressing the corresponding enzymes expression (Mulholland and Lomas 2008). It is also worth to note the inhibition may not be caused by the $NH_4^+$ alone, rather by the intermediate products from downstream nitrogen assimilation pathway. When ammonium assimilation reached equilibrium, the hydrolysis of urea decreased, and then the stabilized ammonium extracellular pool was observed for ADLU and ADTU (Figure 5.10).

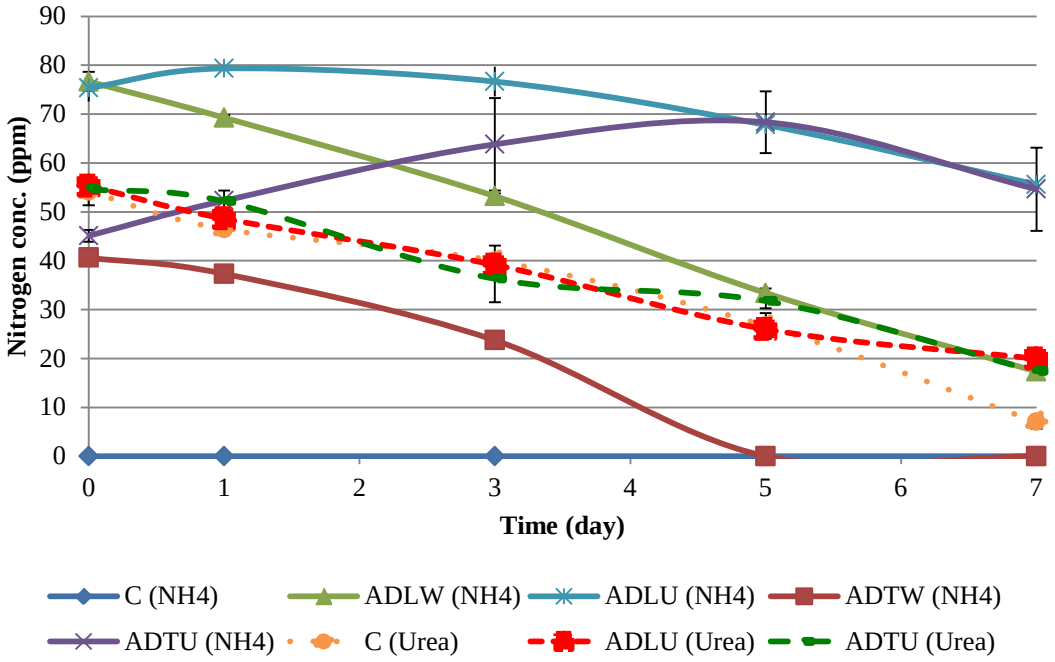


**Figure 5.10 Ammonium (solid lines) and urea (dotted lines) consumption in the media with addition of liquid digestate or total digestate. Error bars represent standard errors (n=3).**

Potassium is another essential element to support algae growth. But its consumption curve was not exactly the same for all samples (Figure 5.11). Apparently the control and four samples started with different amounts of potassium in the media. During the first five days of cultivation, there was no significant potassium decrease in any sample. In day 7, samples starting with a low level of potassium (ADLW and ADTW) used all the potassium; the control also showed a decrease in potassium from 32 to 25 ppm; but samples starting with higher concentrations of potassium (ADLU and ADTU) appeared to have no potassium decline.

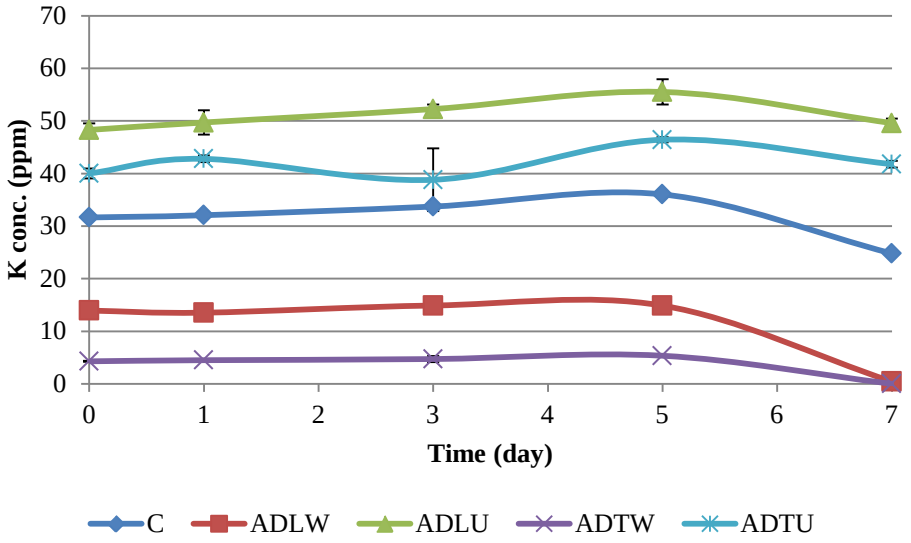


**Figure 5.11 Potassium concentration in the media with addition of liquid digestate or total digestate. Error bars represent standard errors (n=3).**

Phosphate plays an important role in nucleic acids and ATP formation. Samples started with varying levels of phosphate, and during the first five days, no significant reduction was observed except for the control where phosphate concentration decreased approximately 6 ppm (Figure 5.12). In day 7, like potassium, all samples had a pronounced phosphate decrease ranging from 10 to 40 ppm. The phosphate consumption rate increased as the initial concentration increased.

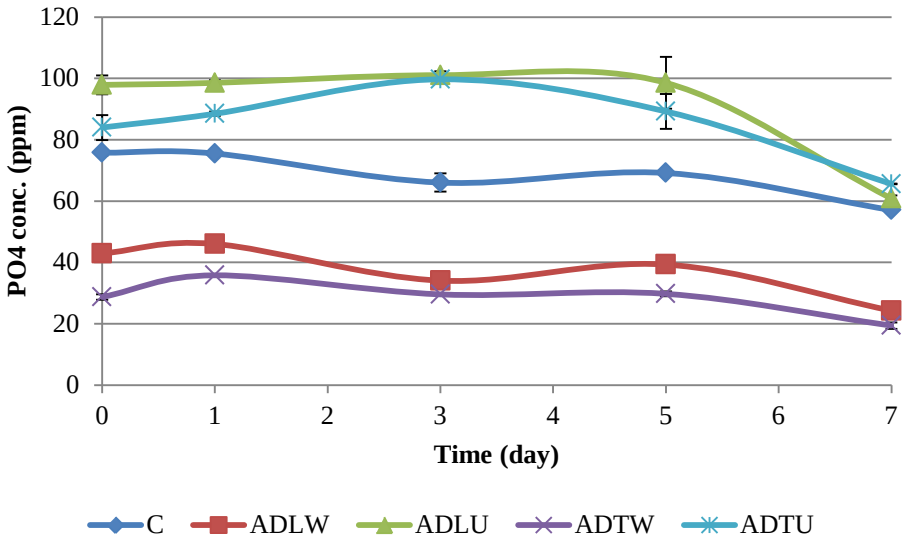


**Figure 5.12 Phosphate concentration in the media with addition of liquid digestate or total digestate. Error bars represent standard errors (n=3).**

Magnesium is the metallic center of chlorophylls, which harvests light energy during photosynthesis. As an autotroph, algae growth is essentially the process of converting light energy to chemical energy, which makes magnesium a critical element for algae cultivation. All samples had various initial magnesium ion concentrations (Figure 5.13). There was not much magnesium consumption during 7 days of cultivation for the control, ADLW and ADTW, and latter two contained the lowest initial magnesium. Significant decrease of magnesium occurred in ADLU and ADTU where both urea and ammonium served as the nitrogen source. With initial concentration of 26 ppm in ADTU, 13 ppm of magnesium was consumed by day 7, meanwhile growth curves (Figure 5.9) showed 0.7 g/L biomass accumulation. Data in Table 5.1 indicated that *Scenedesmus* contained 0.35% of magnesium, which corresponded to 2.7 ppm of magnesium in 0.7 g/L biomass. Clearly magnesium was overdosed in all media, and algal cells were able to pick up more than average when abundant magnesium was provided in the media.

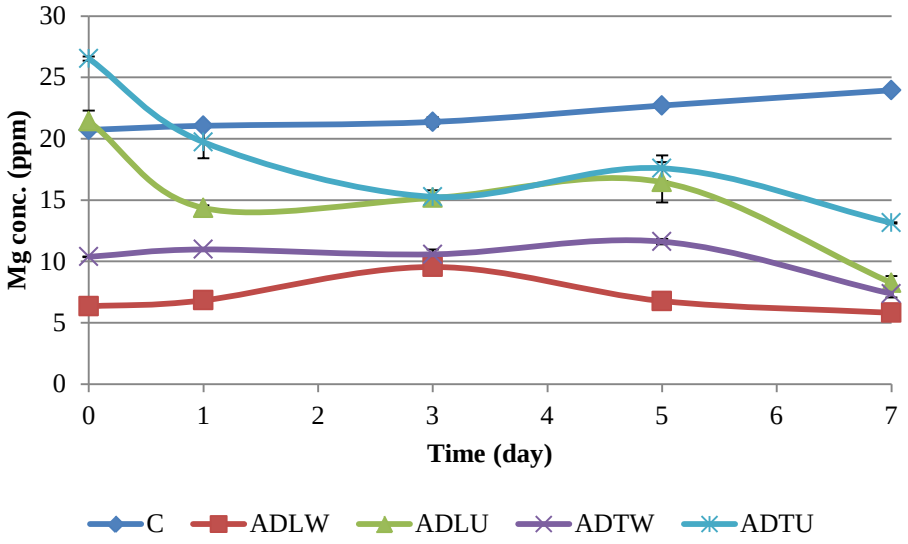


**Figure 5.13 Magnesium concentration in the media with addition of liquid digestate or slurry. Error bars represent standard errors (n=3).**

In the next experiment, total digestate was added to used media as a nutrient source. The prolonged lag phase was observed (Figure 5.14) and after 3 days exponential growth started. The lag phase can be due to the high initial turbidity as well as the low initial biomass inoculation compared to samples fed with total digestate in the previous discussion (0.02 vs. 0.09 g/L). The growth curves of the control and used media were very similar, and the use of used media did not impose any deleterious effects to the growth compared to the control. The control was fed with the mixture of Urea media and total digestate. The initial urea concentration in the control was about 140 ppm, and after 9 days cultivation, 20 ppm urea was left in the system (Figure 5.15).

For samples without addition of total digestate, 140 ppm urea was normally exhausted when the accumulative biomass reached 0.75 g/L (Figure 5.4). But with the addition of digestate containing ammonium, there was leftover urea as the accumulative biomass reached 0.9 g/L at the end. The initial ammonium dosage ranged from 13 to 18 ppm in all flasks. In control flasks, there was an increase of ammonium as urea concentration went down in the first four days. At the end, urea was not exhausted as it was seen in the previous case where urea was the sole nitrogen source (Figure 5.4). The ammonium curve of control flasks fluctuated around 25 ppm during the exponential growth phase. In treatment flasks, ammonium was exhausted on the fourth or fifth day, and then nitrogen became a growth limiting factor. However it seemed the depletion of nitrogen did not slow down or inhibit the growth at all (Figure 5.14). Clearly for a short period of time, algal cells are able to self-sustain on the intracellular nitrogen pool. The presence of extracellular ammonium seemed to inhibit the urea uptake (Figure 5.15), which can be due to the feedback regulation from the intracellular ammonium pool as aforementioned (Healey 1977, Mulholland and Lomas 2008).

