INTEGRATED NUTRIENT REMOVAL AND BIOGAS PRODUCTION USING MICROALGAL AND ANAEROBIC MICROBIAL CULTURES

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNIAL UNIVERSITY

BY

AYŞE ÖZGÜL ÇALICIOĞLU

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
ENVIRONMENTAL ENGINERING

DECEMBER 2013

Approval of the thesis:

INTEGRATED NUTRIENT REMOVAL AND BIOGAS PRODUCTION USING MICROALGAL AND ANAEROBIC MICROBIAL CULTURES

Submitted by **AYŞE ÖZGÜL ÇALICIOĞLU** in partial fulfillment of the requirements for the degree of **Master of Science in Environmental Engineering Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen _____
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. F. Dilek Sanin _____
Head of Department, **Environmental Engineering**

Prof. Dr. Göksel N. Demirer
Supervisor, **Environmental Engineering Dept., METU** _____

Examining Committee Members:

Prof. Dr. Celal F. Gökçay _____
Environmental Engineering Dept., METU

Prof. Dr. Göksel N. Demirer _____
Environmental Engineering Dept., METU

Assist. Prof. Dr. Tuba H. Ergüder _____
Environmental Engineering Dept., METU

Assist Prof. Dr. Barış Kaymak _____
Environmental Engineering Dept., METU

Prof. Dr. Metin Duran _____
Civil and Environmental Engineering Dept., Villanova University

Date: 03.12.2013

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

Name, Last Name : Ayşe Özgül Çalıcıoğlu

Signature:

ABSTRACT

INTEGRATED NUTRIENT REMOVAL AND BIOGAS PRODUCTION USING MICROALGAL AND ANAEROBIC MICROBIAL CULTURES

Çalıcıoğlu, Ayşe Özgül
M.Sc., Environmental Engineering Department
Supervisor: Prof. Dr. Göksel N. Demirer

December 2013, 151 pages

Following industrialization and urbanization, there have been significant impairments in key nutrient cycles, affecting both ecosystems and human well-being. Urban sewage is rich in nutrients such as nitrogen and phosphorus, which cause eutrophication in receiving water bodies, if not removed. Microalgal nutrient removal is a viable alternative for biological wastewater treatment, considering their high nutrient uptake capabilities of microalgae. These systems are also advantageous in terms of nutrient recycling and conversion into microalgal biomass, which, in turn, is a beneficial resource for biofuel production.

In this study, a semi-continuous photo-bioreactor was operated for investigation of nutrient removal efficiency of unialgal culture, *Chlorella vulgaris*. Maximum N and P removal efficiencies of 99.6% and 91.2% were achieved in the photobioreactor.

Biogas production from biomass obtained from semi-continuous photobioreactor was investigated via Biochemical Methane Potential (BMP) assays. The results illustrated that maximum biogas yield of 442 mL/g VS added could be achieved in untreated microalgal slurry reactors.

Evaluation of pretreatment options indicated that the highest biomethane yield could be achieved after heat pretreatment. However, considering that autoclave

pretreatment is less energy intensive, this method was found to be more feasible for enhanced biogas production.

The results of BMP assay conducted for co-digestion of microalgal slurry with model kitchen waste or waste activated sludge indicated that maximum biogas yield of 785 mL/ g VS added could be achieved when model kitchen waste was used as co-substrate.

Outcomes of this study reveal that microalgal biotechnology is a feasible alternative for integrated nutrient removal and biofuel production applications.

Keywords: *Chlorella vulgaris*, Photobioreactor, Anaerobic digestion, Pretreatment, Co-digestion.

ÖZ

MİKROALGAL VE ANAEROBİK MİKROBİYAL KÜLTÜRLER İLE ENTEGRE BESİYER MADDE GİDERİMİ VE BİYOGAZ ÜRETİMİ

Çalıcıoğlu, Ayşe Özgül
Yüksek Lisans, Çevre Mühendisliği Bölümü
Tez Yürütücüsü: Prof. Dr. Göksel N. Demirer

Aralık 2013, 151 sayfa

Endüstrileşme ve kentleşmeyi takiben, anahtar besiyer madde döngülerinde ekosistemleri ve insan sağlığını etkileyen olumsuz değişimler görülmektedir. Şehir atık suyu, giderilmedikleri koşulda alıcı sucul ortamlarda ötrofikasyona sebep olabilecek azot ve fosfor gibi besiyer maddeler yönünden zengindir. Mikroalgal besiyer madde giderimi, kültürün yüksek azot ve fosfor ihtiyacı dolayısı ile biyolojik atık su arıtımı konusunda uygulanabilir bir alternatiftir. Mikroalgal kültürlerin yüksek besiyer madde tutulum kapasitelerinin yanı sıra, söz konusu sistemler besiyer maddenin biyoyakıt kaynağı olan biyokütle biçiminde geri kazanımına olanak sağlamaktadır.

Bu çalışmada, *Chlorella vulgaris* tek hücreli mikroalg kültürü ile besiyer madde giderim veriminin araştırılması amacı ile yarı-sürekli fotobiyoreaktör işletilmiştir. En yüksek azot ve fosfor giderimleri sırası ile %99.6 ve %91.2 olarak kaydedilmiştir.

Yarı-sürekli fotobiyoreaktörde üretilen mikroalg biyokütlesinin biyogaz üretim potansiyeli ise Biyokimyasal Metan Potansiyeli (BMP) testleri yardımı ile araştırılmıştır. Çalışma sonuçları, ön arıtımsız mikroalg çamuru ile 442 mL / g eklenen UKM biyogaz verimi elde edilebileceğini göstermiştir.

Ön arıtım seçeneklerinin değerlendirilmesi sonucunda, ısıl ön arıtımın anaerobik çürütme verimi üzerinde en yüksek olumlu etkiye sahip yöntem olduğu görülmektedir. Ancak, otoklav yönteminin enerjiye daha az duyarlı olması dolayısı ile biyogaz üretiminin ön arıtım ile artırılması konusunda daha uygulanabilir olduğu kaydedilmiştir.

Mikroalg çamurunun model mutfak atığı veya aktif çamur ile birlikte çürütülmesi potansiyelinin araştırılması amacıyla yürütülen BMP testleri sonucunda, en yüksek biyogaz verimi olan 785 mL/ g eklenen UAKM değeri, mikroalg çamurunun ve model mutfak atığının birlikte çürütülmesi sonucunda elde edilmiştir.

Çalışma sonuçları, mikroalg biyoteknolojisi ile entegre besiyer madde giderimi ve biyoyakıt eldesi uygulamalarının gerçekleştirilebilir olduğunu göstermektedir.

Anahtar Kelimeler: *Chlorella vulgaris*; Fotobiyoreaktör, Anaerobik çürütme, Ön arıtım, Birlikte çürütme.

ACKNOWLEDGEMENTS

In the first place, I would like to express my deep gratitude to my supervisor Prof. Dr. Göksel N. Demirer for his patient guidance, encouragement and critical evaluation throughout this research. Owing to his supervision, I learned how to plan, manage and evaluate my own work.

I gratefully acknowledge the time, recommendations, help and encouragement provided by Assoc. Prof. Dr. Sibel Uludağ-Demirer, Assist. Prof. Dr. Tuba Hande Ergüder and Assist. Prof. Dr. Barış Kaymak, throughout this research.

I am particularly grateful for the financial support of The Scientific and Technological Research Council of Turkey (TÜBİTAK), without which the present study could not have been completed.

I would like to extend my thanks to the members of our research group, Gece, Burak, Eray, Engin and Güneş for their patience and support. It was great to spend long hours with you, while working in the lab.

Finally, I wish to thank my husband, parents and brother for their love, trust and support.

TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

ABBREVIATIONS

μ	:	Specific Growth Rate, (d^{-1})
AD	:	Anaerobic Digestion
ATP	:	Adenosine Triphosphate
BCP	:	Batch Cultivation Photobioreactor
BM	:	Basal Medium
BMP	:	Biochemical Methane Potential
BNR	:	Biological Nutrient Removal
BOD	:	Biochemical Oxygen Demand
C	:	Carbon
CH_4	:	Methane
CO_2	:	Carbon Dioxide
CO_3^{2-}	:	Carbonate Ion
COD	:	Chemical Oxygen Demand
DNA	:	Deoxyribonucleic Acid
DO	:	Dissolved Oxygen
EBPR	:	Enhanced Biological Phosphorus Removal
F/M	:	Food-to-Microorganism Ratio
H_2CO_3	:	Carbonic Acid
H_2O	:	Water
HCO_3^-	:	Bicarbonate Ion
HRPs	:	High Rate Algal Ponds
HRT	:	Hydraulic Retention Time
KW	:	Kitchen Waste
LCFA	:	Long Chain Fatty Acids
MSW	:	Municipal Solid Waste
N	:	Nitrogen
NADPH	:	Nicotinamide Adenine Dinucleotide Phosphate
NH_3	:	Free Ammonia
NH_4^+	:	Ammonium Ion
NH_4^+-N	:	Ammonium - Nitrogen
NO_3^-	:	Nitrate Ion
NO_3^--N	:	Nitrate - Nitrogen
NO_x	:	Nitrogen Oxide
O_2	:	Oxygen
OD	:	Optical Density
OFMSW	:	Organic Fraction of Municipal Solid Waste
o-PO_4^{3-}-P	:	Orthophosphate- Phosphorus
P	:	Phosphorus
p	:	Productivity, (mg/(L.d))
PAOs	:	Phosphate Accumulating Organisms
PAR	:	Photosynthetically Active Radiation, (nm)
PO_4^{3-}-P	:	Phosphate Ion

sCOD	:	Soluble Chemical Oxygen Demand
SCP	:	Semi-Continuous Cultivation Photobioreactor
S/X	:	Substrate-to-Inoculum Ratio
TAN	:	Total Ammonifiable Nitrogen
tCOD	:	Total Chemical Oxygen Demand
TKN	:	Total Kjeldahl Nitrogen
TN	:	Total Nitrogen
TP	:	Total Phosphorus
TS	:	Total Solids
TSS	:	Total Suspended Solids
VFA	:	Volatile Fatty Acids
VDS	:	Volatile Dissolved Solids
VS	:	Volatile Solids
VSS	:	Volatile Suspended Solids
vvm	:	Volume Gas per Volume of Broth per Minute
WAS	:	Waste Activated Sludge

CHAPTER 1

INTRODUCTION

Following industrialization, there have been significant impairments in key nutrient cycles, including but not limited to nitrogen and phosphorus, due to anthropogenic activities. These manipulations have brought negative consequences affecting both ecosystems and human well-being (Berhe et al., 2005).

One of the negative feedback of industrialization and urbanization on the ecosystems is the elevated concentrations of atmospheric carbon dioxide, mainly due to fossil fuel consumption (IPCC, 2007) which leads to global climate change and sequestration of carbon in ecosystems (Berhe et al., 2005). Another example for human manipulation on natural ecosystems is the increased nutrient loads to fresh waters and marine environments, which occurred mainly after introduction of sewage and artificial fertilizers (UNEP, 2006).

Nutrient pollution affects atmosphere, groundwater, and surface waters; as a result, creates risk for environmental health, public health and economy. Eutrophication, a phenomenon which is defined as “an increase in the rate of supply of organic matter in an ecosystem” (Nixon, 1995) is an example of human manipulation on ecosystems.

In aquatic environments, eutrophication causes algal blooms, oxygen depletion, increase in undesired vegetation, loss of plant beds, fish, coral reef and other species. Eventually, the water bodies become unavailable for agricultural,

recreational, industrial use and drinking purposes (Carpenter et al., 1998). Discharge of domestic sewage introducing high levels of nutrients to water bodies is one of the main causes of eutrophication (Girard, 2009). Secondary level wastewater treatment may not be adequate for removal of these nutrients. In fact, tertiary treatment methods have been developed for their removal (Tchobanoglous et al., 2003).

Considering their high nitrogen and phosphorus requirements, growth of algae is an alternate method for biological wastewater treatment with advanced nutrient removal. In addition, photosynthetic activity during treatment process requires carbon dioxide, which may be provided from atmosphere or flue gas. In turn, microalgal wastewater treatment aids sequestration of atmospheric carbon dioxide.