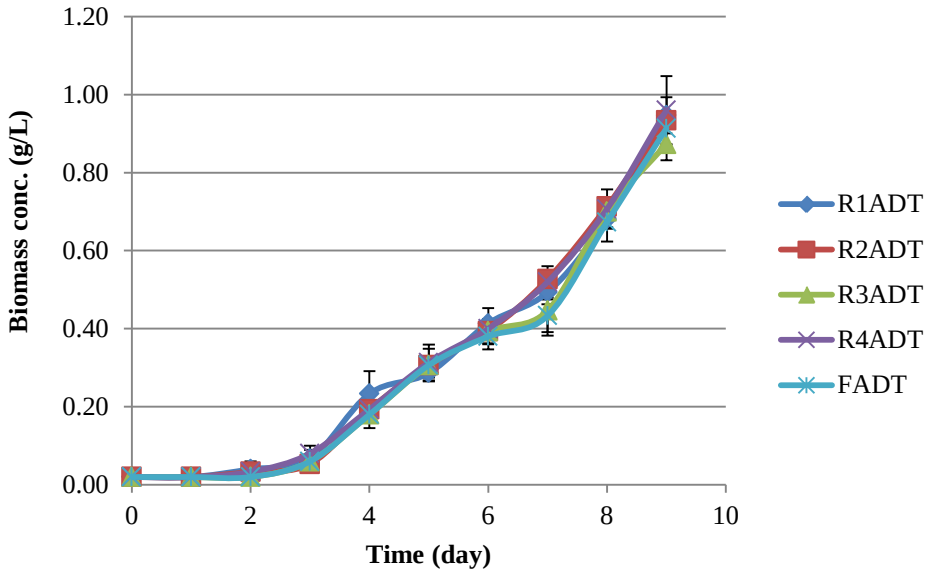


**Figure 5.14 The growth curve of algae in fresh and used media with addition of total digestate. Error bars represent standard errors (n=3).**

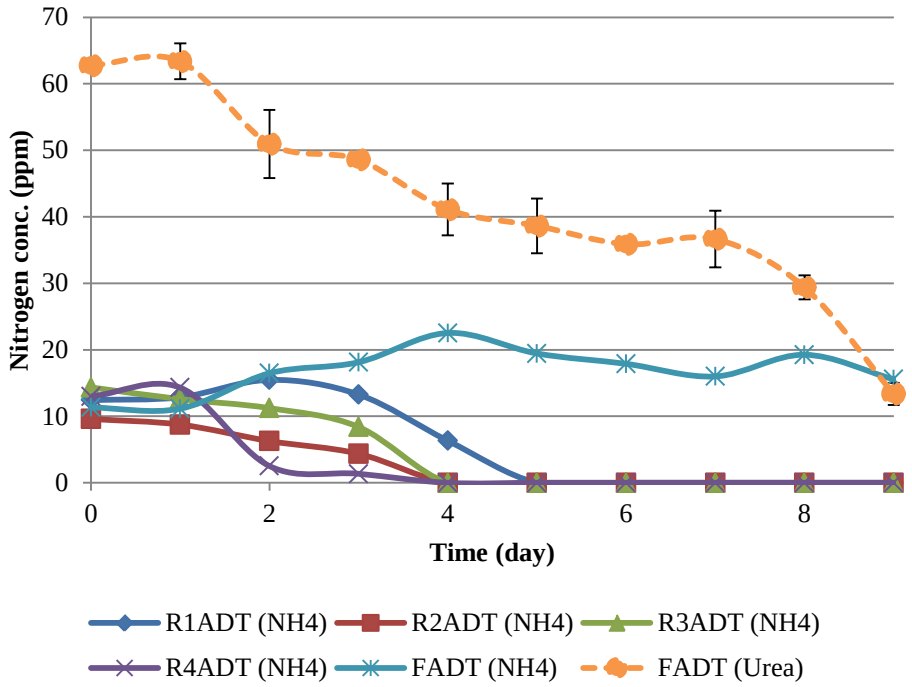


**Figure 5.15 Ammonium (solid line) and urea (dotted line) consumption in used media with addition of total digestate. Error bars represent standard errors (n=3).**

To eliminate the lag phase yet maximally replace urea with ammonium from the digestate, solids were centrifuged out from the digestate, and the liquid fraction was used as nutrient source. The lag phase decreased from 3 days to 1 day (Figure 5.16). But compared to used media with total digestate (R1ADT-R4ADT) (Figure 5.14), the specific growth rate decreased from 0.1 g/L/day to 0.085 g/L/day. So there were benefits of using the total digestate, despite of the longer lag phase. The algae growth rate in liquid digestate-used media was slower than the control within the 7-day exponential phase. The maximum biomass accumulation for both control and treatments were 0.90 and 0.60 g/L. Used media recycled for 1 to 4 times did not show different effects on the algae growth. In control flasks, urea formula was added along with the liquid digestate, but only 16% of urea was consumed (Figure 5.17). Ammonium was exhausted in most flasks at the end, except the R4ADL had 15 ppm remaining. Again ammonium imposed an inhibition on urea assimilation, however urea did not appear to have the similar effect on ammonium uptake. Apparently nitrogen was not the limiting factor on the growth since all flasks had sufficient amount of it. Therefore there should be other factors driving the faster growth in the control (FADL) compared to treatments. Other nutrients (K, P and Mg) consumptions curves were shown in Figure 5.18, Figure 5.19 and Figure 5.20. Apparently

nutrient concentrations were not changed much over 7 days cultivation, and in some cases they were even gradually increased due to the slight vapor evaporation (< 5%).

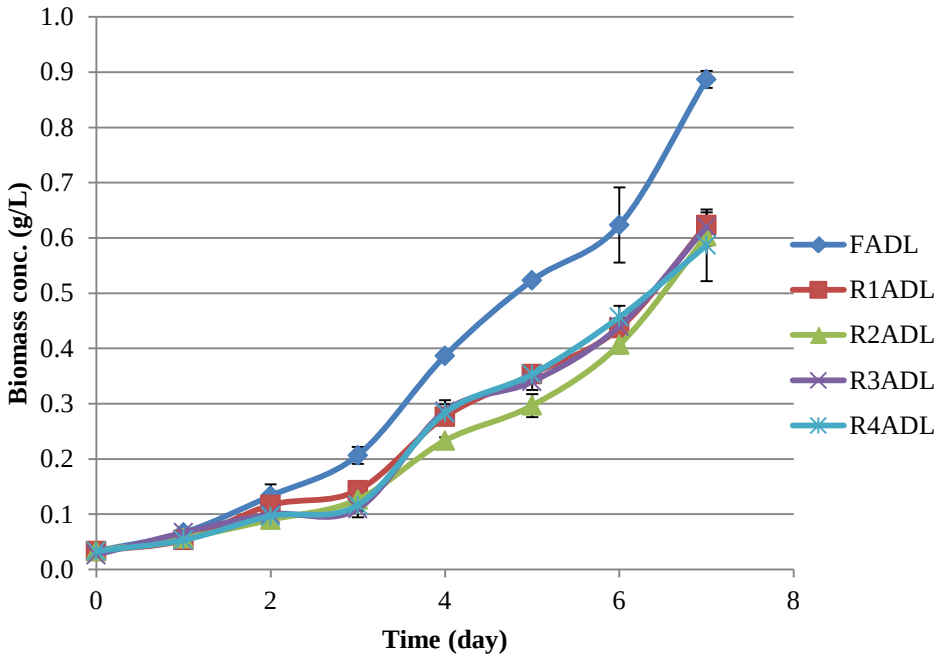


**Figure 5.16 The growth curve of algae in fresh and used media with addition of liquid digestate. Error bars represent standard errors (n=3).**

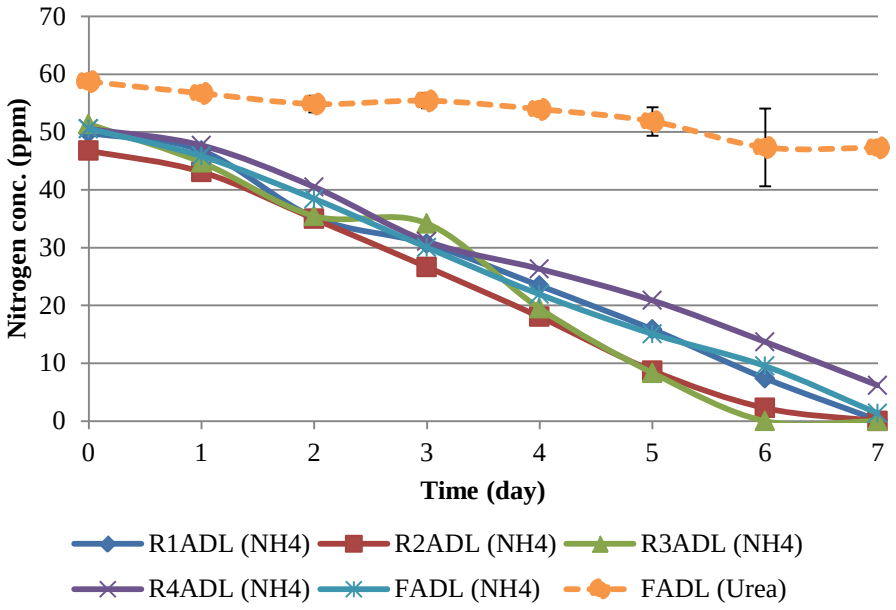


**Figure 5.17 Ammonium (solid line) and urea (dotted line) consumption in used media with addition of liquid digestate. Error bars represent standard errors (n=3).**

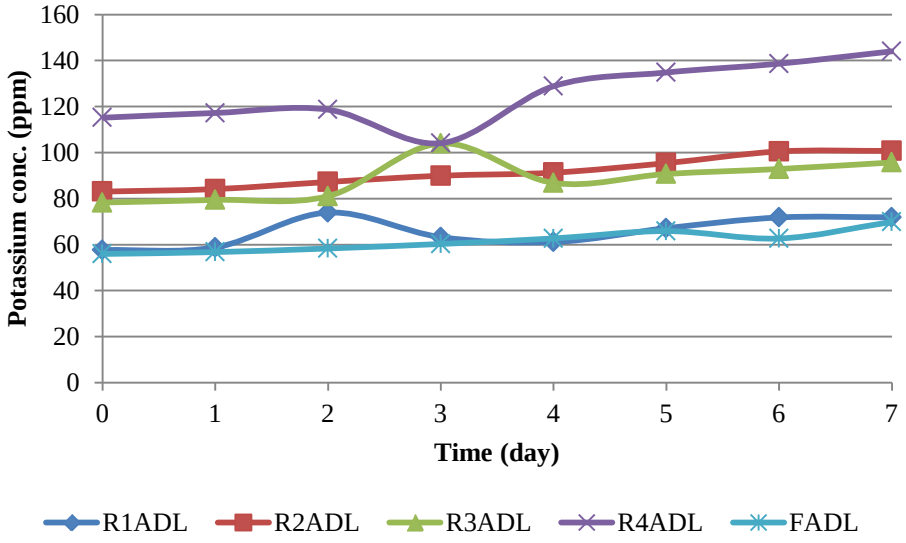


**Figure 5.18 Potassium concentration in used media with addition of liquid digestate. Error bars represent standard errors (n=3).**

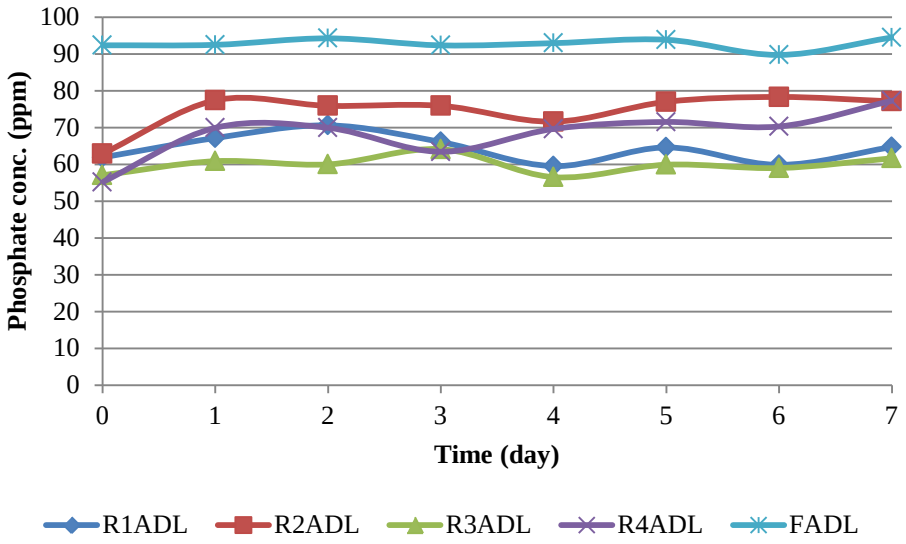


**Figure 5.19 Phosphate concentration in used media with addition of liquid digestate. Error bars represent standard errors (n=3).**

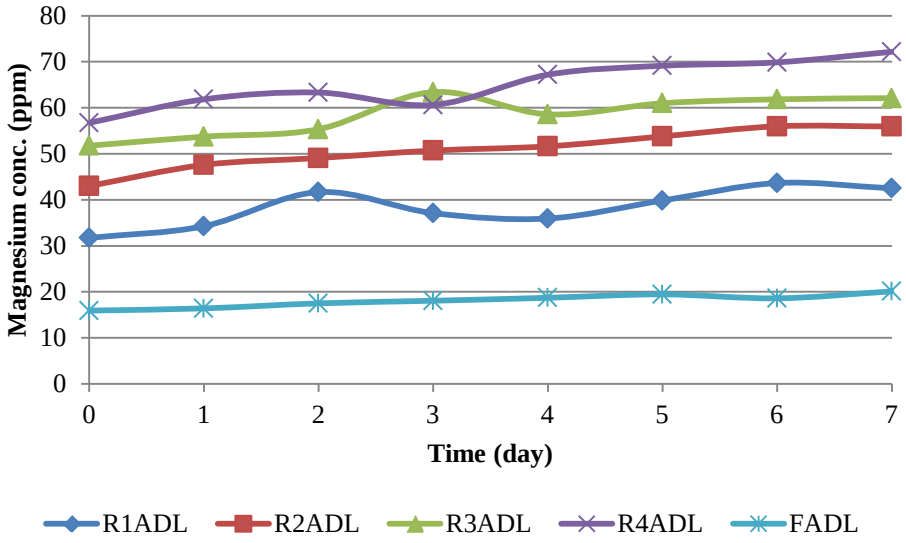


**Figure 5.20 Magnesium concentration in used media with addition of liquid digestate. Error bars represent standard errors (n=3).**

## 5.5. Conclusions

This study provides evidence that used media from algae biomass harvesting and dewatering can be recycled back to the biomass cultivation at least four times without showing any deleterious effect. Algae biomass was anaerobically decomposed, and became the promising nutrient source for algae cultivation. Results specifically showed that:

(1) Nitrogen was the only nutrient being depleted during media recycling, and nitrogen deprivation impaired chlorophyll synthesis.

(2) Replenishment was critical in using used media as growth media.

(3) Total digestate can cause high turbidity, therefore less of it can be applied in order to keep high light penetration; adding liquid digestate does not cause high turbidity, therefore less dilution is required;

(4) Ammonium from anaerobic digestate proved to be an efficient nitrogen source for algae growth; however it showed certain inhibition on urea assimilation.

(5) The consumption of potassium, phosphate and magnesium was not as drastic as nitrogen. This can be simply due to the overdose of all three nutrients. In future experiments, P, K and Mg dosages should be adjusted to a lower level.

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# Chapter 6: Life cycle assessment on a *Scenedesmus* photosynthesis system for $CO_2$ mitigation from coal-fired power plant

## 6.1. Summary

During the development of an algae based bio-$CO_2$ mitigation system, a life cycle assessment was conducted to evaluate the potential environmental impacts of utilizing algae followed by anaerobic digestion for $CO_2$ recycling. The proposed *Scenedesmus* based mitigation system is coupled with a typical coal-fired power plant in an effort to reduce $CO_2$ emissions. The $CO_2$ mitigation system consists of tubular photobioreactors coupled with settling bunkers followed by anaerobic digestion. Results indicated that in order to sequestrate 22.76 ton of $CO_2$ per day ($CO_2$ emitted by producing 1 MW electricity), the culture volume was projected to be 82728 $m^3$. To take advantage of the substantial amount of heat rejected by the power plant, the coolant water circulating in the condensers was used as the heating source for the anaerobic digesters and pretreatment heat exchangers. When the algae biomass was processed by anaerobic digesters, the net $CO_2$ mitigation rate was 9.14 ton per day; when the downstream processing of biomass was not considered, the net $CO_2$ mitigation rate was 11.93 ton per day. The sensitivity analysis showed that the specific growth rate and carbon content of the microalgae have significant effects on the quantity of $CO_2$ mitigated.

***Keywords:*** Greenhouse gas, $CO_2$, emission, mitigation.

## 6.2. Introduction

The growing greenhouse gases emission challenges the society with dramatic increases in energy consumption and demand. Electricity, as the most common form of energy, sustains most human activities from daily life styles to large-scale industrial operations. Electricity can be produced from fossil fuels including coal, petroleum, and natural gas, nuclear power plants, or renewable energy including hydro, wind, solar, geothermal and biomass. Approximately 45% of the electricity in the U.S. comes from coal-fired power plants (EIA 2011). The primary by-products derived from the coal-fired combustion process are flue gases including carbon dioxide ($CO_2$), nitrogen oxides ($NO_x$) and sulfur oxides ($SO_x$). $CO_2$ and $NO_x$ are well-known greenhouse gases, which impose potential threats to the global climate and a potential future regulatory obstacle. $CO_2$ from energy-related emissions accounted for 81.3% of the total U.S. greenhouse gas emissions (GHGE) in 2008 (EIA 2009). Coal-fired power plants emit about 2.1 billion metric tons of $CO_2$ annually, which are 36.5% of the energy-related $CO_2$ emissions (EIA 2009). Thus the environmental impact from coal-fired electricity generation draws significant attention.

$CO_2$ mitigation can be achieved by natural sequestration process or direct injection of $CO_2$ into ocean or underground reservoirs (Yamasaki 2003). Terrestrial and aquatic vegetation has the potential to fix $CO_2$ and convert it to organic forms through photosynthesis. Another major $CO_2$ fixation process in nature is chemical weathering of rocks, soil and sands (Yamasaki 2003). But the reaction rate is extremely slow, which makes it less promising for practical operations. Prior to the direct injection into reservoirs, $CO_2$ has to be captured during pre-combustion, post-combustion or oxygen-rich combustion. Then the liquefied or pressurized $CO_2$ is transported to storage sites via pipelines or trucks. To capture $CO_2$ a number of techniques have been investigated including absorption, adsorption, and membrane separation. The proposed storage sites include ocean and underground locations (unminable coal seams and depleted oil or gas fields) (Yamasaki 2003, EIA 2009). Introducing large amounts of $CO_2$ to the ocean can potentially alter the pH of the seawater and disrupt the marine ecosystem. Underground storage sites are less sensitive to the environmental changes that $CO_2$ injection could cause and in some cases pumping $CO_2$ could be beneficial (enhanced oil recovery that has been employed in oil fields in Texas and New Mexico) (EIA 2009). However the viability of underground sequestration is highly location-dependent. If there are no ideal storage sites near the power plant, a pipeline network has to be constructed for $CO_2$ transportation, which significantly increases the cost. Putting this aside, massive $CO_2$ leakage can be a risk factor for underground injection (Yamasaki 2003).

Microalgae $CO_2$ mitigation systems have been proposed to alleviate GHGE. Lab scale experiments have shown that the microalgae $CO_2$ mitigation system is feasible in terms of utilizing flue gases and generating microalgal biomass. However, the documentation of the net energy consumption and impacts on GHGE are still needed. To better understand the impacts of $CO_2$ mitigation using microalgae, it is necessary to execute a life cycle assessment (LCA) on the overall system. Life cycle assessments have been already employed to investigate and evaluate the environmental impacts of the production of algae based biofuels (Collet et al. 2011 and Hulatt et al. 2011).

## 6.3. Goal and Scope

The goal of this study was to investigate the energy consumption and net greenhouse gas emissions from a microalgae based $CO_2$ mitigation system coupled with a coal-fired power plant. The system boundary (Figure 6.1) included the coal-fired power plant, algae photobioreactors (PBRs), anaerobic digesters (ADs), digestate recovery and nutrient recycling. Algae biomass was considered as a co-product in the LCA analysis. Through anaerobic digestion, algae biomass was

decomposed to biogas (a mixture of $CH_4$ and $CO_2$) and mineralized nutrients, and latter were utilized as recycled nutrients to sustain the algae growth. The primary functional unit was one day of operation, since the power plant, PBR, and anaerobic digesters operated on a daily basis.

The coal-fired power plant was assumed to be located in Kentucky with a 1 MW capacity, and ran 24 hours per day for 350 days per year. Photobioreactors (PBRs) and anaerobic digesters (ADs) were constructed in the vicinity of the power plant to minimize the energy consumption due to flue gas transportation. Sunlight was the only light source. The biogas upgrading which involved various techniques was not included in this study. The system ran 350 days per year, and 24 hours per day continuously. The preliminary tests indicated that PBR was operated at steady state with a biomass concentration of 1 g/L, and an average growth rate of 0.15 g/L/day (Chapter 3).