Chlorella vulgaris, microalga from genus of single-cell green algae, *Chlorella*, has high photosynthetic efficiency, productivity and adaptable to severe environmental conditions. Owing to these properties, *Chlorella vulgaris* is a viable alternative for wastewater treatment systems, in order to provide system flexibility against variations in wastewater compositions.

Microalgal biotechnology has been developed not only for wastewater treatment, but also for a variety of consumer products such as pharmaceuticals (García-Casal et al., 2009) and nutrient supplements (Harun et al., 2010). Apart from consumer products, microalgal biomass can be used for production of biofuels. Extracted oil from microalgal biomass can be converted into biodiesel (Mata et al., 2010). The cell residues can then be converted into biomethane (Vergarafernandez et al., 2008), bioethanol (Sukahara and Awayama, 2005) or biohydrogen (Mussgnug et al., 2010).

Biomethane production from microalgal biomass has received attention, since biogas obtained from anaerobic digestion can be used for electricity generation (Holm-Nielsen et al., 2009). In addition, biomass residues after anaerobic

digestion can be converted into fertilizers. Utilization of these fertilizers provide sustainable agriculture and reduce production costs of microalgae.

Production cost of methane from microalgae is higher than other feedstocks, due to microalgae cultivation costs involved in these systems. Therefore, integrated wastewater treatment and biomethane production can be the most feasible approach to reduce cost of both wastewater treatment and energy production. Moreover, when they are coupled with carbon dioxide sequestration, microalgae can provide possible solutions to environmental problems and simultaneously create valuable consumer products and biofuels (Harun et al., 2010). This process was first discussed by Oswald and Gotaas (1957), where algae was used for wastewater treatment in ponds and then harvested for methane production through anaerobic digestion.

Although cellulose and lignin contents of microalgae are almost zero and anaerobic process stability is high, hydrolysis of microalgal cell wall is problematic. The biodegradation of algal biomass can be improved by different pretreatment methods. such as microwave pretreatment (Passos et al., 2013); ultrasound pretreatment (Alzate et al., 2012); thermal pretreatment including drying (Mussgnug et al., 2010), heating (Chen and Oswald, 1998), thermal hydrolysis (Alzate et al., 2012) or high pressure thermal hydrolysis (Keymer et al., 2013); chemical pretreatment (Chen and Oswald, 1998) and biological pretreatment (Alzate et al., 2012).

Apart from difficulties in cell biodegradability, the low carbon to nitrogen (C/N) ratio of microalgae, resulting in high total ammonia nitrogen release and high volatile fatty acids (VFAs) accumulation, is a serious problem for anaerobic digestion (AD) process. Therefore, algae can be subjected to anaerobic digestion not only as the sole substrate but can also be co-digested with other substrates with higher carbon contents such as sewage sludge (Chen, 1987), organic fraction of municipal solid waste (Spierling, 2011) and waste paper (Yen and Brune, 2007). Considering their compositions, low cost and availability, kitchen

waste and waste activated sludge are two viable alternatives to be co-digested with microalgae.

The objectives of this study are to investigate nutrient removal potential of green alga, *Chlorella vulgaris*, from primary effluents of municipal wastewater treatment plants and investigation of anaerobic digestibility of the produced microalgal biomass to produce biogas.

The tasks undertaken are:

- Laboratory- scale continuous photobioreactor operation for microalgal culture with primary effluents of municipal wastewater treatment plants, as well as investigation of nitrogen and phosphorus removal efficiencies,
- Determination of anaerobic digestibility of microalgal slurry produced in a photobioreactor through Biochemical Methane Potential (BMP) assays,
- Evaluation of heat, autoclave and thermochemical pretreatment options for the improvement of anaerobic digestibility of microalgal slurry through BMP assays,
- Investigation of waste activated sludge and model kitchen waste as alternative co-substrates for anaerobic digestion of microalgal slurry through BMP assays.

CHAPTER 2

THEORETICAL BACKGROUND

2.1. Global Nutrient Cycles

Nutrients can be defined as "inorganic or organic compounds, other than carbon dioxide and water, whose presence in the cell is necessary for cellular function" (Neenan et al., 1986). Many compounds, which are composed of more than twenty elements, can be considered as inorganic nutrients. However, the term "nutrient" often refers to nitrogen (N) and phosphorus (P), which are the limiting elements for cellular growth in most cases.

Nutrient balance, adequacy and availability regulate the life on Earth, having a direct effect on any ecosystem services. Since 19th century, there have been significant impairments in key nutrient cycles, including but not limited to nitrogen and phosphorus, due to anthropogenic activities. These manipulations have brought not only positive, but also negative consequences affecting both ecosystems and human well-being (Berhe et al., 2005). For instance, one of the negative feedback of industrialization on the ecosystems is the elevated concentrations of atmospheric carbon dioxide, mainly due to fossil fuel consumption (IPCC, 2007) which leads to global climate change and sequestration of carbon in ecosystems (Berhe et al., 2005). Another example for human manipulation on natural ecosystems is the increased nutrient loads to fresh waters and marine environments, which occurred mainly after introduction of artificial fertilizers (UNEP, 2006).

Nutrients, especially phosphorus is often limiting to ecosystems and crops. Large amounts of P are mined from nonrenewable resources, such as P-rich geological deposits, to produce fertilizers. However, much of this fertilizer reach to water bodies, cause eutrophication or become unavailable for recycling (Elser, 2012).

In this section, a summary of nitrogen and phosphorus cycles has been given. Consequences of their alteration by anthropogenic impacts and strategies implemented for their mitigation are also provided.

2.1.1. Nitrogen Cycle

Nitrogen is an element, which is required by any living organism to develop and grow. Therefore, its availability in any ecosystem is essential and it is transformed into different forms in the biosphere. Although nitrogen gas is abundant in air, it can only be fixed by a few bacterial species and nitrogen fixing crops, thus its natural conversion into forms available for aquatic and terrestrial ecosystems is limited (Galloway et al., 2004).

Nitrogen cycle has been drastically altered by human activities. After industrialization, the annual nitrogen flux from atmosphere to terrestrial and aquatic ecosystems has been increased from 90-130 to 290- 330 teragrams per year. The difference is mainly a consequence of synthetic fertilizer utilization, planting of nitrogen fixing crops and deposition of nitrogen containing air pollutants. Current nitrogen flux is therefore not in equilibrium with denitrification rate. Despite of the fact that this elevation causes an increase in food yields, it accounts for the decrease in water quality in marine and fresh water environments (Berhe et al., 2005).

2.1.2. Phosphorus Cycle

Phosphorus is an essential element for any living organism, since it is the basic component of genetic and energy carrier molecules, namely, deoxyribonucleic acid (DNA) and adenosine triphosphate (ATP). It is also an important component of photosynthesis, nitrogen fixation, crop quality and growth

(Ashman and Puri, 2009). Introduction of phosphorus into biosphere mostly after dissolution of phosphate rocks (Olsen, 1975; Ashman and Puri, 2009).

Annual accumulation rate of phosphorus in ecosystems has been increased from 1-6 to 10.5 – 15.5 teragrams, compared to that of preindustrial time. The majority of the resultant flux is caused by phosphorus mining for agricultural purposes. After phosphorus accumulates in land, it is transported into water bodies and lowers fresh water quality. It is expected that the negative effect of phosphorus on ecosystems will increase; since high amounts of phosphorus which has already accumulated in soil gradually reaches water ecosystems (Berhe et al., 2005).

2.2.Nutrient Pollution

Changes in nitrogen and phosphorus cycles and their negative feedback are of high importance in environmental management. Nutrient pollution affects atmosphere, groundwater, and surface waters; as a result, create risk for environmental health, public health and economy.

2.2.1. Atmospheric Pollution

Main sources of atmospheric nutrient pollution is atmospheric deposition. As a result of fossil fuel burning and other combustion processes, gaseous and particulate nitrogen oxide (NO_x) are released into the atmosphere. These emissions are originated from mobile and stationary sources.

Excessive nitrogen loadings in the atmosphere cause formation of harmful nitrogenous compounds and ozone, which decrease visibility, impair plant growth and cause breathing problems. After NO_x is converted into nitric acid in the air, it is removed from the atmosphere by wet (rain, snow etc.) or dry (without precipitation) deposition and delivered to lithosphere, hydrosphere and biosphere. Acid rain has corrosive impact on urban structures and natural environments. It also contributes to nutrient loadings into soils and water bodies (EPA, 2009).

2.2.2. Groundwater Pollution

Nutrient pollution in groundwater may occur by its contamination with either point sources such as intentional or accidental spills of sewage from septic tanks or diffuse sources such as agricultural activities.

Utilization of nutrient- contaminated groundwater for drinking purposes may cause severe health problems. For example, elevated nitrate concentrations in groundwater cause methemoglobinemia, i.e. reduction of oxygen –carrying capacity of blood. Methemoglobinemia causes death in infants. Such high nitrate levels are usually associated with intensive farming (EPA, 2009).

2.2.3. Eutrophication

Eutrophication, a phenomenon which is defined as "an increase in the rate of supply of organic matter in an ecosystem" (Nixon, 1995) is a direct consequence of human manipulation on ecosystems.

Discharge of domestic sewage, which introduces high levels of N originating from human wastes and P originating from detergents, to water bodies are main causes of eutrophication (Girard, 2009). Agriculture and excessive usage of fertilizer are also important direct causes. Indirectly, deforestation is also a cause of eutrophication, since it increases surface runoff and therefore accumulation rate of nutrients (UNEP, 2006).

In aquatic environments, eutrophication causes algal blooms, oxygen depletion, increase in undesired vegetation, loss of plant beds, fish, coral reef and other species. Eventually, the water bodies become unavailable to utilize for agricultural, recreational, industrial and drinking purposes (Carpenter et al., 1998).

Eutrophication is a trans-boundary problem in many parts of Europe, North America and other parts of the world (WHO, 1999). For instance, in Eastern Europe, several studies have demonstrated anthropogenic impact by revealing reduced nutrient loads originated from fertilizers in rivers as a result of economic

collapse in Eastern Europe and Soviet Union in early 1990's (Carstensen et al., 2006). On the other hand, in last decades, the diversity of marine species of Black Sea sharply declined and phytoplankton communities began to predominate. Global International Water Assessment (GIWA) declared this situation to be the most critical environmental issue, which is a consequence of agricultural runoffs and sewage discharges. Other than in Black Sea case, harmful algal blooms have been increasing in size and number within the last few decades (UNEP, 2006).

2.2.4. Municipal Wastewater as a Source of Nutrient Pollution

Raw municipal wastewater contains nutrients, which are still present after secondary treatment. These nutrients have been considered as main causes of eutrophication receiving in water bodies.

Nitrogen compounds present in municipal wastewater are organic nitrogen in either soluble or particulate forms, sodium nitrate ($NaNO_{3)}$ and ammonium ion (NH_4^+) (Kladitis et al., 1999). Nitrite (NO_2^-) is very scarce in wastewater and its concentration seldom exceeds 1 mg/L. Organic nitrogen in wastewater is usually referred as the difference between Total Kjeldahl Nitrogen (TKN) and NH_4^+-N concentrations. Soluble portion of organic nitrogen is readily converted into nitrate (NO_3^-) and ammonia. Typically, ammonia nitrogen concentration in untreated municipal wastewater is 25 mg/L NH_4^+-N and nitrate concentration 0 mg/L NO_3^--N, although concentrations up to 20 mg/L are observable. Typical TKN value for municipal wastewater is around 40 mg/L (Tchobanoglous et al., 2003).

Phosphorus compounds present in municipal wastewater are organic and inorganic phosphorus in either soluble or particulate forms. A large portion of organic phosphorus is solubilized in the form of orthophosphate- phosphorus (o-PO_4^{3-}-P) during treatment, which is the form that can be assimilated by phytoplankton (Griffiths, 2010). Municipal wastewaters may contain from 4 to 16 mg/L of total phosphorus where the typical value is 7 mg/L (Tchobanoglous et al., 2003).