## 6.4. Inventory

### 6.4.1. Coal-fired power plant

Based on EIA 2010 report, on average a Kentucky coal-fired power plant emitted 2.3 kg sulfur dioxide, 0.86 kg nitrogen oxide and 948.3 kg carbon dioxide for each MWh electricity produced. This study focused on $CO_2$ emission since microalgae can effectively assimilate $CO_2$. Plant efficiency varied according to the design of the electric power generation system. The efficiency of current pulverized coal systems using steam boilers had approached the maximum efficiency of 36% (Beer 2007). Given the capacity and efficiency of the targeted power plant, the condensers were projected to receive nearly 42.7 MWh/day rejected heat generated from a typical Rankine cycle. This significant amount of rejected heat can be reused for algal thermal pretreatment and maintenance of the optimal anaerobic digester temperature. Assuming the power plant operated 350 days per year and 24 hours per day, the net electricity generation per year was 8400 MWh, which generated 22.76 metric ton of $CO_2$ per day. The emissions and impacts of coal mining and transportation were neglected in this study since they will be equivalent for a plant with or without bio-$CO_2$ mitigation system.

### 6.4.2. Photobioreactor (PBR)

Under the current project, a series of closed tubular photobioreactors (PBRs) were used for microalgae cultivation. Tubular reactors were constructed with polyethylene terephthalate (PET) tubes (Table 6.1 and Table 6.2). $CO_2$ emission of PBRs was primarily from the material acquisition, liquid media pumping and algae harvesting. To simplify the scenario, we neglected flue gas conditioning that adjusted the temperature and composition of the inlet gas before being injected to PBRs. PET tubes were assumed to last for 5 years, and $CO_2$ emission from PET

acquisition was evenly distributed through its life span. PBR sizing was computed based on the capacity of coal-fired power plant, the specific growth rate and the elemental composition of *Scenedesmus* (Table 6.3). The goal of current project was to capture 22.72 ton $CO_2$/day from the power plant that would require a PBR volume of approximately 82728 $m^3$. The average growth rate of *Scenedesmus* was about 0.15 g/L/day at 3% of $CO_2$ with 16 h light and 8 h dark cycle (Chapter 3). Previous studies had shown that semi-continuous cultivation can keep microalgae growth in the exponential phase that will maximize biomass production (Krichnavaruk et al. 2005). In this case, to maintain 1 g/L concentration, algae biomass was harvested daily and stored in the settling bunkers where algae were precipitated through a two-stage settling process. *Scenedesmus* cells had good sedimentation properties with a sedimentation velocity of 3.575 m/d (Collet et al. 2011). Taking advantage of this, harvested algae stock was initially settled by gravity in the concrete bunker (Table 6.2) for one day. Once the algae concentration increased to about 7 g/L, the algae-free supernatant was pumped back to the PBR to compensate the water lost during harvesting, and then flocculants (cation polyacrylamide) were added at the dosage of 10 ppm to the rest of the algae stock. The second stage settling lasted for one day, and the final concentration of the slurry was about 40 g/L, which was ready for anaerobic digestion. Unlike centrifugation, solid-liquid separation by gravity and flocculation was relatively slow but required little or no energy input.

**Table 6.1 Inventory of raw material used in the $CO_2$ bio-mitigation system and $CO_2$ emission factor of raw material acquisition.**

| Raw material | Value (ton/day or ton/life time) | Emission factor (kg $CO_2$/ton product) | Life time | Reference or Description |
|---|---|---|---|---|
| Tap water | 1653 | 0.5 | - | DEQ (Oregon) 2009 |
| Waste water | 1463 | 0.23 | - | DEQ (Oregon) 2009 |
| Urea | 1.20 | 1000 | - | IFA 2007. If nutrients from anaerobic digestate is reused, only 1.83 ton of urea is required daily. |
| TS Phosphate | 0.75 | 52 | - | IFA 2007. Or 1.17 ton TS phosphate is required. |
| Potash | 0.11 | 330 | - | IFA 2007. Or 0.17 ton Potash is required. |
| PET | 900 | 1270 | 5 years | EPA-WARM #12, recycling process is included. |
| PVC | 4708 | 2230 | 20 years | EPA-WARM #12, no recycling, but landfill emission is included. |
| Steel | 1090 | 1060 | 20 years | Global Average, IPCC,2006 |
| Mineral wool | 19 | 260 | 20 years | EPA-AP-42, Cupola |
| Concrete | 2062 $m^3$ | 0.273 ton $CO_2/m^3$ concrete | 20 years | Flower and Sanjayan 2007. Standard strength (32 MPa), contains 25% fly ash. |

**Table 6.2 The sizing of photo-bioreactor, settling bunker, and anaerobic digester.**

| Components | Value | Unit |
|---|---|---|
| Photo-bioreactor (Tube) | | |
| Working volume | 82728 | $m^3$ |
| Diameter of photo-bioreactor tube (PET) | 0.127 | m |
| Length of photo-bioreactor tube (PET) | 2.44 | m |
| Thickness of photo-bioreactor tube (PET) | 0.00027 | m |
| Number of PET tubes | 2.68 | $10^6$ |
| | | |
| Settling bunker (Cylinder with a hopper bottom) | | |
| Working volume | 94.25 | $m^3$ |
| Height of cylinder | 4 | m |
| Height of hopper | 2.4 | m |
| Diameter | 5 | m |
| Thickness | 0.1 | m |
| Number of bunkers | 229 | $10^0$ |
| | | |
| Anaerobic digester (Cylinder) | | |
| Working volume | 3200 | $m^3$ |
| Height | 15 | m |
| Diameter | 16.5 | m |
| Number of digesters | 3 | $10^0$ |

**Table 6.3 Elemental composition of *Scenedesmus* used in this study (by weight)[a].**

| Element | % |
|---|---|
| Carbon (C) | 50.02 |
| Oxygen (O) | 25.98 |
| Hydrogen (H) | 7.43 |
| Nitrogen (N) | 7.80 |
| Phosphorus (P) | 2.20 |
| Potassium (K) | 0.82 |
| Magnesium (Mg) | 0.39 |
| Sulfur (S) | 0.69 |
| Iron (Fe) | 0.43 |
| Calcium (Ca) | 1.3 |
| Manganese (Mn) | 0.0023 |
| Copper (Cu) | 0.0021 |
| Zinc (Zn) | 0.0053 |

a. The algae sample was centrifuged and washed with DI water, and oven dried at 60 °C prior to the analysis. The elemental composition was determined by ICP-OES.

The algae culture needs to be well mixed to assure that nutrients, $CO_2$ and light were evenly distributed, so electrically powered pumps (70% efficiency) were used to maintain a flow rate of 0.0015 $m^3/s$ through each PET tube that ensure turbulent conditions. The pumps were also employed to transfer liquid culture from PBR to settling bunkers, and from settling bunkers to anaerobic digesters. To minimize the energy consumption, the pump lines for liquid transfer were designed to be short and have limited elevation. The pump lines used for liquid transfer were made of polyvinyl chloride (PVC) assumed to last for 20 years.

The algal medium comprised 0.138 g/L of urea (46% N), 0.14 g/L of triple super (TS) phosphate (20% P), 0.026 g/L of iron chelate (10% Fe), and 0.068 g/L of potash (50% K) (Chapter 3). The $CO_2$ emission rates from fertilizer manufacturing and transportation were included in this analysis (Table 6.1).

### 6.4.3. Anaerobic Digestion (AD)

Anaerobic digestion was operated in a continuous feeding and intermittently stirred tank reactor (CSTR) with a hydraulic retention time of 15 days and a loading rate of 2.5 g VS algae/L/day. The operation temperature was 38 °C and intermittent stirring was set to be 2.5

hour/day. Due to the resistance of algae cell walls to biodegradation, thermal pretreatment can be employed to improve the yield of biogas and mineralized nutrients (Chen 1987). In this study, thermal pretreatment was assumed at a temperature of 50 °C for 60 minutes. The energy source for thermal pretreatment and additional heat for the anaerobic digesters was the waste steam from the Rankine cycle or the coolant water from condensers. Therefore no extra energy was required for these two operations. To model the empirical equation of the bio-chemical reactions taking place in the digester, an empirical formula for *Scenedesmus* was established based on its elemental composition (**Table 6.3**). Assuming the major metabolic products were $CO_2$, $CH_4$ and $NH_3$, the empirical equation of the anaerobic digestion of *Scenedesmus* was formed as (Khanal 2009):

$$CH_{1.78}O_{0.39}N_{0.13} + 0.4575H_2O \rightarrow 0.5763CH_4 + 0.4238CO_2 + 0.13NH_3 \qquad (6.1)$$

If each gram of *Scenedesmus* was completely digested by the anaerobic bacteria, the yields of $CO_2$ and $CH_4$ were 0.46 or 0.63 L. Preliminary data indicated the algae degradation rate was 45% (Chapter 4). The nutrient profile of the digestate was listed in Table 6.4. The digestate had a solid and liquid fraction. The soluble nutrients in the liquid fraction can be recycled to the PBR, which significantly reduced the usage of commercial fertilizers. The dewatered solid digestate was also rich in mineralized nutrients, which makes it a promising alternative for agricultural fertilizers (Moller and Muller 2012). In this study, the impacts of this allocation on the $CO_2$ emission were considered and included.

**Table 6.4 The yield and nutrient profile of gas, liquid and solid from the anaerobic digester (degradation rate = 45%)[a].**

| Components | Value | Unit |
|---|---|---|
| Gas Phase | | |
| $CO_2$ | 0.21 | L/g Dry Algae |
| $CH_4$ | 0.28 | L/g Dry Algae |
| Liquid Phase | 50% | V liquid/V slurry |
| Nitrogen (N) | 0.033 | kg/kg Dry Algae |
| Phosphorous (P) | 0.0099 | kg/kg Dry Algae |
| Potassium (K) | 0.0037 | kg/kg Dry Algae |
| Solid Phase | 93% | moisture content |
| Nitrogen (N) | 6.1* | $kg/m^3$ wet digestate |
| Phosphorous (P) | 1.5* | $kg/m^3$ wet digestate |
| Potassium (K) | 0.27* | $kg/m^3$ wet digestate |

a. Data was calculated based on the assumption that degradation rate is 45%. The wet digestate contains 93% of water.

Four anaerobic digesters were built with a height of 15 m and a diameter of 16.5 m (Table 6.2). The wall of the anaerobic digester contained three layers: a stainless steel inner layer (50 mm), a mineral wool insulation layer (100 mm), and galvanized sheet steel shell (1 mm). The reactor temperature was maintained at 38 ˚C using the effluent coolant from the condenser and agitation (0.05 $kW/m^3$) was 2.5 hours per day.