Secondary wastewater treatment plant effluents contain nutrients such as NH_4^+-N, NO_3^--N and PO_4^{3-}-P. The predominant nitrogen and phosphorus species in treated wastewater nitrate (Tchobanoglous et al., 2003) and orthophosphate (Griffiths, 2010) respectively.

There are plenty of studies that model the relationship between nutrient concentrations and phytoplankton growth in both fresh water and marine environments (Correll, 1998; Harrison and Cota, 1991). These outcomes reveal the necessity of nutrient removal before that the treated or untreated wastewaters reach water bodies, in order to assure water quality (Ruiz-Marin et al., 2010).

2.3.Nutrient Removal Technologies

Wastewater produced by communities contain nutrients and compounds, which may have triggering and toxic effect on aquatic flora growth. These chemicals may also cause mutations or may be carcinogenic. In order to prevent deteriorations in public health and environment, it is necessary to separate abovementioned constituents before they reach natural environments.

Nutrient treatment methods can be separated into physical, chemical and biological processes. Biological nutrient removal (BNR) is a process incorporated in activated sludge systems for the purpose of total nitrogen (TN) and total phosphorus (TP) removal, using different microorganisms which favor different environmental conditions (Tchobanoglous et al., 2003).

2.3.1. Nitrogen Removal Technologies

Nitrogen can be treated physically, chemically or biologically. There are three basic physical-chemical techniques, namely, ammonia stripping, selective ion exchange and breakpoint chlorination (U.S. EPA, 1993). These techniques remove nitrogen in ammonia form and do not require its conversion into nitrates as in biological nitrogen removal processes, which involve nitrification and consecutive denitrification (Tchobanoglous et al., 2003). On the other hand, these techniques have drawbacks, which are discussed further in this section.

Ammonia stripping is the only physical-chemical process that has been used in large-scale treatment facilities. This process consists of increasing the pH up to 11 and agitation by circulation of air in a tower. Fate of removed ammonia by the usage of stripping method is often questionable, since this process may be considered as conversion of a water quality problem into an air quality problem. Moreover, dissolution of the stripped ammonia in water bodies nearby may also be possible due to precipitation.

The selective ion exchange process involves utilization of a zeolite (resin), which has higher affinity for ammonia than for other cations. Wastewater is passed through the zeolite bed to achieve ammonia removal. The zeolite must be regenerated when ammonia leakage occurs. During the regeneration process, concentrated salt or alkaline solutions are required. This requirement increases the cost of the process. In addition, it is not possible to regenerate the resin unlimitedly and handling of spent resin is another concern.

Breakpoint chlorination is a process that involves addition of chlorine into wastewater. As a result, chloramines are formed. Chloramines are converted into nitrogen gas and stripped out after further addition of chlorine. Wastewater constituents may not be sufficient to neutralize the acid produced by addition of chlorine gas. In this case, external alkalinity source is required. The breakpoint process is therefore not feasible for treatment of wastewaters solely; it can be used for "polishing" purposes (U.S. EPA, 1993).

Biological nitrogen removal process consists of nitrification and denitrification. The first step, nitrification, covers the oxidation process of ammonia to nitrite by *Nitrosamonas* and nitrite to nitrate by *Nitrobacter*. The second step, denitrification, is the conversion of nitrate to nitric oxide, nitrous oxide and nitrogen gas. This reaction is carried out commonly by *Pseudomonas sp.* (Tchobanoglous et al., 2003). Nitrification is the rate-controlling step in biological nitrogen removal process, since growth rate of nitrifiers is lower. Sole nitrification is not sufficient for nitrogen removal; rather, denitrification is the step that converts nitrogen into gaseous form. In order to achieve nitrification,

oxygen is necessary. On the other hand, anoxic (oxygen free) conditions should be favored for denitrifiers to function (Jeyanayagam, 2005).

2.3.2. Phosphorus Removal Technologies

Phosphorus can be treated physically, chemically or biologically. These processes can be coupled in order to achieve desired effluent phosphorus concentrations (Griffiths, 2010).

Physical treatment of phosphorus involves primary treatment, i.e. sedimentation, floatation and filtration methods. These processes remove particulate organic and particulate inorganic phosphorus species (Wang et al., 2006).

Chemical treatment of phosphorus includes precipitation of soluble species, with the aid of metal- phosphate complex formation. For this purpose, divalent or trivalent metal salts, such as iron and aluminum, are added to wastewater and pH is raised by lime addition in order to form insoluble phosphate salts. Usually, chemical treatment is coupled with physical treatment, in order to remove residual precipitates of phosphorus after chemical treatment. Chemical treatment produces large amounts of inorganic sludge, which is difficult to handle and usually have to be landfilled.

Phosphorus can also be removed by means of a biological process termed as Enhanced Biological Phosphorus Removal (EBPR). This process involves luxury uptake of phosphorus by Phosphate Accumulating Organisms (PAOs), which is enhanced by cyclic anaerobic and aerobic conditions. Once PAOs are enriched with phosphorus in amounts more than stoichiometrically necessary for microbial growth, they are separated via sedimentation. This process avoids formation of large quantities of chemical sludge (Morse et al., 1998).

2.3.3. Nutrient Removal Using Microalgae

Use of algal cultures is an alternate method for biological wastewater treatment, considering their high nitrogen and phosphorus requirement for nucleic acids, phospholipids and protein syntheses. A portion of nutrients is also removed by

ammonia stripping and phosphorus precipitation at elevated pH values due to photosynthetic activity.

It has been reported that microalgal species such as *Botryocoocus*, *Chlamydomas*, *Scenedesmus* and *Chlorella* have high potential of nutrient removal. Mixed microalgal cultures have also been reported to achieve nutrient removal. In addition, these systems for microalgal- bacterial symbiotic treatment of wastewater are present (Zhou, 2010).

There are basically two wastewater treatment systems incorporating microalgae, namely, facultative waste stabilization ponds (lagoons) and high rate algal ponds (HRPs) (Griffiths, 2010).

Facultative lagoons are shallow ponds in which minimal or no mixing is provided. Oxygen required for bacteria to consume Biochemical Oxygen Demand (BOD) is supplied by atmospheric aeration and microalgal photosynthesis (Woertz et al., 2009). Although facultative lagoons are cost-effective, they are not suitable for wastewater treatment at large quantities (EPA, 2002). In addition, these systems are not specifically designed to enhance optimal microalgal growth (Muñoz and Guieysse, 2006).

Oswald (1957) has developed HRPs beginning in 1950's as alternatives to unmixed facultative lagoons for treatment of wastewater constituents such as nutrients, BOD, solids and pathogens (Oswald and Gotaas, 1957). HRPs are raceway-shaped shallow ponds with gentle mixing provided by paddle wheels (Woertz et al., 2009b). Ammonium and phosphorus removal efficiencies of these systems are reported to be around 89% and 49% respectively (Green et al., 1995). In HRPs, quality of microalgae can be controlled better than in facultative lagoons (Griffiths, 2010).

Microalgal wastewater treatment can provide economically and ecologically effective removal of nutrients compared to physical and chemical processes (Ristenson, 2011). These systems recycle nutrients and convert into beneficial resources as stated in Section 2.12.4.7.

2.4.Microalgae

Microalgae can be defined as a heterogeneous group of unicellular, and photoautotrophic, either eukaryotic or prokaryotic microorganisms (Olaizola, 2003). Although microalgae are mostly aquatic, they can be found in a wide habitat range and symbiotic systems. Their systematic classification is based on pigment components. The largest systematic groups are green algae, brown algae, dinoflagellates, golden-brown algae, diatoms and red algae (Harwood and Guschina, 2009). In this section, physiology and environmental factors controlling growth of green algae are discussed.

2.4.1. Physiology

Green algae are eukaryotic and have a similar cell structure with basic plant cells. Simplicity of their structure allow adaptation to a variety of environments. Eukaryotic algae can be autotrophic, heterotrophic, or both (mixotrophic). Autotrophic microalgae utilizes inorganic carbon such as carbon dioxide, inorganic salts such as nutrients and light for hydrocarbon synthesis. Heterotrophic microalgae capture organic carbon and utilize them as carbon source. Mixotrophic microalgae can use both organic and inorganic carbon sources. Photosynthesis is the key process that enables survival of autotrophic microalgae (Brennan and Owende, 2010; Edberg, 2010).

2.4.2. Growth Kinetics

In a simple homogeneous batch culture, algal growth passes through six phases: (i) adaptation (lag phase); (ii) accelerating growth; (iii) exponential growth (log phase); (iv) decreasing log growth (linear growth); (v) stationary phase; (vi) death. These growth phases are depicted in Figure 2-1 (Becker, 2008).

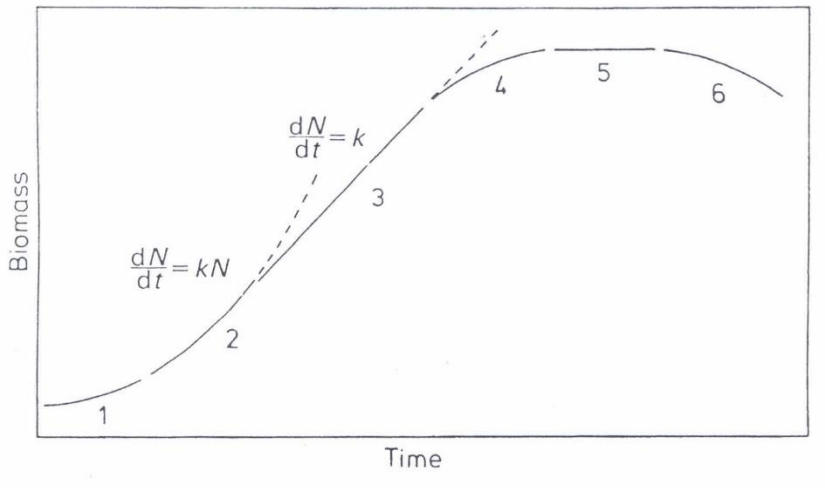


Figure 2-1: Growth Phases of Microalgae (Becker, 2008)

Adaptation phase is usually observed after inoculation, when cultivation conditions differ from that of the original environment. Lag phase is the period when microalgae adjusts to the new environment. In this stage, sensitivity of the culture to alteration in conditions such as temperature is higher than it is in latter phases.

After adaptation, algae enters exponential growth phase through accelerating growth. During exponential growth phase, light limitation does not occur and effect nutrient uptake on nutrient concentration is negligible. Since there is no substrate limitation, the amount of algae in a time increment in the batch system is proportional to the biomass concentration at the specified time interval, i.e. the cells divide with a constant rate. The overall biomass productivity, *p*, can be calculated using Equation 2-1.

$$p(\mathrm{mg/L.d}) = \frac{\mathrm{dC}}{\mathrm{dt}} \qquad (2\text{-}1)$$

0,It ca,n easily be seen that *p* is the slope of the exponential growth phase. A plot of log concentration values versus time gives a straight line as well, slope of

which is equal to the specific growth rate, μ, and can be calculated using Equation 2-2.

$$\mu\,(d^{-1}) = \frac{dC}{C\,dt} \qquad (2\text{-}2)$$

After logarithmic growth phase, cells begin to shade each other and specific growth rate starts to decrease. During this phase, there is a linear relationship between growth rate and time. In nutrient rich medium, this phase may remain over a period.

When light supply becomes limited and respiration starts to predominate, culture achieves an equilibrium between growth and decay. This stage is called stationary phase. During stationary phase, the maximum concentration that can be reached is attained.

Eventually, death rate predominates and culture enters to death phase due to nutrient and light limitation as well as over-age of culture (Becker, 2008).

2.4.3. Photosynthesis

Photosynthesis is the process in which carbon dioxide ($CO_{2)}$ is converted into sugar by plants and algae, using water (H_2O) and light (Kumar et al., 2011). The overall reaction of photosynthesis is given in Equation 2-3.

$$CO_2 + H_2O + light \rightarrow (CH_2O)_n + O_2 \qquad (2\text{-}3)$$

Required electrons for reduction of carbon dioxide to sugars are obtained from H_2O. Pigments such as chlorophylls and carotenoids absorb solar energy, which is required for breakup of water. When electrons are taken, oxygen is produced and excess protons remain. (Vunjak-Novakovic et al., 2005) These excess electrons are used during ATP synthesis.