## 6.5. Results and Discussion

### 6.5.1. Mass flow and balance

The mass flow within the system was displayed in Figure 6.1. The total number of the PBR tubes was projected to be approximately 2.68 million, which required nearly 63.7 acres of land (10.38 tubes/$m^2$). For a better control and easier construction, 2.68 million tubes were divided into a number of subdivisions. This can minimize the pressure drop within PBR. In each subdivision, tubes were arranged in rows and columns (Figure 6.2). Algae media and $CO_2$ traveled through several rows of tubes in a parallel flow. Within the same row of tubes, the liquid flowed in the serpentine pattern. By doing so, the elevation was nearly zero. To effectively sustain algae growth and ensure a stable $CO_2$ assimilation rate, fertilizers had to be added to supply nitrogen, phosphorous and potassium. Based on the specific growth rate (0.15 g/L/day) and the elemental composition of *Scenedesmus* (Table 6.3), the quantities of N, P and K required

for the daily growth of algae should be 0.98, 0.28 and 0.10 ton per day, which were equivalent to 2.10, 1.37 and 0.20 ton/day of urea, TS phosphate and potash. Algal biomass was subjected to anaerobic digestion later on, and one of the by-products was the liquid digestate, which was rich in mineralized N, P and K (Moller and Muller 2012). The daily yield of N, P and K was 0.41, 0.12 and 0.04 ton per day, which were equivalent to 0.90, 0.61 and 0.09 ton of urea, TS phosphate and potash. It was apparent that by recycling the nutrients from the liquid digestate, the usage of commercial fertilizers was significantly reduced, which improved the sustainability of the algae PBR cultivation.

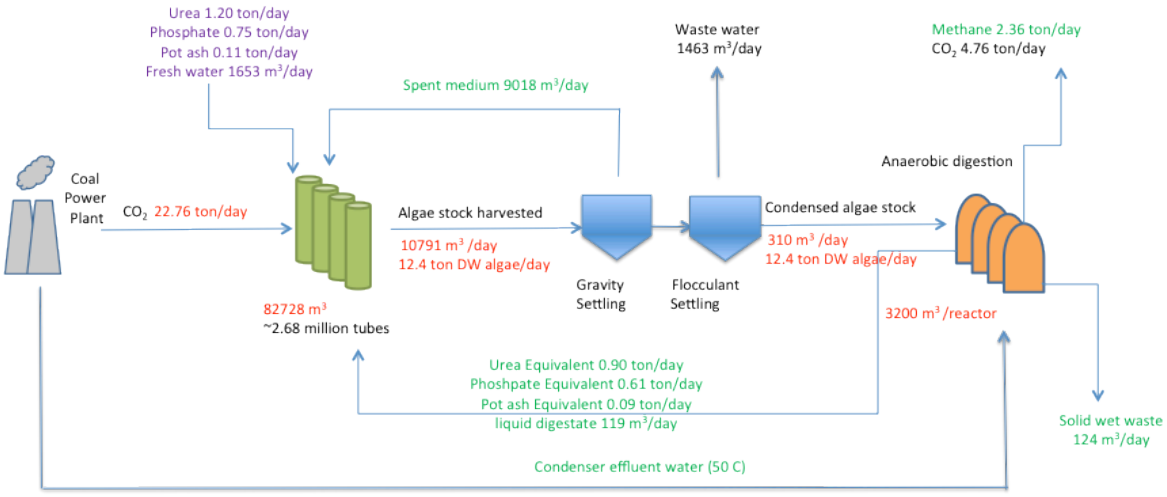


**Figure 6.1 The overview of the microalgae $CO_2$ mitigation system.**

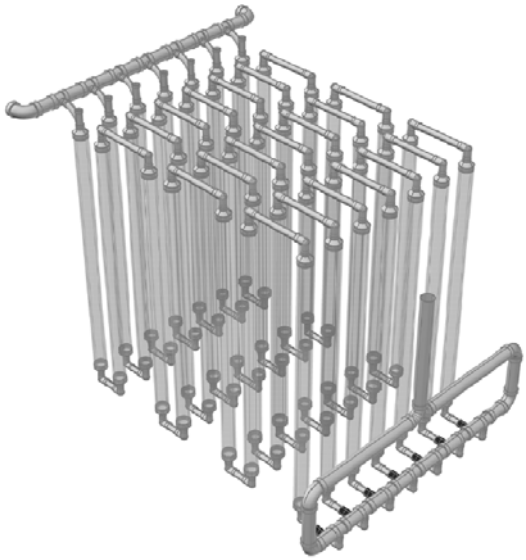

**Figure 6.2 The subdivision of the photobioreactor (PBR).**

To maintain the algae concentration in the PBR at 1 g/L, 10791 $m^3$ of algae stock had to be harvested daily. As aforementioned, settling was performed prior to any further processing of algae biomass, and in this system, settling was achieved by a two-stage process: gravity settling followed by flocculation settling. During gravity settling, algae stock was pumped into 229 settling bunkers, and after one day's settling, the algae stock was condensed to 7 g/L, whereas 9018 $m^3$ supernatant (used media) was available to add back to the PBR. The thicker algae stock

was exposed to the flocculants, and the final concentration reached 40 g/L after the second day. The drawback of using flocculants was the potential contamination of the used media, which caused the generation of a substantial amount of wastewater (1463 $m^3$/day) after the secondary settling.

The algae slurry (310 $m^3$/day) was the feedstock for the continuous anaerobic digesters. Assuming 45% of the algae substrate decomposed, the yields of methane and carbon dioxide from the digesters were projected as 2.36 and 4.76 ton/day. The volume of effluent was 80% of the influent (310 $m^3$/day), therefore 248 $m^3$ digestate was withdrawn from the digesters daily. The volume fraction of the wet solid digestate was 0.5, which meant 124 $m^3$ of the wet digestate was yielded along with the same volume of liquid digestate.

### 6.5.2. $CO_2$ emission

The overall profile of $CO_2$ emission from the algae based $CO_2$ mitigation system was depicted in Figure 6.3. The major emitters of this system were PBR and AD. $CO_2$ emission of PBR was attributed to the raw material acquisition, water, fertilizers, $N_2O$ emission and pumps. The photobioreactors were made of polyethylene terephthalate (PET) tubes and polyvinyl chloride (PVC) fittings. The WARM model (EPA 2012) provided the $CO_2$ emission factors of raw material acquisition for both PET (2.49 $MTCO_2E$/ton) and PVC (2.18 $MTCO_2E$/ton), which included the $CO_2$ emission from manufacturing process and transportation. Recycling or landfill was the waste management of PET or PVC respectively, and taking this into consideration, the adjusted $CO_2$ emission factors for PET and PVC were 1.27 and 2.23 $MTCO_2E$/ton. PET's life span was assumed to be 5 years, whereas PVC's life span was assumed to be 20 years. When distributing the $CO_2$ emission of raw material acquisition throughout the life span, the carbon footprint for PET tubes and PVC fittings was 0.63 and 1.44 $MTCO_2E$/day. PVC lasted longer than PET, but it had no recycling potential, which imposed a larger carbon footprint. Fertilizers and water were consumable inputs in this system, so the $CO_2$ emission brought by them were inevitable. The production of fertilizers required significant amounts of energy and had a significant carbon footprint. To fulfill algae daily nutrient requirement, urea, TS phosphate and potash had to be added, which represented 2.64 $MTCO_2E$/day $CO_2$ emission; however if nutrients from digestate replaced substantial amount of commercial fertilizers, the $CO_2$ emission will be offset (Figure 6.3). Aquatic microalgae cultivation was often a $N_2O$ emission source, and low culture oxygen level can cause the increase of $N_2O$ emission (Fagerstone et al. 2011). The closed PBR was supplied with continuous moist flue gas to provide $CO_2$, and limited air sparging to minimize the water evaporation. During the light period (12 hours/day) when $O_2$ was sufficiently

generated by photosynthesis, the $N_2O$ emission rate was estimated as 0.002% of the nitrogen fertilizer (in this case it was urea) added in the media; during the dark period (12 hours/day), the aquatic system was anoxic due to the algae respiration, the $N_2O$ emission rate was about 0.39% of the nitrogen added in the media (Fagerstone et al. 2011). Therefore the daily $N_2O$ emission from PBR accounted for 1.23 $MTCO_2E$/day. Since PBR was a closed system with limited air sparging, the primary water loss was from algae harvesting other than water evaporation. Gravity settling essentially condensed the algae biomass and separated the used media out; the latter was recycled back to the PBR to compensate the water loss. This used media recycling strategy reduced water loss by nearly 90%. The $CO_2$ emission from processing fresh water and treating wastewater was 0.83 $MTCO_2E$/day. The most influential $CO_2$ emitter within PBR was the hydraulic pump. Even though the serpentine design minimized the elevation head to zero, energy loss due to pressure drop (friction, expansion and contraction) in the PET tubes and PVC fittings was pronounced. It turned out to maintain the flow rate inside the PET tubes at 0.0015 $m^3/s$, pumps with 70% efficiency consumed 4.21 MWh/day of energy, which corresponds to 3.99 $MTCO_2E$/day of $CO_2$ emission.

Anaerobic digestion decomposed algae biomass and produced methane and mineralized nutrients. Along with the production of methane, significant quantities of $CO_2$ were released from this operation as well (Figure 6.3). More accurately, the anaerobic respiratory $CO_2$ accounted for the majority of the carbon footprint from the anaerobic digesters. Calculated from the empirical equation of the algae anaerobic decomposition, 4.76 tons of $CO_2$ were generated along with the production of 2.36 tons of $CH_4$ daily. $CO_2$ and $CH_4$ were vented as a gaseous mixture and upgrading the biogas to remove $CO_2$ was frequently required. However biogas upgrading was beyond the scope of this study. The anaerobic digesters were made of steel and insulated by mineral wool, which emitted 0.16 $MTCO_2E$ of $CO_2$ per day over the 20 year life span. The electricity to power the agitators emitted 0.54 $MTCO_2E$ per day. These two $CO_2$ emitters were directly related to the size of the digesters: smaller digesters required less building material per ton of methane produced and less energy consumption, and then lower $CO_2$ emission. The minimum volume of AD digesters was determined by the ratio of organic loading rate (OLR) to the substrate (S). At steady state, algae biomass production rate was constant, which meant the mass flux of substrate (S) into the AD was fixed. Therefore higher organic loading rates would result in smaller digesters. But over loaded anaerobic digester can be inactivated and had poor biogas production rates (Chen 1987). To enhance the digestibility of *Scenedesmus*, thermal pretreatment was conducted prior to the digestion. To maintain a robust reactor and optimal digestion rates, temperatures should be held around 38 °C. Pretreatment and maintenance of the

anaerobic digester temperature required a fairly large amount of heat which was assumed to be obtained from the waste steam or coolant water from the power plant. These recycled heat loads yield zero $CO_2$ emissions.

The microalgae culture consumed 22.76 tons of $CO_2$ per day, and taking all operations' carbon footprint into consideration, at the end the net $CO_2$ mitigation was 9.14 tons per day. To understand the efficiency of the system based on every MWh produced by the coal-fired power plant, the $CO_2$ emission can be expressed as kg $CO_2$/MWh produced (Table 6.5). For every MWh of electricity produced, PBR fixed 948.3 kg of the emitted $CO_2$ into algal biomass by photosynthesis. The hydraulic pumps to create a turbulent flow inside the photo bioreactor tubes released 166.18 kg of $CO_2$ back to the atmosphere when every 948.3 kg of $CO_2$ was fixed by the PBR. Another process emitting substantial amount of $CO_2$ was anaerobic digestion of the biomass. The anaerobic respiratory $CO_2$ was about 198.52 kg/MWh produced. Due to the long life span, the $CO_2$ emission from building materials was less significant. Manufacturing the fertilizers also had carbon footprint, however the allocation of AD digestate to fertilizers for algae cultivation or agricultural application offset the $CO_2$ emission from commercial fertilizer. Overall the algal mitigation system had the net $CO_2$ emission of -380.89 kg/MWh produced, and for PBR alone the net $CO_2$ emission was -497.13 kg/MWh produced.

If the algae biomass was not anaerobically digested, the net $CO_2$ mitigation rate solely from PBR was about 11.93 $MTCO_2E$/day, which was about 51% of the $CO_2$ sequestrated by the algae photosynthesis. Anaerobic digestion essentially broke down the organic carbon in the algae biomass into methane and $CO_2$. As a matter of fact, any further downstream processing and utilization of the algal biomass as an energy feedstock would eventually convert the organic carbon back to $CO_2$. Compared with AD digestion, algal biofuel production heavily involved dewatering and oil extraction, which made it an energy intensive process (Grima et al. 2003). Anaerobic digestion not only generated $CH_4$, but also converted algae into mineralized nutrients. Specifically for this system, the liquid digestate was considered a co-product used as a nutrient source for microalgae cultivation. Therefore AD was an ideal downstream processing, even though it emitted a large quantity of $CO_2$ and decreased the overall mitigation efficiency of the system.

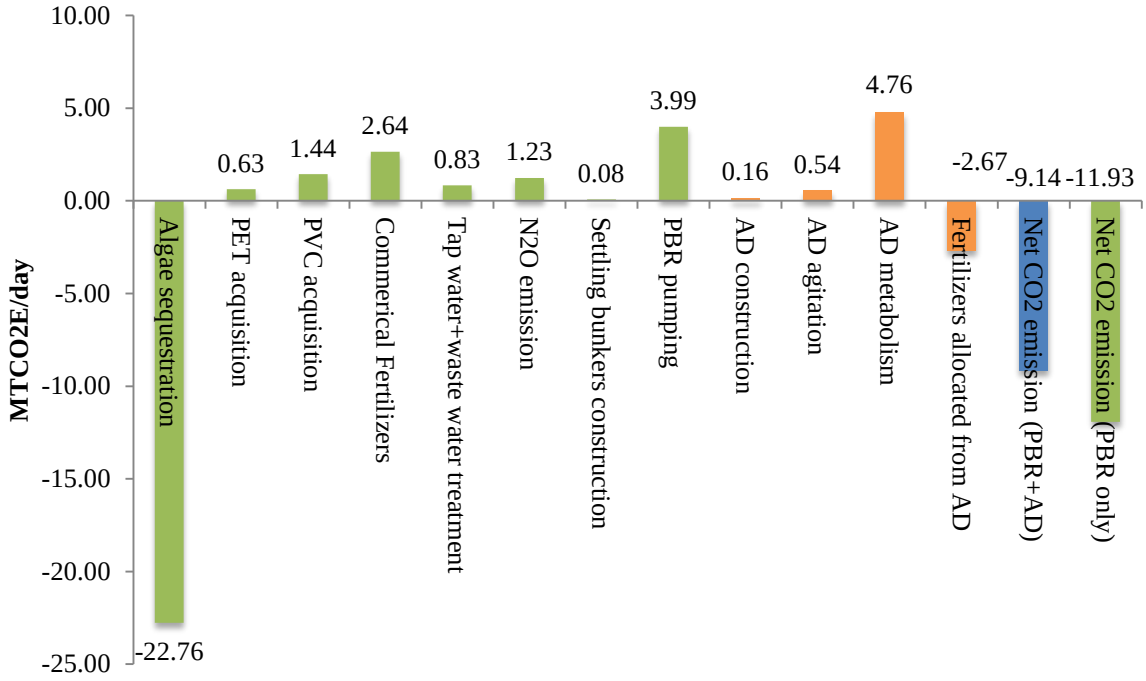


**Figure 6.3 The $CO_2$ emission of the microalgae $CO_2$ mitigation system. PBR is the photobioreactor, and AD is the anaerobic digester. The amount of $CO_2$ sequestrated by algae photosynthesis is the assumption. Green columns represent all emissions from the PBR, and orange columns represent all emissions from AD.**

**Table 6.5 $CO_2$ emission of the microalgal $CO_2$ bio-mitigation system (kg $CO_2$ per MWh electricity produced by the coal-fired power plant).**

| | |
|---|---|
| $CO_2$ mitigated by algae photosynthesis | -948.30 |
| PET acquisition | 26.07 |
| PVC acquisition | 59.85 |
| $N_2O$ emission from PBR | 51.21 |
| Commercial fertilizers | 110.19 |
| PBR pumping | 166.18 |
| Tap water+waste water treatment | 34.46 |
| Settling bunkers construction | 3.21 |
| AD construction | 6.62 |
| AD agitation | 22.50 |
| AD metabolism | 198.52 |
| Fertilizers allocated from AD | -111.40 |
| Net $CO_2$ mitigation (PBR+AD) | -380.89 |
| Net $CO_2$ mitigation (PBR) | -497.13 |

### 6.5.3. Energy Inventory

The operation of PBR and AD inevitably consumed electrical energy, which had an associated $CO_2$ emission. However to fulfill the goal of mitigating $CO_2$, any process required some energy input (Yamasaki 2003). The photobioreactors faced the same challenge (Figure 6.4). The circulation pumps consumed 4.21 MWh/day of electricity. Algae pretreatment and AD heating required 11.11 MWh/day of energy. Assuming the efficiency of the power plant was 36%, the net energy output (electricity) from the plant was 24 MWh/day, and the rejected heat was about 42.67 MWh/day. Channeling 26% of the rejected heat for pretreatment and temperature maintenance for the anaerobic digester can meet the energy demand, and meanwhile reduce the carbon footprint of the operation. Methane was the primary product from anaerobic digestion. The daily production of methane was about 3526.94 $m^3$, which contained 38.50 MWh energy (Figure 6.4), assuming the HHV of methane was 10.9 $kWh/m^3$ (Klass 1998). The total energy input and output were 15.88 and 38.50 MWh, and the energy return was 2.42.

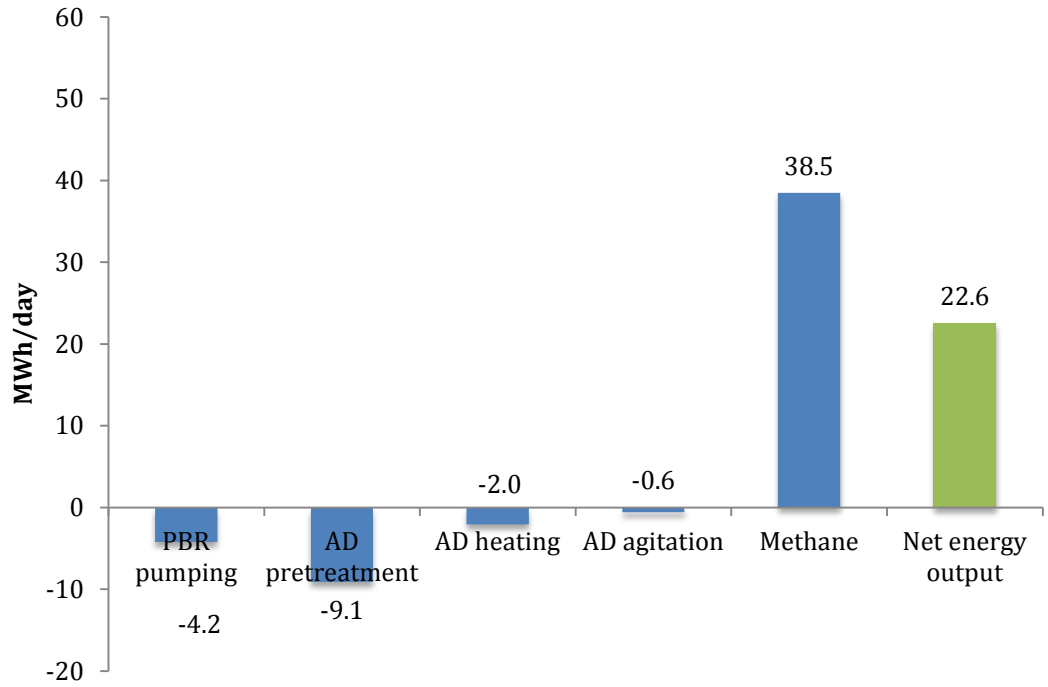


**Figure 6.4 The energy input and output of the microalgae $CO_2$ mitigation system.**

### 6.5.4. Sensitivity Study

$CO_2$ mitigation rate was directly linked to the growth rate of *Scenedesmus*, and the latter determined the size of the PBR. The highest *Scenedesmus* growth rate reported was 0.292 g/L/day (Ho et al. 2010), and as for the sensitivity study, four growth rates were chosen: 0.10, 0.15, 0.20 and 0.30 g/L/day (Table 6.6). When the $CO_2$ mitigation rate was held constant at 22.76 metric

ton/day, increasing the algal growth rate from 0.10 to 0.30 g/L/day can reduce the number of PET tubes used for the PBR from about 4 to 1.3 million. In addition, the power consumed due to pumping decreased from 6.31 to 2.10 MWh/day. However the mass of algae harvested daily from the PBR did not change, which led to the same anaerobic digestion efficiency and methane production. When the specific growth rate increased to 0.30 g/L/day, the net $CO_2$ mitigation of the whole system (PBR and AD) was projected to be 14.56 $MTCO_2E$/day compared to 9.14 $MTCO_2E$ in the base case (0.15 g/L/day). Neglecting downstream processing of the algal biomass, the PBR mitigated 11.93 $MTCO_2E$/day with a specific growth rate of 0.15 g/L/day; and doubling the growth rate would increase the net $CO_2$ mitigation rate to 17.35 $MTCO_2E$/day.