Photosynthesis consists of two stages. In the light-dependent first step, which is termed as “light reactions” or “Phase I reactions”, energy carrier molecules namely ATP and NADPH are produced. Production of these molecules is achieved via photophosphorylation. In the light- independent second step, the

energy stored during light reactions are consumed for construction of covalent bonds between carbon atoms of hydrocarbons. Incorporation of carbon into sugars and biomass is realized in a cyclic process termed as Calvin- Benson Cycle. (Vunjak-Novakovic et al., 2005) Photosynthesis process is schematically summarized in Figure 2-2.

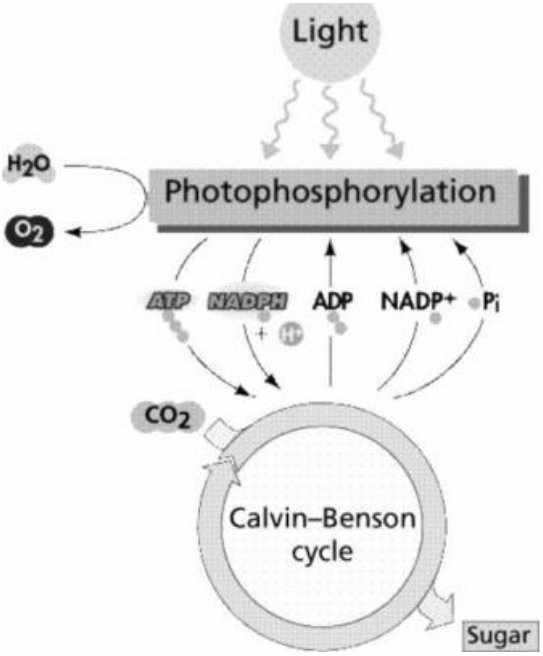


Figure 2-2: Schematic Diagram of Photosynthesis (Vunjak-Novakovic et al., 2005)

2.4.4. Environmental Factors Controlling Algal Growth

Environment in which microalgae grow has high influence on culture. Therefore, environmental factors including nutrient and carbon dioxide availability, pH, temperature and light should be at optimum levels for enhanced algal growth. (Oncel and Sukan, 2008)

2.4.4.1.Nutrient Availability

Nutrients account for 45% to 60% of dry microalgal weight (Muñoz and Guieysse, 2006). General nutrient demand of the algae can be estimated stoichiometrically, using Redfield ratio of an algae cell on an average basis, which equals to $C_{106}H_{263}O_{110}N_{16}P$. This ratio can be used to derive the

formulation given in Equation 2-4 (Anderson, 1995) and indicates that N/P ratio of algal mass is 7.2 g N/g P on average.

$$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4)+138O_2 \rightarrow 106CO_2+16HNO_3+H_3PO_4+122H_2O \quad (2\text{-}4)$$

However, cell composition and nutrient ratios usually differ, depending on the nutrient availability in the media where microalgae grow. For example, P is limiting in freshwaters and N is limiting in marine environments. These variations are especially due to the anthropogenic effects. When human manipulations were much less, N and P co-limitation was more probable and cell compositions were comparable to Redfield ratio (Moss et al., 2012). Typically, nitrogen content of microalgal cell varies between 1% and 14 %, whereas phosphorus content is between 0.05% and 3.3% (Zhou, 2010).

2.4.4.2.Carbon dioxide Availability

Dissolved carbon dioxide in water exists as an equilibrium of inorganic carbon species, namely, carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) is achieved. Concentration of each species depend on pH and temperature. Microalgae primarily uptake HCO_3^-, rather than CO_2 (Carvalho et al., 2006). General stoichiometric carbon dioxide requirement of microalgae is 1.85 g CO_2/ g biomass or higher, considering carbon fraction of biomass varying from 0.45 to 0.8 (Posten, 2009).

Carbon dioxide concentrations exceeding a certain level are inhibitory for microalgae due to reduced biological CO_2 sequestration capacity and decreased pH as a result of bicarbonate buffer accumulation (Kumar et al., 2011). Tolerable carbon dioxide concentration limits vary among microalgal species.

2.4.4.3.Oxygen Saturation

Carbon dioxide uptake is not only related to its availability, but also partial pressure gradient of oxygen, which in turn related to photosynthetic efficiency (Posten, 2009). During photosynthesis, oxygen molecule released after water is

split into its elements. Molecular oxygen remains in water, causes photo-bleaching and eventually photosynthetic efficiency of microalgae decreases (Kumar et al., 2011). Therefore, stripping of oxygen is necessary in algae cultivation systems to avoid oxygen levels above 200% (for some species, above 120 %) of air saturation level (Posten, 2009), in order to achieve efficient carbon dioxide uptake.

2.4.4.4.pH

Variation of pH has multiple effects on growth of microalgae, since physiological state of algae as well as distribution and availability of inorganic carbon species, nutrients and trace elements depend on pH (Chenl and Durbin, 1994). The pH range for growth of many microalgal species is between 7 and 9 where optimum range is often 8.2 – 8.7 (Bitog et al., 2011). However, there are some species which have higher (Su et al., 2010) or lower (De Morais and Costa, 2007) growth optima.

In poorly buffered systems, microalgal growth can change pH abruptly (Chenl and Durbin, 1994). Consumption of carbon dioxide (Chisti, 2008) and nitrate (Hulatt and Thomas, 2011) by algae causes an increase in pH. On the other hand, utilization of ammonium ions may cause pH to drop down to 3 (Larsdotter, 2006).

2.4.4.5.Temperature

In general, optimum temperature range for microalgae is 15-25 ^{o}C (Raven and Geider, 1988). Deviations above or below this range cause adverse effects on metabolism rate and enzymatic activity (Raven and Geider, 1988). However, temperature optima vary among microalgal species. Thus, when nutrient uptake is of major concern, it is possible to utilize different microalgal species in different climatic regions in order to achieve removal (Powell et al., 2008).

2.4.4.6.Light

Efficient utilization of light is closely related to its wavelength, intensity, diurnal illumination regime and light/dark cycles in medium.

Photosynthesis can be carried out by photosynthetic algae within a certain range of visible spectrum, which is termed as photosynthetically active radiation (PAR) and corresponds to wavelengths from 400 to 700 nm. Each microalgal species may utilize a certain portion of this wavelength range (Pecegueiro do Amaral, 2012).

Photosynthetic algae are typically cultured in the laboratory under light intensities in the range of 100-200 μmol sec^{-1} m^{-2}, which is approximately equal to 5-10% of full daylight (Cormier, 2010). Due to light absorption and self-shading in high-density cultures where biomass concentration exceeds 0.1 g/L (Chiu et al., 2011), light intensity is inversely proportional with the distance from irradiated surface. This situation leads to formation of light gradient and dark zones in culture media. As a result of mixing, each algal cell is transported across the light and dark zones and exposed to light/dark cycles. This intermittent exposure to light improves photosynthetic efficiency, compared to continuous light and is called "flashing light effect" (Barbosa et al., 2003).

In nature, photosynthesis is a diurnal process and occurs only when sunlight is available. Therefore, many studies mimicked environment and practiced 16:8 (Janssen et al., 2000; Barbosa et al., 2003; Perner-Nochta et al.,2007), 15:9 (Powell et al., 2008), 14:10 or 12:12 (Watanabe and Saki, 1997) day: night cycles for cultivation of microalgae. On the other hand, there are several studies which reveal efficiency of continuous illumination (Joel and Hall, 1995; Watanabe et al. 1997; De Godos et al., 1998; Tredici and Zittelli, 1998; González et al.,2009; Wang et al., 2010; He et al., 2012), which is an illumination regime that can be used for photobioreactors with artificial light source, but not feasible for large-scale algal ponds (Stewart and Hessami, 2005).

2.4.5. Properties of *Chlorella vulgaris* Cultures

Chlorella vulgaris is a spherical microalga from genus of single-cell green algae, *Chlorella*. It has a diameter of 2 to 10 μm and has no flagella. It has high photosynthetic efficiency, productivity and adaptable to severe environmental conditions. Nutrient availability, carbon dioxide availability, pH, temperature and light are important environmental factors for *Chlorella vulgaris* growth.

2.4.5.1.Nutrient Availability

Stoichiometric equation of *C. vulgaris* growth was reported by Roels (1983) as:

$$CO_2 + {}_{0.148}NH^+_4 + 0.006\ H_2SO_4 + 0.10\ H_3PO_4 + 0.57\ H_2O \rightarrow$$

$$CH_{1.776}O_{0.459}N_{0.148}S_{0.006}P_{0.010} + 1.088O_2 \qquad (2\text{-}5)$$

Considering the outcome of Equation 2-5, Optimum N/P ratio on mass basis for growth of *Chlorella vulgaris* is 7; slightly lower than Redfield Ratio of 7.2 on mass basis.

Chlorella vulgaris can utilize nitrogen sources such as NH_4^+ and NO_3^- . Among nitrogen sources, ammonia is primarily consumed (Smith and Thompson, 1971).

2.4.5.2.Carbon dioxide Availability

Dissolved CO_2 concentrations in growth medium directly affect growth rates of *Chlorella vulgaris*. can survive in atmospheres with carbon dioxide levels of 0.03 to 40% carbon dioxide (Hirata et al., 1996). It has been found that the optimum carbon dioxide concentration for *Chlorella vulgaris* is 10% by volume (Powell et. al., 2009).

2.4.5.3.pH

Optimum pH range for the cultivation of *Chlorella vulgaris* is 5.5 to 7. However, effect of higher pH values on growth is minimal (Powell et al., 2009). On the other hand, at pH 4, growth rate of *Chlorella vulgaris* decreases. These species are not able to grow at all at pH 2 (Edberg, 2010).

2.4.5.4.Temperature

Chlorella vulgaris has an optimum temperature range of 20 – 30 ^{0}C (Hirata et al., 1996), where the growth rate is maximum at 30 ^{0}C and decreases above this value.

2.4.5.5.Light

Optimum light spectra for *Chlorella vulgaris* are blue (420 – 450 nm) and red (660 – 700). Among many available light sources, fluorescent lamps are the most common light source used for the cultivation of *Chlorella vulgaris*, since they are cost effective, efficient and stable (Sasi, 2009).

2.4.5.6.Nutrient Removal Potential of Chlorella vulgaris

Chlorella vulgaris has been reported to utilize nitrogen and phosphorus from Industrial, agricultural and municipal wastewaters, when they are used as medium source of nutrients. Several studies have been conducted to investigate nutrient removal potential of *C. vulgaris*.

Yun et. al. (1997) investigated cultivation of *Chlorella vulgaris* in steel-making plant wastewater. It was revealed that *Chlorella vulgaris* was able to remove 100% of ammonia, by addition of external phosphate salts. After ammonia was depleted, 50% nitrate removal was also achieved.

Wang et. al. (2010) investigated semi-continuous cultivation of *Chlorella vulgaris* in undigested and digested dairy manures and demonstrated that the species are efficient in terms of nutrient removal. Results of the study revealed that 99.7%, 89.5%, 92.0% and 75.5% NH_4^+-N, TN, TP and COD removal from undigested dairy manure was achieved respectively, at a hydraulic retention time (HRT) of 5 days. For digested dairy manure, 100% NH_4^+-N, 93.6% TN, 89.2% TP and 55.4% COD removal was achieved at 20 day HRT.

Another study conducted by Wang et al. (2010) determined nutrient removal potential of *Chlorella vulgaris* from different wastewaters of municipal

wastewater treatment plant. The results of this study showed that *Chlorella vulgaris* is capable of treating 82.4% NH_4^+-N , 68.4% TN, 83.2% TP and 50.9% COD from the wastewater before primary settling. Percent removal values from wastewater after primary settling were 74.4, 68.5, 90.6 and 56.5 for NH_4^+-N, TN, TP and COD, respectively. Effluent from aeration tank was also treated using *Chlorella vulgaris* and NO_3^--N, TN, TP and COD removal percentages were found to be 62.5, 50.8, 4.69 and 22.7, respectively. Finally, nutrient removal potential from centrate from sudge centrifuge was reported as 78.3, 82.8, 85.6 amd 83.0 for NH_4^+-N, TN, TP and COD respectively.