**Table 6.6 The effect of microalgal specific growth rate on the $CO_2$ mitigation rate.**

| Algae Specific growth rate g/L/day | Algae Specific growth Rate $g/m^2$/day | Mitigated $CO_2$ ton/day | PET tube number | PBR-pump power required MWh/day | Algae biomass ton DW/day | Net $CO_2$ mitigation (PBR+AD) $MTCO_2E$/day | Net $CO_2$ mitigation (PBR) $MTCO_2E$/day |
|---|---|---|---|---|---|---|---|
| 0.10 | 32.08 | 22.76 | 4014716 | 6.31 | 12.41 | 3.73 | 6.52 |
| 0.15 | 48.13 | 22.76 | 2676477 | 4.21 | 12.41 | 9.14 | 11.93 |
| 0.20 | 64.17 | 22.76 | 2007358 | 3.15 | 12.41 | 11.85 | 14.64 |
| 0.30 | 96.25 | 22.76 | 1338239 | 2.10 | 12.41 | 14.56 | 17.35 |

The base case was based on experimental results that indicated *Scenedesmus* had a carbon content of 50%. However Mandalam and Palsson (1998) reported that *Chlorella*, another member of Chlorophyta, had carbon contents between 51.4 to 72.6%. Carbon contents for *Scenedesmus* could be higher based on data from its higher carbon relatives. The effects of carbon content on the system efficiency were shown in Table 6.7. When the specific growth rate was fixed at 0.15 g/L/day, carbon rich algae led to an improved system in terms of $CO_2$ mitigation. Since there was more carbon assimilated per gram of algae, less algae biomass was needed to fix the same quantity of $CO_2$. When the carbon content increased from 50% to 72%, the volume of the PBR was reduced by 30%, and the power required for pumping decreased an equal percentage. The net $CO_2$ mitigation of the system (PBR and AD) increased from 9.14 to 13.30 $MTCO_2E$/day, and for PBR only, it increased from 11.93 to 15.24 $MTCO_2E$/day.

**Table 6.7 The effect of carbon content of microalgae on the $CO_2$ mitigation rate.**

| Carbon% in Microalgae | Algae specific growth rate g/L/day | Mitigated $CO_2$ ton/day | PET tube number | Algae biomass ton DW/day | PBR-pump power required MWh/day | Net $CO_2$ mitigation (PBR+AD) $MTCO_2E$/day | Net $CO_2$ mitigation (PBR) $MTCO_2E$/day |
|---|---|---|---|---|---|---|---|
| 50% | 0.15 | 22.76 | 2677548 | 12.41 | 4.21 | 9.14 | 11.93 |
| 60% | 0.15 | 22.76 | 2231290 | 10.35 | 3.50 | 11.41 | 13.74 |
| 65% | 0.15 | 22.76 | 2059652 | 9.55 | 3.24 | 12.28 | 14.43 |
| 72% | 0.15 | 22.76 | 1859408 | 8.62 | 2.92 | 13.30 | 15.24 |

## 6.6. Conclusion and future work

This LCA analysis was based on state-of-the-art engineering. The principle goal was to realize the energy consumption and $CO_2$ emissions from a *Scenedesmus*-based $CO_2$ mitigation system, and evaluate its benefits and drawbacks. Electricity required for pumping in the PBR and $CO_2$ released during anaerobic digestion were the two major emitters within the system. The net $CO_2$ mitigation was 9.14 $MTCO_2E$ per day for the system, or 11.93 $MTCO_2E$ per day for PBR, which complied with the mission of biologically and environmental-friendly sequestrating the $CO_2$ emission from electricity generation. Meanwhile 38.50 MWh/day of methane was generated by anaerobic decomposition of *Scenedesmus*, and this valuable bio-fuel can be used for electricity generation, syngas formation and engine combustion. Nevertheless future work is still required to optimize the energy and $CO_2$ mitigation efficiency of the whole process chain. Anaerobic digestion requires more evaluation to determine ideal operation conditions, which ensures the optimal methane production and minimum energy consumption. System optimization based on $CO_2$ emission and energy consumption is highly anticipated. Biogas upgrading should also be studied and included in LCA.

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# Chapter 7: Conclusions and future work

Microalgae-based $CO_2$ mitigation systems are projected to be a potential solution for reducing the $CO_2$ emission from coal-fired power plants. Cultivated in a closed photobioreactor, microalgae growth is restricted by nutrient concentration, light diffusion, $CO_2$ and $O_2$ transfer rate, contaminations, as well as pH and temperature. Nutrients embedded in the microalgae biomass and the used media were studied to be potentially used in the microalgae cultivation. The project was separated into three main parts: pretreatment and anaerobic digestion of microalgae biomass, nutrient recycling in microalgae cultivation, and life cycle assessment for the overall process. Mild pretreatment at 50 °C can help to improve the digestibility of *Scenedesmus* cells, and the anaerobic digestion can be conducted in a traditional digester. In the low cell density culture (1 g/L), media can be continuously recycled with nutrients replenishment, and growth inhibitors were not observed. Mineralized nutrients (N, K and P) from anaerobic digestate were proved to sufficiently support the algal growth. Overall it was proved to be possible to recycle the nutrients and used media in the microalgae-based $CO_2$ mitigation system, and LCA demonstrated that recycling nutrients and water improve the sustainability of the system.

## 7.1. Thermal-chemical pretreatment of *Scenedesmus*

Biodegradation of *Scenedesmus* is quite a challenge due to the cellulosic cell wall. The degradation of cell wall prior to anaerobic digestion can improve the bioconversion rate. In this project, three factors were considered to determine the pretreatment efficiency: heating temperature (50°C and 90°C), heating duration (10, 30 and 60 min) and NaOH dosage (0, 3, 6 and 12%). Three types of algae biomass harvested by centrifugation, flocculation or flocculation and oven-drying were subjected to various pretreatment conditions. The cell wall degradation rate was determined by fluorescent microscopic images and soluble organic matter (SOM). Three types of algae biomass reacted distinguishingly to the thermal-chemical treatment. The pretreatments increased the SOM contents for all algae biomass, especially, 6% to 15% for fresh algae, 6% to 17% for flocculated algae, and 0% to 11% for oven-dried algae. Oven-dried algae showed certain cell wall destruction before the pretreatment and mild treatment conditions failed to further impair the cell wall. But intense treatment (heating at 90°C with 12% of NaOH) induced more SOM release and further cell wall break down. In terms of most effective pretreatment, for fresh algae, heating at 50°C with 3% NaOH or at 90°C with 12% gave the highest SOM increase; for flocculated algae, heating at 90°C with 12% NaOH caused the most SOM leaching. Duration in general did not show significant effect on cell wall destruction.

## 7.2. Anaerobic digestion of *Scenedesmus*

Bio-methane potential (BMP) test demonstrated that *Scenedesmus* can be used as anaerobic digestion substrate, and the specific gas production rate was approximately 200 mL/gVS for both raw and pretreated algae biomass. But pretreated algae displayed a quicker start-up compared to raw algae. The digester came to the stationary phase after 10 days. A continuous stirring tank reactor (CSTR) was used to study the relation between organic loading rate (OLR) and specific gas production rate. Eight OLRs were compared, and the medium OLR (0.75 kgVS/$m^3$/d) resulted the highest specific gas production rate (529 L/kgVS/d).

## 7.3. Used media recycling

Used media was generated through algal biomass harvesting, and was a vehicle of both unused mineralized nutrients and metabolic wastes. In the semi-continuous cultivating mode, with nutrient replenishment, used media had been recycled four times with no apparent deleterious effects on the algal growth. Nitrogen was depleted fairly quick compared to other nutrients, so without replenishment, used media failed to provide sufficient nitrogen for the healthy growth. It was feasible to repeatedly use used media for the low cell density algae cultivation (0.8 g/L to 1 g/L).

## 7.4. Using anaerobic digestate as nutrient sources

Anaerobic bacteria can use *Scenedesmus* cells as food source and mineralize cell components including carbohydrate, protein, fat and other minor molecules. Data had shown the digestate was rich in ammonium, phosphate and potassium, which are three major nutrients for algae to grow. Meanwhile digestate was two-phase slurry including liquid and insoluble particles (dead algae, bacteria cells and metabolic sediments). When directly using the digestate without removing any solid, prolonged lag phase was resulted if the culture started with the low inoculation rate (0.02 g/L), but not for the culture with the high inoculation rate (0.09 g/L). If solid was removed from the digestate, higher dosage can be added to supple sufficient nutrients so no addition commercial fertilizers were needed. The digestate can be used in used media, and resulted similar algae growth curve compared to the control. Nitrogen was the only nutrient became depleted at the end of cultivation, whereas phosphate, potassium, and magnesium showed slight consumption, and had great amount of excess at the end.

## 7.5. Life cycle assessment

The microalgae-based $CO_2$ mitigation system consisted of closed tubular photo-bioreactor (PBR), harvesting bunkers, anaerobic digesters and nutrient recycling system. The

assumed working volume for the PBR in this analysis was 82728 $m^3$, which was responsible for mitigate 22.76 ton $CO_2$ per day. Given the $CO_2$ emission by the system itself, the net $CO_2$ mitigation was 9.14 ton $CO_2$ per day, or 11.93 ton $CO_2$ per day prior to anaerobic digestion. The algal biomass productivity and carbon content were directly linked to the system scale and $CO_2$ mitigation rate. Doubling the productivity can downsize the PBR by half, or increase the $CO_2$ mitigation rate by 50%. Increased the carbon content from 50% to 72%, the size of PBR was reduced by 30%.

## 7.6. Future work

### 7.6.1. Nutrient study

Potassium and phosphorous are two essential elements for algae growth besides nitrogen. The consumption trend of these two elements was not yet clearly shown within current study. To fill the gap, experiments should be conducted to understand how efficient algae cells consume potassium and phosphorous, and how these two elements impact the growth rate and overall algae culture health. Magnesium and iron are relatively expensive ingredients in the formula. It is important to realize their significance for a healthy algal culture, especially when the semi-batch culture has a long retention time.

### 7.6.2. Inhibitor study

As indicated by others' studies, growth inhibitors were pronounced in the high algae cell density culture. However no inhibition was observed in the current low cell density culture. As media are recycled more times, inhibitors can impose potential threat to the culture, and lead to a system crash. Given the reality that the system will run in a continuous mode, a study is needed to focus on the inhibition effect in the low cell density culture during long term growth and recycling.

### 7.6.3. Anaerobic digestion efficiency

Studies are needed to provide more guidance on how to improve the process efficiency. High organic loading rates require a smaller digester and less energy, so if it renders a similar digestion efficiency compared to low organic loading rates, it is beneficial to use the high loading rates. The correlation between pretreatment and specific methane production need to be determined, the ultimate goal is to improve the digestion efficiency with minimum pretreatment. Another approach to enhance the digestion is co-digestion. Algae biomass is protein-rich feedstock, and to balance the ratio of C/N carbohydrate-rich feedstock can be added. Several carbohydrate-rich substrates can be potentially co-digested with algae biomass, including crop

residues (e.g. corn stover and wheat straw) and energy crops (e.g. switchgrass). The correlation between C/N ratio and specific gas production should be investigated.

# Appendix I: LCA computation data

## 1. Emission factor

**Table 1. $CO_2$ emission factors of materials.**

| Emitter | $CO_2$ emission | Unit | Reference |
|---|---|---|---|
| Electricity | 984.3 | kg/MWh | EIA 2009 Kentucky power plant $CO_2$ emission |
| Tap water | 0.5 | $kg/m^3$ | Franklin Associates 2009 |
| Waste water | 0.23 | $kg/m^3$ | Franklin Associates 2009 |
| PET | 1.15 | ton $CO_2$/short ton | EPA WARM 12 |
| PVC | 2.02 | ton $CO_2$/short ton | EPA WARM 12 |
| Steel | 1.06 | ton/ton | IPCC Metal Industry Emission 2006 |
| Mineral wool | 0.26 | ton/ton | EPA-AP-42, Cupola, 1993 |
| Concrete | 0.273 | $ton/m^3$ | Flower and Sanjayan 2007 |
| Urea | 1 | ton/ton | IFA 2007 |
| TS Phosphate | 0.052 | ton/ton | IFA 2007 |
| Pot Ash | 0.33 | ton/ton | IFA 2007 |

## 2. Photobioreactor (PBR)

**Table 2. The calculation of $CO_2$ emission from PBR.**

| | Variable | Value | Unit |
|---|---|---|---|
| Algae | Growth rate | 0.15 | g/L/day |
| | Cell conc. | 1 | g/L |
| PBR Design | Size | 82,728 | $m^3$ |
| | Diameter of PET tube | 0.127 | m |
| | Length of PET tube | 2.44 | m |
| | Thickness of PET wall | 0.00027 | m |
| | NO. of tubes | 2,676,477 | |
| Media Preparation | Initial Fertilizer Required | | |
| | Urea | 14.03 | ton |
| | TS photophate | 9.10 | ton |
| | Pot ash | 1.36 | ton |
| | Daily Fertilizer Required after allocating nutrients from anaerobic digestate | | |
| | Urea | 1.20 | ton/day |
| | TS photophate | 0.75 | ton/day |
| | Pot ash | 0.11 | ton/day |
| Pumping | Flow rate | 0.0015 | $m^3$/S |
| | Head | 0 | m |
| | Friction loss | 3.14 | MWh/day |
| | Pump efficiency | 75% | |
| | Power input for pumps | 4.19 | MWh/day |
| | $CO_2$ emission | 3.99 | ton/day |
| $N_2O$ | $N_2O$ emission during light growth | 0.002% | % of urea by weight |
| | $N_2O$ emission during dark growth | 0.39% | % of urea by weight |
| | $N_2O$ daily emission | 0.004 | ton/day |
| | Equivalent $CO_2$ daily emission | 1.23 | ton/day |

| | | | |
|---|---|---|---|
| Water | Tap water $CO_2$ emission | 0.83 | ton/day |
| | Waste water $CO_2$ emission | 0.0003 | ton/day |

## 3. Friction loss in PET tubes and PVC fittings

**Table 3. The calculation of friction loss in PBR.**

| | Variable | Value | Unit |
|---|---|---|---|
| Flow properties | Flow rate (PET and PVC fittings) | 0.0015 | $m^3/s$ |
| | PET Velocity | 0.122 | m/s |
| | Inner diameter of PET tube | 0.125 | m |
| | PET Reynolds number | $1.55\times10^4$ | |
| | PVC Velocity | 0.191 | m/s |
| | Inner diameter of PVC fittings | 0.1 | m |
| | PVC Reynolds number | $1.94\times10^4$ | |
| Friction loss (F) in pipes<br>$f=0.25/(\log_{10}(\varepsilon/(3.7D)+2.51/(Re*f^{0.5})))^2$<br>$F=f(L/D)(V^2/(2g))$ | Roughness factor (ε) | $1.50\times10^{-6}$ | m |
| | PET friction factor (f) | 0.025 | |
| | PVC friction factor (f) | 0.026 | |
| | F per PET tube | 0.00037 | m |
| | F per PVC connecting tube | 0.00018 | m |
| Friction loss (F) in fittings (elbow)<br>$F=K(V^2/2g)$ | Friction loss factor (K) | 0.25 | |
| | F per elbow | 0.00046 | m |
| Friction loss (F) due to contractions<br>$F=K(V^2/2g)$ | Friction loss factor (K) | 0.05 | |
| | F per contraction | $9.31\times10^{-6}$ | m |
| Friction loss due to enlargement<br>$F=(V_1-V_2)^2/2g$ | F per enlargement | 0.00024 | m |

## 4. Friction loss in PVC feeding lines and liquid transferring lines

**Table 4. The calculation of friction loss during liquid transferring.**

| | Variable | Value | Unit |
|---|---|---|---|
| Flow properties | Length of feeding lines | 312,256 | m |
| | Length of transferring lines | 7,306 | m |
| | Inner diameter of feeding lines | 0.15 | m |
| | Inner diameter of transferring lines | 0.25 | m |
| | Flow rate of feeding lines | 0.009 | $m^3/s$ |
| | Flow rate of transferring lines | 0.027 | $m^3/s$ |
| Friction loss in pipe<br>$f=0.25/(\log_{10}(\varepsilon/(3.7D)+2.51/(Re*f^{0.5})))^2$<br>$F=f(L/D)(V^2/(2g))$ | Roughness factor (ε) | $1.50\times10^{-6}$ | m |
| | Friction factor for feeding lines (f) | 0.019 | |
| | Friction factor for transferring lines (f) | 0.016 | |
| | F for feeding lines | 523.43 | m |
| | F for transferring lines | 2.94 | m |
| Friction loss due to elbows (feeding line)<br>$F=K(V^2/2g)$ | Friction factor (K-elbow) | 0.25 | |
| | F for all elbows in feeding lines | 147.58 | m |
| Friction loss due to constrictions (feeding line)<br>$F=K(V^2/2g)$ | Friction factor (K) | 0.05 | |
| | F for all constrictions in feeding lines | 24.91 | m |
| Friction loss due to Tees (transferring line)<br>$F=K(V^2/2g)$<br>$D_{Tee} = D_{PVC}$, no contraction or enlargement | Friction factor (K) | 1.5 | |
| | F for all Tess in transferring lines (360 for 4 million PET tubes) | 0.69 | m |

## 5. Algae biomass harvesting

**Table 5. Processing factors for algae biomass harvesting.**

| | Variable | Value | Unit |
|---|---|---|---|
| First settling | Harvesting rate (Working volume) | 10,791 | $m^3$/day |
| | Algae conc. after settling | 7 | g/L |
| | Used media | 9018 | $m^3$/day |
| | Algae-rich slurry | 1773 | $m^3$/day |
| Second settling | Working volume | 1773 | $m^3$/day |
| | Algae conc. after settling | 40 | g/L |
| | Waste water | 1463 | $m^3$/day |
| | Algae stock for anaerobic digestion | 310 | $m^3$/day |
| | Harvested dry algae | 12.4 | ton/day |

## 6. Anaerobic digestion (CSTR)

**Table 6. Design and yields of the anaerobic digester.**

| | | Variable | Value | Unit |
|---|---|---|---|---|
| Reactor Design | Overall | Height | 15 | m |
| | | Diameter | 16.5 | m |
| | | Volume | 3200 | $m^3$ |
| | Steel wall | Height | 15 | m |
| | | Length | 51.8 | m |
| | | Thickness | 0.05 | m |
| | | Volume | 49.5 | $m^3$ |
| | Insulation | Height | 15 | m |
| | | Length | 51.8 | m |
| | | Thickness | 0.1 | m |
| | | Volume | 99.0 | $m^3$ |
| | Sheet steel covering | Height | 15 | m |
| | | Length | 103.5 | m |
| | | Thickness | 0.001 | m |
| | | Volume | 1.8 | $m^3$ |
| Operation factors | Organic loading rate (OLR) | | 2.5 | kgVS/$m^3$/day |
| | Hydraulic retention time (HRT) | | 15 | day |
| | Feeding rate (mass) | | 17 | tonVS/day |
| | Feeding rate (volume) | | 310 | $m^3$/day |
| | Discharge rate (volume) | | 248 | $m^3$/day |
| Energy consumption | Agitation | Specific power | 0.05 | kW/$m^3$ |
| | | Operation time | 2.5 | h/day |
| | | Energy | 0.85 | MWh/day |
| | Heat loss* | $T_{amb}$ | 25 | °C |
| | | $T_{reactor}$ | 38 | °C |
| | | Energy | 2.67 | MWh/day |
| Product yields<br>Assume digestion rate is 45%. | Gas | $CH_4$ | 0.28 | L/g dw algae |
| | | $CO_2$ | 0.21 | L/g dw algae |
| | Soluble nutrients | N | 414.90 | kg/day |
| | | P | 122.85 | kg/day |

| | | | | |
|---|---|---|---|---|
| | | K | 45.79 | kg/day |
| | Insoluble nutrients | Wet digestate 93% MC | 124.09 | $m^3$/day |
| | | N | 753.98 | kg/day |
| | | P | 185.02 | kg/day |
| | | K | 33.88 | kg/day |

- Heat loss is detailed in section 7.

## 7. Heat loss in CSTR

Assumptions:

1. Steel and sheet steel do not contribute to any thermal resistance.
2. Insulation material is mineral fiberboard.
3. Neglect the radiation.
4. An unheated floor loses heat primarily from its perimeter rather than from the interior part of the building.
5. Calculations is based on one CSTR.

**Table 7. The calculation of heat loss from anaerobic digester.**

| | Variable | Value | Unit |
|---|---|---|---|
| Known | $T_{amb}$ | 25 | °C |
| | $T_{reactor}$ | 38 | °C |
| | Thickness of insulation | 0.1 | m |
| | $K_{mineral\ fiberboard}$ | 0.049 | W/m/K |
| | $K_{steel\text{-}AISI302}$ | 15.1 | W/m/K |
| Heat loss from side insulation $q''=(T_{reactor}-T_{amb})/R$ | $R_{insulation}$ | 0.015 | $m^2K/W$ |
| | $h_{surface}$ | 3.0 | $W/m^2/K$ |
| | Heat flux | 37.3 | $W/m^2$ |
| | Heat loss | 0.70 | MWh/day |
| Heat loss from top insulation $q''=(T_{reactor}-T_{amb})/R$ | $R_{insulation}$ | 2.04 | $m^2K/W$ |
| | $h_{surface}$ | 1.30 | $W/m^2/K$ |
| | Heat flux | 4.63 | $W/m^2$ |
| | Heat loss | 0.024 | MWh/day |
| Heat loss from floor $q = F\times(T_{reactor}-T_{amb})\times Pi\times D$ | Heat loss factor (F) | 1.5 | W/m/K |
| | Heat loss (q) | 0.024 | MWh/day |

# Appendix II: Tap water composition

**Table 1. Tap water elemental composition**

| mg/L | In tap water |
|---|---|
| Ca | 42 |
| K | 4.44 |
| Na | 9.66 |
| Mg | 8.02 |
| Fe | 0.09 |
| P | 1.98 |
| S | 14 |
| Mn | 0.03 |
| Cu | 0.07 |
| Zn | 0.07 |
| Co | <0.01 |
| Mo | <0.01 |
| V | <0.01 |

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Crofcheck C, E XY, Shea A, Montross M, Crocker M, Andrews R (2013) Influence of media composition on the growth rate of *Chlorella vulgaris* and *Scenedesmus acutus* utilized for $CO_2$mitigation. *Journal of Biochemical Technology* 4(2): 589-594.

**Presentations**

E XY, Crofcheck C, Aurandt J (2013) Nutrients recycling strategies for microalgae-based CO2 bio-mitigation system. 2013 Annual conference of the Institute for Biological Engineering, NC.

E XY, Crofcheck C, Montross M (2012) Life cycle assessment for a microalgae-based CO2 bio-mitigation system. 2012 Annual conference of American Society of Agricultural and Biological Engineers, TX.

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