Changling Li et al. (2013) conducted batch, modified semi- continuous and continuous cultivation experiments of *Chlorella vulgaris* in municipal effluent. It was found that NH_4^+-N , total nitrogen, total phosphorus, COD and BOD_5 can effectively be removed by 98.0%, 90.3 – 93.6%, 89.9 – 91.8%, 60.7, 90.0% and 83.4 – 88.4% respectively.

Results of another study, in which free and immobilized *Chlorella vulgaris* were used, revealed that free and immobilized microalgae respectively could achieve 60.1% and 80% ammonia removal from urban wastewater. (Ruiz-Marin et al., 2010)

2.4.6. Microalgae Cultivation Systems

Cultivation of algae can be provided in both open and closed systems. Most commonly, wastewater treatment systems are open, whereas closed systems are preferred for production of unialgal or controlled mixed-algal species. In this section, basic properties of open and closed microalgae cultivation systems, as well as their most common types are presented.

2.4.6.1.Open Systems

Open ponds are commonly used for microalgae cultivation. Types of ponds that are most commonly used in research and commercialized are raceway, shallow, circular and closed ponds and tanks. Since open pond systems are not well-

controlled, environmental conditions where a pond is located determines the amount of light for photosynthesis, temperature and pH; in turn, contributes to the success of cultivation. Open ponds are usually limited in terms of light, temperature, pH and oxygen concentration. In addition, there is a high risk of contamination by predators. Therefore, only some strains, which favor severe conditions, can be cultivated successfully; such as *Chlorella* species, which require high nutrition. On the other hand, open ponds are cost effective in terms of operation and maintenance, less technical and more stable, compared to closed systems (Harun et al., 2010).

2.4.6.2.Closed Systems

Photobioreactors can be defined as the systems, which are used for production of phototrophic microorganisms. Closed- system photobioreactors enable production of high-density microbial cultures by provision of nutrients, water, ideal temperature, light and pH level and mixing regime, which are necessary for microalgae. Different geometries and operation methods have been developed considering local and economic conditions and the desired end products (Posten, 2009).

Despite high investment cost and technical problems faced during sterilization, there are also advantages of microalgae cultivation in photobioreactors such as high efficiency under controlled conditions, high surface – to – volume ratio, gas transfer control, decrease in evaporation, equal temperature distribution, better protection against external pollution, less contamination (Pulz, 2001).

2.4.6.2.1. Important Factors Influencing Microalgal Growth in Closed Systems

Light penetration, gas injection and mixing are the key factors for growth of microalgae.

Light penetration:

Inner illumination of photobioreactors affects biomass composition, growth rate and end products (Bitog et al., 2011).

Gas Injection:

In photobioreactors, provision of sufficient carbon dioxide to microalgae and removal of the produced oxygen away from the medium is important. Carbon-dioxide demand of a culture can be calculated stoichiometrically, considering the carbon content of the biomass. The required amount of carbon dioxide is usually supplied with carbon dioxide enriched gas bubbles (Posten, 2009). This application helps removal of oxygen from the medium, as well as mixing within the photobioreactor (Bitog et al., 2011). The gas used during aeration process usually contains 5-10 % of carbon dioxide (v/v) (Ho et al., 2011).

Mixing:

Mixing plays an important role in distribution of optical density, pH and carbon dioxide. In addition, mixing is necessary to avoid microalgal accumulation and growth on the surface of the photobioreactor. Mixing also helps even light and nutrition exposure of microalgal cells and provides enhanced air and gas exchange between media (Hu et al., 1996; Luo and Al-Dahhan, 2004; Carvalho et al., 2006).

In order to develop higher performance photobioreactors, the following conditions should be provided (i) minimization of non-illuminated part volumes; (ii) even illumination of culture surface; (iii) rapid mass transfer of carbon dioxide and oxygen; (iv) frequent mechanical cleaning and sterilization; (v) high mass transfer rate; (vi) minimization of illumination and mixing energy requirements (Harun et al., 2010).

2.4.6.2.2. Photobioreactor types

Most commonly used photobioreactors for laboratory scale and large scale cultivation of microalgae are flat plate, tubular and vertical column photobioreactors.

Flat Plate Photobioreactors:

Due to their high illumination surface area, flat plate photobioreactors drew attention for photosynthetic microorganism cultivation. In order to gain maximum benefit from solar energy, Flat plate photobioreactors are constructed using transparent materials (Ugwu et al., 2008). Despite culture temperature control and scaling up problems and potential of excessive hydrodynamic stress for some microalgal species, utilization of flat plat photobioreactors have various advantages including high illumination surface area, suitability for outdoor cultivation, high biomass productivity and photosynthetic efficiency, low cost, ease of cleaning and low oxygen deposition.

Tubular Photobioreactors:

Tubular photobioreactors are one of the most suitable photobioreactors for outdoor cultivation, owing to their high illumination surface areas and low investment costs. On the other hand, mass transfer in these reactors are relatively poor. In addition, tubular photobioreactors have large land requirement (Ugwu et al., 2008). This reactors, easily reached high oxygen levels but temperature control is difficult (Molina et al., 2001). Tubular photobioreactors are present in horizontal / helical (Chaumont, 1993; Molina et al., 2001), vertical (Tredici and Zittelli, 1998) horizontal, conical (Watanabe and Saki, 1997) and inclined (Ugwu et al., 2008) forms.

Vertical Column Photobioreactors:

Vertical Column photobioreactors are compact, easily operated and low cost reactors (Sanchez et al., 2000). In addition, high mass transfer, low energy

consumption, high scale – up potential, effective mixing, ease of sterilization and low photo inhibition risk and photo oxidation are the basic advantages of vertical column photobioreactors. On the other hand, low illumination area and quality of construction material can be considered as the limitations of this photobioreactor type (Ugwu et al., 2008).

Many photobioreactors can be operated in laboratory, only a few of them could be transferred into outdoor conditions. For example, bubble column and flat plate reactors are used in laboratory conditions; vertical tubular reactors are also present in pilot scale. Culture compartment size, height, diameter and number increase, scale of photobioreactors can be increased (Ugwu et al., 2008). In Tubular photobioreactor, transparent tubes can be expanded in large-scale applications such as bioreactors in different shapes, i.e. increasing numbers of vertical tubes larger scales. At the present, only tubular reactors are commercially available, since technical limitations prevent other types from large-scale applications.

2.4.7. Use of Microalgal Biomass as a Renewable Energy Source

Microalgae biotechnology has been developed for a variety commercial applications including food and cosmetic purposes (Spolaore et al., 2006) , pharmaceutical industries (García-Casal et al., 2009) and nutrient supplements (Harun et al., 2010). Apart from consumer products, owing to their high lipid contents, microalgae can be used for production of biofuels. Extracted oil from microalgal biomass can be converted into biodiesel (Mata et al., 2010). The cell residues can then be converted into biomethane (Vergarafernandez et al., 2008), bioethanol (Sukahara and Awayama, 2005) or biohydrogen (Mussgnug et al., 2010).

2.4.7.1.Biodiesel

Biodiesel is obtained from vegetable oils and animal fats with triglycerides, which consist of three fatty acid chains and a glycerol molecule. Biodiesel is formed by replacement of the glycerol molecule with methanol. Glycerol is then

removed from biodiesel by phase separation. In order to become a substitute for fossil fuel, biodiesel should have several properties such as (i) adequacy of commercial feedstock production; (ii) comparability to conventional fossil fuel costs; (iii) satisfaction of standard fuel quality. Considering these properties, microalgae are smart alternatives as biodiesel feedstock, owing to their high growth rate and oil content (Song et al., 2008, Harun et al., 2010).

2.4.7.2.Bioethanol

Biomass is commonly converted into bioethanol: (i) biochemically (fermentation) and (ii) thermochemically (gasification). Most commonly used biomass feedstocks for bioethanol production are corn and sugar cane. Apart from biofuel applications, these feedstocks have high food values and land requirements. Therefore, production and utilization capacity of these feedstocks are limited (Sun and Cheng, 2002). Ethanol production from microalgae fermentation is therefore gaining interest, considering high carbohydrate and protein content of the biomass. However, amount of research conducted on bioethanol production from microalgae is rather limited.

Fermentation process is simpler and less energy intensive than biodiesel production process. In addition, carbon dioxide produced as a result of fermentation process can be recycled into the system for microalgal uptake. However, bioethanol production technology from microalgae is under development and has not been commercialized (Harun et al., 2010)

2.4.7.3.Biomethane

Biomethane production from microalgal biomass has received attention, since biogas obtained from anaerobic digestion can be used for electricity generation (Holm-Nielsen et al., 2009). In addition, biomass residues after anaerobic digestion can be converted into fertilizers. Utilization of these fertilizers provide sustainable agriculture and reduce production costs of microalgae.

Since cellulose and lignin contents of microalgae are almost zero, anaerobic process stability and digestion efficiency are high. However, production cost of methane is still higher than other feedstocks. Therefore, integrated wastewater treatment and biomethane production can be the most feasible approach to reduce production cost (Harun et al., 2010). This process was first discussed by Oswald and Gotaas (1957), where algae was used for wastewater treatment in ponds and then harvested for methane production through anaerobic digestion. This system could eliminate negative effects of nutrients such as eutrophication on receiving environments, providing efficient nutrient treatment.

In conclusion, when coupled with carbon dioxide sequestration and wastewater treatment, microalgae can provide possible solutions to environmental problems and simultaneously create valuable consumer products and biofuels (Harun et al., 2010).

2.4.7.4. Microalgae Harvesting for Biofuel Production

Efficient harvesting of biomass is essential for mass production of biofuels from microalgae. The major harvesting techniques include centrifugation, flocculation, filtration and screening, gravity sedimentation, flotation, and electrophoresis techniques (Uduman et al., 2010). The cost of algae harvesting can be high, since the mass fractions in culture broth are generally low, while the cells normally carry negative charge and excess algogenic organic matters (AOM) to keep their stability in a dispersed state (Danquah et al., 2009). The selection of harvesting technique is dependent on the properties of microalgae.

Golueke and Oswald (1965) compared algae removal using filtration, flotation, centrifugation, precipitation, ion exchange, passage through a charged zone, and ultrasonic vibration. They concluded that only centrifugation and chemical precipitation are economically feasible options, with centrifugation being marginally better. (Chen et. al, 2011).

2.5. Anaerobic Digestion

Anaerobic digestion (AD) is a process that involves organic matter (substrate) decomposition in the absence of molecular oxygen (O_2) by microorganisms, which results in production of methane (CH_4), CO_2 and inorganic nutrients (Mccarty, 1964a). Anaerobic treatment of wastes typically end up with conversion of organic matter into biogas, which consists of 20-30 % of CO_2, 60-79 % of CH_4, 1-2% of hydrogen sulfide (H_2S) and other gases (Parkin and Owen, 1987; Sperling, Andreoli, and Von, 2007; Verma, 2002; Yılmazel, 2009). In these systems, up to 90 percent of the organic portion of waste can be converted into methane (Mccarty, 1964b).

AD consists of four consecutive steps, namely, hydrolysis (liquefaction), acidogenesis, acetogenesis and methanogenesis, which involve bacterial flora adapted to anaerobic environment.

The hydrolytic bacteria excrete hydrolytic enzymes that break down complex organics into simpler forms, such as sugars, long chain fatty acids (LCFA) and amino acids. This step is rate limiting for substrates with high solid contents. In this stage, stabilization of waste does not occur (Parkin and Owen, 1987).

After the organic matter is solubilized in the first stage, Fermentative Acidogenic Bacteria provide conversion of hydrolyzed waste into acetic, propionic, butyric and other short chain fatty acids, as well as alcohols.

Fermentative acetogenic bacteria convert volatile fatty acids synthesized in the previous phase into hydrogen, acetate and carbon dioxide. Elevated hydrogen concentrations cause inhibition of methane formation and increase in organic acid concentrations, therefore play an essential role in methane formation (Parkin and Owen, 1987).

Acetogens are slow-growing bacteria and not resistant to abrupt organic load or physical changes. (Parawira et al., 2004). In the final stage of AD, Methanogens simultaneously produce biogas from the end product of previous stage.

Methanogens are strict anaerobes and sensitive to environmental conditions (McCarty, 1964a; Speece, 2008)

Figure 2-3 illustrates sequential stages of AD (McCarty, 1964a)**.**

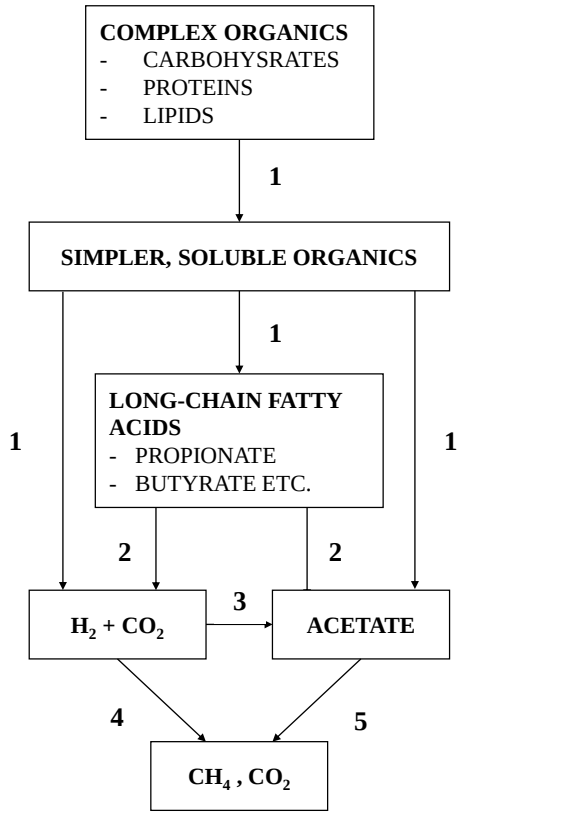


Figure 2-3: Sequential stages of AD process

2.5.1. Important Environmental Factors in Anaerobic Digestion

Anaerobic degradation efficiency is directly related to the balanced microbial activity during AD process. Operating parameters, which must be controlled to achieve optimum growth of microbial flora, are discussed in this section.

2.5.1.1.Nutrients

Inorganic nutrients are essential for growth and maintenance of both aerobic and anaerobic microorganisms. Major nutrients that must be supplied in sufficient amounts are N and P. Nitrogen is required basically for protein and amino acid synthesis, where phosphorus is necessary for the synthesis of nucleic acid and energy structures. Hence, nutrients, especially nitrogen and phosphorus, must be provided sufficiently for a balanced AD (Speece, 2008). The optimum C/N ratio for AD is 20/1 to 30/1 (Yen and Brune, 2007) and N/P ratio is 5/1 to 7/1 (Parkin and Owen, 1987). Other than nitrogen and phosphorus, there are other nutrients such as iron, nickel, cobalt, sulfur, calcium, and some trace organics, which are required in lower amounts. Compared to aerobic systems, anaerobic systems usually require less nutrient supply, however, in some cases, external source may be necessary.

Another parameter that describes nutrient availability in anaerobic digestion process is COD/N/P ratio. For lightly loaded systems, COD/N/P ratio of 350/7/1 is recommended, where this ratio is 1000/7/1 for highly loaded systems. High C/N or COD/N ratio in anaerobic digesters may cause rapid acidification, if the biodegradable portion of waste is high. On the other hand, low C/N or COD/N ratio may cause ammonia accumulation is the system, which may cause toxicity and inhibit AD process (Speece, 2008). Free ammonia (NH_3) is the toxic form in high ammonia loaded systems and its concentration is a function of total ammonia concentration ($NH_4^+ + NH_3$), temperature, pH and pressure (CO_2). Therefore, any increase in pH or temperature causes increase in free ammonia fraction; increase in gas pressure decrease this fraction since pH is lowered due to CO_2. In systems with no acclimation, inhibitory concentration of free ammonia can be as low as 80- 200 mg/L. Inhibition is also linked total ammonia concentration. In processes with total ammonia-N concentrations of 1.5 – 7 g/L has been revealed to be toxic; however, up to 3 – 4 g/L total ammonia-N concentrations can be tolerated after adaptation (Nielsen and Angelidaki, 2008).

2.5.1.2.pH

AD process involves a variety of microorganisms, majority of which is inhibited by acidic conditions. Especially, growth of methanogens is strictly dependent upon pH. Optimum pH interval for AD process is 6.6 – 7.6 (McCarty, 1964a). Unless system is well- buffered, high amounts of organic acid deposition create a tendency towards pH levels lower than 6, which is inhibitory for methanogenic activity.

In AD process, bicarbonate system is the predominant alkalinity species that create buffering capacity, which suppress pH drop. On the other hand, excessive presence of alkalinity may also cause damage by favoring ammonia-N toxicity (Parkin and Owen, 1987). Alkalinity concentrations between 2000 and 4000 mg/L is typically sufficient to sustain neutral pH (Tchobanoglous et al., 2003).

2.5.1.3.Temperature

Three temperature ranges are used for anaerobic digesters, namely, psychrophilic (0-20 ^{o} C), mesophillic (30-38 ^{o} C) or thermophillic (50-60 ^{o} C) (McCarty, 1964a). Most conventional digesters are operated in mesophillic range (Parkin and Owen, 1987).

2.5.1.4.Toxicity

Toxicity of a substance depends on its nature, concentration and acclimation of system. Generally, many substances are tolerable at low concentrations but become inhibitory as their concentrations increase. Alkali and alkaline earth-metals, heavy metals, ammonia-nitrogen, sulfide and some other inorganic and organic compounds such as sodium, potassium, calcium, magnesium, copper, chromium, nickel, formaldehyde, chloroform, ethyl benzene, ethylene dichloride, kerosene, and detergents are toxic to anaerobic digestion. Microorganisms can improve their resistance to toxic compounds by acclimation (Parkin and Owen, 1987).

2.5.1.5.Mixing

Mixing has dual effect in AD. First, in anaerobic digesters formation of scum and dead zones can be avoided by mixing. Second, mixing assures that methanogens are exposed to the substrate (Speece, 2008).

2.5.2. Pretreatment Methods for Enhanced Anaerobic Digestion

Hydrolysis of the particulate organic matter is usually the rate-limiting step in anaerobic digestion. Aim of pretreatment is to facilitate hydrolysis of wastes with high solids contents. Pretreatment methods include but not limited to single or combined application of : (i) physical pretreatment , such as milling and grinding; (ii) physicochemical pretreatment; such as steam, thermal, hydrothermolysis and wet oxidation; (iii) chemical pretreatment, such as alkali, acid or oxidizing agents; (iv) biological and electrical pretreatment.

Each pretreatment method brings various benefits and shortcomings. However, thermal pretreatment and its combination with alkali pretreatment has been proven to be effective for many feedstocks such as corn stover and municipal organic wastes and complex materials (Liu, 2010).

2.5.3. Anaerobic Co-digestion of Wastes

Apart from environmental factors, waste characteristics strongly influence the efficiency of anaerobic digestion process. If initial waste characteristics, such as the ratio between carbon and nitrogen are not suitable for achieving desired level of treatment, co-digestion of wastes can improve AD efficiency.

Co-digestion is the simultaneous digestion of more than one substrate as a homogeneous mixture, with the aim of improving AD efficiency (Alvarez et al., 2010). Most commonly, major amount of a substrate is mixed with additional substrates in minor amounts. Digestion of co-substrates usually improve biogas yield (Mata-Alvarez et al., 2000) and decrease the cost (Callaghan et al., 2002) of AD process.

Anaerobic co-digestion process was investigated for animal wastes (Callaghan et al., 2002; Güngör-Demirci and Demirer, 2004; Misi and Forster, 2001;

Umetsu et al., 2006; Yılmazel and Demirer, 2009), food industry wastes (Carucci et al., 2005), organic fraction of municipal solid waste (Sundarajan et al., 1997), fish wastes (Mshandete et al. 2004), potato waste (Parawira et al., 2004) and wastewater sludge (Carrère et al., 2010). Studies conducted on co-digestion of algae are given in Section 2.5.4.2.

2.5.4. Anaerobic Digestion of Microalgae

The technical feasibility data on the anaerobic digestion of algal biomass have been reported for many species of microalgae. The following cultures have been successfully used for the production of methane: the mixed culture of *Scenedesmus spp*. and *Chlorella spp*., the mixed culture of *Scenedesmus spp.*, *Chlorella spp*., *Euglena spp.*, *Oscillatoria spp.*, and *Synechocystir sp.*, the culture of *Scenedesmus sp*. alone, and together with either *Spirulina sp*., *Euglena sp*., *Micractinium sp*., *Melosira sp.*, or *Oscillatoria sp*.(Mussgnug et al., 2010).

Gouleke et al. (1957) studied AD of mixed microalgal culture obtained from a wastewater treatment pond. Results of this study revealed that biogas yield of 412 mL/g VS with methane content of 61.1% can be achieved with 30 days HRT and at 35^{o}C digestion temperature.

In another study conducted by Chen (1987) it was reported that biogas yield and methane content obtained by AD of microalgae as 418.3 mL/g VS and 73% respectively for mesophillic digestion at 35^{o}C with an HRT of 28 days. In the same study, anaerobic digestion of microalgae at 40^{o}C revealed biogas yield and methane content as. 493.2 mL/g VS and 62.1% respectively.

Mussgnug et al. (2010) used several microalgal species for mesophillic AD at 35^{o}C. The results were as follows for microalgal species (biogas yield ; methane content): *Arthrospira platensis (*481 ± 13.8 mL/g VS; 61%), *Chlamydomonas reinhardtii (*587 ± 8.8 mL/g VS; 66*%), Chlorella kessleri (*335 ± 7.8 mL/g VS; 65%*), Dunaliella salina (*505 ± 24.8 mL/g VS; 64%*), Euglena gracilis (*485 ± 3 mL/g VS; 67%*), Scenedesmus obliquus (*287 ± 10.1 mL/g VS ; 62%*).*

2.5.4.1.Pretreatment

The biodegradation of algal biomass can be improved by different pretreatment methods. Some researchers investigated different pretreatment methods for enhanced methane production such as microwave pretreatment (Passos et al., 2013); ultrasound pretreatment (Alzate et al., 2012); thermal pretreatment including drying (Mussgnug et al., 2010), heating (Chen and Oswald, 1998), thermal hydrolysis (Alzate et al., 2012) or high pressure thermal hydrolysis (Keymer et al., 2013); chemical pretreatment (Chen and Oswald, 1998) and biological pretreatment (Alzate et al., 2012).

Passos et al. (2013) conducted a study evaluate the effect of microwave pretreatment on the solubilization and anaerobic digestion of microalgae obtained from high rate algal ponds. In BMP assays, final biogas yields increased between 12–78% after pretreatment. Solubilization and methane production were found to have correlation. It was concluded that microwave irradiation improved disintegration and digestibility potential of microalgae.

Alzate et al. (2012) compared three pretreatment methods, namely, thermal, ultrasound and biological, in terms of improvement potentials in methane production. For this purpose, variety of microalgal species were subjected to pretreatment. Samples were heated by 9 bar steam injection into a stainless vessel for thermal pretreatment; energy was supplied to biomass with an ultrasonic probe for ultrasound preteratment; and biological pretreatment was performed by incubation of samples for 24 h after being placed in glass bottles and capped. Results of this study revealed that the effect of biological pretreatment on enhancement of methane production was minor; whereas the highest improvement of 46-62% was observed among samples subjected to thermal hydrolysis. Ultrasound pretreatment resulted in 6-24% increase in methane productivity. It was concluded that direct correlation between solubilization and methane enhancement did not exist and concluded that anaerobic digestion of algae after thermal pretreatment is a promising technology for renewable energy production.

Chen and Oswald (1998) investigated heat, chemical and thermochemical pretreatment of microalgae obtained from effluents of high-rate stabilization pond for enhanced methane production during AD process. The variables of the study were pretreatment temperature, duration, concentration, and dosage of sodium hydroxide (NaOH). They found that the heat pretreatment of algal sludge at 100 °C for 8h could improve the efficiency of methane fermentation a maximum at 33%. However, the improvement on the methane energy produced would not be economically competitive to the energy lost on the heat pretreatment.

Keymer et al., (2013) subjected algae to high-pressure thermal hydrolysis. Pretreatment was applied to both raw algae and after lipid extraction was performed to algae. High-pressure thermal hydrolysis increased methane yield by 81%.

Mussgnug et al. (2010) investigated drying as a pretreatment for algae before anaerobic digestion. They indicated that drying decreased the amount of biogas production by 20 % and complete drying at high temperatures should be avoided since the biogas potential decreases significantly. In general, they concluded that cell disintegration was not efficient without pretreatment.

2.5.4.2.Co-digestion

The low C/N ratio of microalgae is a serious problem for AD process. Low C/N ratio in digesters may result in inhibition caused by high total ammonia nitrogen release and high volatile fatty acids (VFAs) accumulation. Therefore, algae can be subjected to anaerobic digestion not only as the sole substrate but also co-digested with other substrates with higher carbon contents such as sewage sludge (Chen, 1987), organic fraction of municipal solid waste (Spierling, 2011) and waste paper (Yen and Brune, 2007).

Yen and Brune (2007) co-digested microalgae (*Scenedesmus* spp. And *Chlorella* spp.) with waste paper with 10 days HRT and obtained biogas yield of 100-140 mL/g VS. It was also revealed in this study that the optimum C/N ratio was

20:1 – 25:1 for methane production. Paper addition increased methane content up to 50%.

Considering their compositions, low cost and availability, kitchen waste and waste activated sludge are two smart alternatives for co-digestion of microalgae.

2.5.4.2.1. Kitchen Waste

Kitchen waste (KW) is the one of the major component of municipal solid waste (MSW), with high moisture and organic matter content (Tchobanoglous et al., 2003). AD process is widely used for treatment of organic wastes such as organic fraction of municipal solid waste (OFMSW) and food waste as sole feedstock. Nutrients and buffering agents are significant in AD of cellulose-poor KW, since hydrolysis and acidification occur very rapidly in these systems due to high biodegradable organic content (Vandevivere et al., 2003). Co-digestion is an alternative in order to provide necessary elements, without addition of nutrients or buffering agents (Li et al., 2009). In turn, owing to high COD/N value of KW and low solids deposition in AD systems, co-digestion with microalgae is a feasible alternative for a synergetic effect in AD process (Spierling, 2011).

Zhao and Ruan (2013) investigated co-digestion of Taihu algae with kitchen wastes, in order to improve biogas production potential. The results indicated that the biogas yield reached 388.6 mL/g TS, which was a value 1.29 and 1.18 times higher than algae and kitchen wastes only. It was also reported that optimum C/N for co-digestion was15:1.

2.5.4.2.2. Waste Activated Sludge (WAS)

Due to relatively higher C:N ratio ranging between 6:1 and 16:1 (Tchobanoglous et al., 2003), one potential co-substrate for AD of microalgae is WAS. Although this ratio does not provide optimum condition for AD, availability and low cost of WAS as co-substrate is advantageous.

Chen (1987) co-digested 50/50 sludge and algae at mesophillic conditions with 28 day HRT and reported biogas yield and methane content as 290.9 mL/g VS and 64% respectively. In the same study, thermophillic co-digestion was also

investigated. It was revealed that 449.5 mL/g VS biogas yield could be achieved with 66% methane content at 40^{o}C with 28 day HRT. For the same HRT and co-digestion fraction, 299.7 mL/g VS and 62% methane content was observed at 55^{o}C.

CHAPTER 3

MATERIALS AND METHODS

In this study, the experimental design consists of two parts: (1) Microalgal biomass production and nutrient removal studies, which aim investigation of *Chlorella vulgaris* growth in a photobioreactor using nutrients in municipal wastewater; (2) BMP studies, which aim determination of anaerobic degradability and biogas production potential of raw and pretreated microalgal slurry harvested from photobioreactor effluents, as well as its co-digestion.

Analytical methods, inocula used and experimental sets and procedures followed throughout this study are presented in this section.

3.1. Inocula

3.1.1. Microalgae

Unialgal culture of *Chlorella vulgaris* (CCAP 211/11B) was obtained from Culture Collection of Algae and Protozoa, England (URL:http://www.ccap.ac.uk/ strain_info.php?Strain_No=211/11B). Prior to experiments, culture was enriched as presented in Section 3.3.1.1.1.

3.1.2. Anaerobic Cultures

Mixed anaerobic cultures were obtained from the anaerobic sludge digesters of Greater Municipality of Ankara Tatlar Domestic Wastewater Treatment Plant. Characterization of the seed culture is given on Table 3-1:.

Table 3-1: Characterization of Anaerobic Seed Used in BMP Assay

Parameter	Anaerobic Seed
TS (mg/L)	38900 ± 566
VS (mg/L)	13300 ± 0.0
VS as %TS	32.6
TSS (mg/L)	36586 ± 99
VSS (mg/L)	11913 ± 103
VSS as %TSS	32.6
tCOD (mg/L)	19762 ± 12
TKN (mg/L)	1072 ± 67
TP (mg/L)	350 ± 2.6
sCOD (mg/L)	245 ± 0.8
TAN (mg/L)	146 ± 4.5
ortho-P (mg/L)	67 ± 0.9
COD/TKN	18.4

3.2. Analytical Methods

Throughout this study, pH, temperature, DO, light intensity, OD, TS, VS, TSS, VSS, tCOD, sCOD, TKN, TN, TAN, NO_3^- - N, TP and PO_4^{3-} -P were measured. Apart from measurements, microscopic examination of microalgal cultures, as well as gas and VFA analyses of batch anaerobic digesters were performed.

3.2.1. pH

pH values were measured using a pH meter (Eutech, CyberScan pH510, Nijkerk, The Netherlands) and pH probe (Sensorex, p350, Garden Grove, CA, USA).

3.2.2. Temperature

Temperature values of microalgal growth and nutrient removal photobioreactors were determined using a submerged thermometer (Sensorex, p350, Garden Grove, CA, USA).

3.2.3. Dissolved Oxygen

DO values in photobioreactors were measured using DO Meter (Extech,407510A, Waltham, MA, USA)

3.2.4. Light Intensity

Light intensity in culture medium was measured using a light meter (Li-Cor, 250 A, Lincoln, Nebraska, USA) with an underwater quantum sensor.

3.2.5. Optical Density

Optical density of any sample was measured using macro-cuvettes and spectrophotometer (HACH, DR 2800, Berlin, Germany) at 685 nm wavelength with 1cm light path. The optimum wavelength value for measurement was determined by reading optical density values at different wavelengths within the range of 450 to 800 nm. 685 nm was found to reveal the highest absorbance value within the specified wavelength range (Ras et. al., 2011). Since absorbance values below 0.1 and above 1 are not reliable sample dilution was made if necessary.

3.2.6. Total Solids, Volatile Solids, Total Suspended Solids and Volatile Suspended Solids

TS, VS, TSS and VSS values were determined according to Standard Methods 2540 (APHA, 2004). Suspended portion of any sample was obtained on glass fiber filters (Millipore, AP40, Billerica, MA, USA) using a vacuum filtration unit (Millipore, WP8 11 2250, MA, USA).

3.2.7. Total Chemical Oxygen Demand

tCOD values were determined according to Standard Methods 5220 (APHA, 2004).

3.2.8. Soluble Chemical Oxygen Demand

Soluble portion of any sample was obtained by filtration through 0.45 μm cellulose- acetate filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250, Billerica, MA, USA) sCOD values were determined using Micro-COD method, using medium-range (0 – 1500 mg/L COD) and low-range (0-150 mg/L COD) test kit vials (Lovibond, Aqualytic, Dortmund, Germany). Vials were heated up to 150^{o} C, digested for 120 minutes and cooled to ambient temperature prior to COD value detection using a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany).

3.2.9. Total Kjeldahl Nitrogen

TKN values were determined according to Standard Methods 4500-N_{org} Macro Kjeldahl Method (APHA, 2004).

3.2.10. Total Soluble Nitrogen

Soluble portion of any sample was obtained by filtration through 0.45 µm cellulose- acetate filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, AP40, Billerica, MA, UISA). TN values were determined using test kit vials (Lovibond, Vario 535560, Aqualytic, Dortmund, Germany). After preparation of samples, TN values were detected using a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany).

3.2.11. Total Ammonifiable Nitrogen

Soluble portion of any sample was obtained by filtration through 0.45 µm cellulose- acetate filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250, Billerica, MA, UISA). TAN (NH_4^+-N + NH_3-N) values were determined using test kit vials (Lovibond Vario 535600, Aqualytic, Dortmund, Germany). After addition of samples, TAN values were detected using a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany) within 20 minutes.

3.2.12. Nitrate – N

Soluble portion of any sample was obtained by filtration through 0.45 µm cellulose- acetate filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250). NO_3^- -N values were determined using test kit vials (Lovibond Vario NitraX 535580, Aqualytic, Dortmund, Germany). After addition of samples, NO_3^- -N values were detected using a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany) within ten minutes.

3.2.13. Total Phosphorus

Total Phosphorus analyses were performed according to Standard Methods, 4500-P. Persulfate digestion was applied to each sample and ascorbic acid method was applied for solubilized ortho-phosphate determination (APHA, 2004).

3.2.14. Ortho- Phosphate

Soluble portion of any sample was obtained by filtration through 0.45 μm cellulose- acetate filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250, Billerica, MA, UISA). PO_4^{3-}- P values were determined using Lovibond phosphorus tablet pack (Lovibond, Vario 515810, Aqualytic, Dortmund, Germany). After preparation of samples, PO_4^{3-}- P values were detected using a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany).

3.2.15. Microscopic Analysis

Microscopic analyses were conducted using Automated Inverted Microscope for Life Science Research (Leica, DMI4000 B, Buffalo Grove, IL, USA) using Utermohl Method (Paxinos and Mitchell, 2000).

3.2.16. Gas Analysis

During BMP assays, gas production of each reactor was measured by water displacement method (Demirer and Chen, 2008).

Gas composition analysis was performed by a gas chromatograph (GC) (Agilent 6890N, CA, USA) equipped with a thermal conductivity detector and capillary column CP-Sil 8 (CP8752, Varian) to detect CH_4 content. The temperatures of the oven, injector and detector were 45, 100 and 250°C, respectively. Helium was employed as a carrier gas at pressure of 4.11 psi.

3.2.17. VFA Analysis

VFA compositions including lactic, formic, acetic, propionic, iso-butyric, butyric and iso-valeric acids and their concentrations were determined using a

High Performance Liquid Chromatograph (HPLC) device (SHIMADZU, 20A, Kyoto, Japan) equipped with refractive index detector. Sample volume of 10 μL was used for each measurement. Oven temperature was adjusted to 66ºC. 0.085 M HPLC grade sulfuric acid solution was used as mobile phase and its flowrate was adjusted to 0.4 mL/min. Total VFA concentrations were expressed in terms of total acetic acid (HAc) equivalences, which are calculated by division of each acid concentration by its molecular weight and multiplication of the result with the molecular weight of acetic acid (Yılmaz and Demirer, 2008).

3.3. Experimental Sets and Procedures

3.3.1. Microalgal Biomass Production and Nutrient Removal Studies

3.3.1.1.Characterization of Cultivation Medium and Municipal Wastewater

3.3.1.1.1. Cultivation Medium

In this study, cultivation of microalgae prior to experiments was achieved using Bold's Basal Medium with 3-Fold Nitrogen and Vitamins (3N BBM + V) (Bilanovic et al., 2009). Its constituents are given on Table 3-2.

Table 3-2: 3N BBM + V Constituents

Constituents	Concentration (g/L)
$NaNO_3$	0.75
$CaCl_2.2H_2O$	0.025
$MgSO_4.7H_2O$	0.075
$K_2HPO_4.3H_2O$	0.075
KH_2PO_4	0.175
NaCl	0.025
Na_2EDTA	0.0045
$FeCl_3.6H_2O$	$5.84x10^{-4}$
$MnCl_2.4H_2O$	$2.46x10^{-4}$
$ZnCl_2$	3x10-5
$CoCl_2.6H_2O$	1.2x10-5
$Na_2MoO_4.2H_2O$	2.4x10-5
Vitamin B1	0.0012
Vitamin B12	0.00001

3.3.1.1.2. Municipal Wastewater

Wastewater used throughout this study was obtained from primary clarifier effluents of Greater Municipality of Ankara Tatlar Domestic Wastewater Treatment Plant, located in Ankara, Turkey. Wastewater fed to the photobioreactor was collected as five different batch on January 1st, February 1st, April 1st, April 16th and April 18th, 2013. Depending on meteorological and seasonal variations, each batch differed in characteristics (Tchobanoglous et al., 2003). Each batch was screened through a sieve with pore size of 0.3 mm, in order to remove larger particles, prior to storage at 0^{o}C at dark. Wastewater characterizations and feeding cycles for which they are used are given on Table 3-3.

Table 3-3: Municipal Wastewater Characterization

Constituents (mg/L)	January 1st	February 1st	April 1st	April 16th	April 18th
TAN	25.1 ± 1.01	31.9 ± 1.32	28.8 ± 0.23	20.6 ± 0.18	20.8 ± 0.41
NO_3^--N	< 0.1	< 0.1	2.3 ± 0.08	4.4 ± 0.12	1.5 ± 0.06
PO_4^{3-}-P	4.91 ± 0.03	5.4 ± 0.02	1.9 ± 0.01	1.5 ± 0.01	1.79 ± 0.0
TN	29.4 ± 0.82	36.9 ± 0.66	35.1 ± 1.79	23.9 ± 1.24	25.3 ± 1.24
tCOD	351.1 ± 2.81	368.4 ± 7.96	337.2 ± 1.39	253.2 ± 8.44	266.6 ± 4.29
sCOD	82.2 ± 0.0	84.95 ± 1.06	73.0 ± 1.34	65.5 ± 1.99	± 0.28

3.3.1.2.Reactor Design

3.3.1.2.1. Batch Cultivation Photobioreactor

Microalgal culture was cultivated in a 3300 L cylindrical bubble column reactor with 3000 ml of working volume. Reactor is constructed with 0.3 cm thick glass. The diameter of the reactor is 8 cm. The batch cultivation photobioreactor (BCP) is capped with a glass stopper with a tube for gas exit. An aeration tube with inner diameter of 0.5 cm is submerged into the reactor through the stopper and is connected to an air pump (RESUN AC-9602, China). Gas inlet and outlet of the reactor are equipped with 0.2 μm syringe filters. The BCP is illustrated on Figure 3-1.

Figure 3-1: A photograph of BCP

3.3.1.2.2. Semi-continuous Cultivation Photobioreactor

In order to obtain microalgal biomass for BMP studies and determine nutrient removal potential of *Chlorella vulgaris*, a photobioreactor for wastewater treatment was designed. The reactor has 40 L inner volume with length, width and height values of 32 cm, 25 cm and 50 cm, respectively. The maximum working volume of the photobioreactor is 35L. Semi-continuous cultivation photobioreactor (SCP) was equipped with two identical air pumps (RESUN 9602, China), each connected to the smallest surface of a pair of spargers with dimensions of 25 cm x 2 cm x 2cm. Light was provided to a reactor with eight cool-white 18 W fluorescent lamps (OSRAM, L 18W/685, Korea), placed four by four on largest surfaces of the photobioreactors. The distance between each lamp and the photobioreactor surface is 5 centimeters. Lamps are 8 cm away from and parallel to each other and symmetrically aligned at each surface of the photobioreactor, providing 150 μmol/m^2.s PAR at the surface and 80 μmol/m^2.s in the center of the photobioreactor, when filled with water.

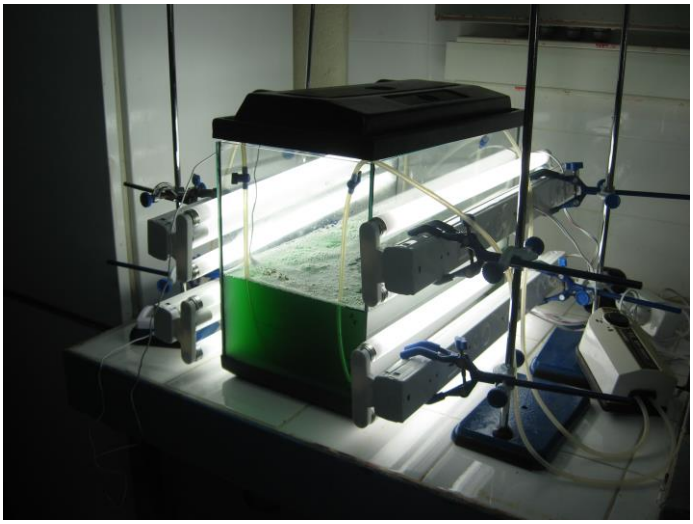


Figure 3-2: A photograph of SCP

3.3.1.3.Operation of Reactors

3.3.1.3.1. BCP Operation

Prior to cultivation, all plastic and glass parts of the photobioreactor, including tubes were autoclaved for 60 minutes at 121^0C and 5 psi for sterilization. The basal medium was also sterilized by the same method. 20 ml of culture was inoculated under sterile conditions, after the photobioreactor and basal medium was cooled. Initial optical density inside the photobioreactor was undetectable.

The BCP was operated with 16 h : 8 h day – night cycle . Day cycle was obtained with eight 18 W cool-white fluorescent lamps (OSRAM, L 18W/685, Korea) in a cabin isolated from ambient light using black curtains. The closest pair of lamps, which provided light intensity of 120 μmol/m^2.s was 10 centimeters away from one side of the reactor. At the opposite side of the reactor, light intensity was 100 μmol/m^2.s. These light intensity values are within the optimum range for *Chlorella vulgaris* cultivation (Filali et al., 2011).

BCP was continuously aerated with 0.5 volume air per volume broth per minute (vvm) air, in order to supply necessary carbon dioxide for growth and adequate mixing for light-dark cycle achievement (See Section 2.4.4.6)

Temperature was 28 ± 2 ^{0}C in culture broth. Initially, pH was set to neutral, whereas elevated up to alkaline levels at the end of each day cycle. On the other hand, due to absence of photosynthesis, pH dropped down to 6 ± 0.5 at the end of each night cycle (See Section 2.4.4.4). Hence, pH was lowered to 7.5 ± 0.2 at the end of day cycles using 1N HCl solution. After a pH control of six consequent days, pH fluctuation in the reactor was insignificant; varying between 6 and 8.

100 ml sample was collected daily from the reactor for pH, temperature and optical density measurements. BCP had been operated for 56 days, until the optical density of the culture reached value of 4 absorbance. After sufficient culture density, which allows high-density culture inoculation (>150 mg/L) in SCP, was reached, Optical Density versus Total Suspended Solids data were determined and a correlation curve was obtained. This calibration curve was used for TSS estimations from optical density measurements (See Section 4.1.1). Microalgae were then transferred into semi-continuous cultivation photobioreactor.

3.3.1.3.2. SCP Operation

The SCP was continuously illuminated (See Section 2.4.4.6) for massive amounts of biomass production, enhancing continuous photosynthesis throughout the day. Aeration was also continuously supplied with 0.5 vvm air (See Section 2.4.5.2). pH was elevated up to alkaline levels, therefore pH was lowered down to 6.0 ± 0.5 using 37% HCl (Merck, Germany) and its increase above 9.3 was avoided, in order to prevent decrease in carbon dioxide uptake capacity and escape of ammonia by stripping (See Section 2.4.4.2).

Feeding Protocol

The photobioreactor was operated for 21 days with varying feeding regimes. For the first four cycles, reactor was operated in fed- batch mode. Afterwards, operation mode was switched to semi-continuous. Rather than setting hydraulic

residence time and performing feeding / wasting operation on diurnal basis, feedback from the reactor was gathered periodically to determine optimum time for wasting and feeding. That is, soluble nitrogen (TAN and NO_3^--N) concentrations in the reactor were measured frequently as a feedback from the system, since wastewater obtained were N limiting. When a minimum of 75% soluble nitrogen removal was achieved on mass basis, photobioreactor was fed with fresh wastewater. Wasting and feeding volumes were determined not only considering the feedback, but also providing a constant ratio between microalgae and soluble nitrogen on a mass basis, at the beginning of each cycle.

Microalgal weight was determined in correlation to its optical density rather than its dry weight, despite the minor interference of wastewater with OD versus TSS calibration curve is a rough assumption. Nevertheless, as feeding protocol suggests, information about instantaneous microalgal biomass concentrations at a specific time must be known, when nutrient concentrations were determined, in order to decide on feeding time. Therefore, nutrient concentration changes within the system during dry weight measurements would bring higher levels of error, since the analysis is time consuming compared to optical density measurements. Table 3-4 summarizes the feeding protocol.

Table 3-4: Summary of Feeding Protocol in SCP

Cycle #	Operation Mode	Wastewater Collection Date	g N/ g TSS_{algae}
Feed 1	Batch	01.01.2013	0.18
Feed 2	Fed-Batch	01.02.2013	0.22
Feed 3	Fed-Batch	01.02.2013	0.18
Feed 4	Fed- Batch	01.02.2013	0.18
Feed 5	Semi- Continuous	01.04.2013	0.18
Feed 5	Semi- Continuous	01.04.2013	0.13
Feed 6	Semi- Continuous	01.04.2013	0.13
Feed 7	Semi- Continuous	01.04.2013	0.13
Feed 8	Semi- Continuous	01.04.2013	0.13
Feed 9	Semi- Continuous	01.04.2013	0.13
Feed 10	Semi- Continuous	01.04.2013	0.13
Feed 11	Semi- Continuous	16.04.2013	0.13
Feed 12	Semi- Continuous	16.04.2013	0.13
Feed 13	Semi- Continuous	16.04.2013	0.13
Feed 14	Semi- Continuous	16.04.2013	0.13
Feed 15	Semi- Continuous	16.04.2013	0.13
Feed 16	Semi- Continuous	18.04.2013	0.13
Feed 17	Semi- Continuous	18.04.2013	0.13
Feed 18	Semi- Continuous	18.04.2013	0.13

In addition to optical density and soluble inorganic nitrogen species, ortho-phosphorus concentrations in the reactor at the beginning and end of each cycle were analyzed.

3.3.2. BMP Assays

3.3.2.1.Basal Medium

In order to provide all necessary micro- and macro-nutrients required for an optimum anaerobic microbial growth, a BM was used during BMP test. The composition of basal medium (BM) used in the experiments was as follows (concentrations of the constituents are given in parentheses as mg/l $MgSO_4 \cdot 7H_2O$ (400), KCl (400), $Na_2S \cdot 9H_2O$ (300), $CaCl_2 \cdot 2H_2O$ (50), $FeCl_2 \cdot 4H_2O$ (40), $CoCl_2 \cdot 6H_2O$ (10), KI (10), $MnCl_2 \cdot 4H_2O$ (0.5), $CuCl_2 \cdot 2H_2O$ (0.5), $ZnCl_2$ (0.5), $AlCl_3 \cdot 6H_2O$ (0.5), $NaMoO_4 \cdot 2H_2O$ (0.5), H_3BO_3 (0.5), $NiCl_2 \cdot 6H_2O$ (0.5), $NaWO_4 \cdot 2H_2O$ (0.5), Cysteine (10), $NaHCO_3$ (6000) (Demirer et al., 2000).

3.3.2.2.Preparation of Substrates for Anaerobic Digestion

Substrates used in BMP studies include raw microalgal slurry, pretreated microalgal slurry, waste activated sludge and organic fraction of municipal solid waste. In this section, their preparation steps are given.

3.3.2.2.1. Microalgal Slurry

Prior to BMP assay, SCP effluents were collected and microalgal biomass grown was harvested daily. In order to determine the effect of pretreatment on anaerobic digestion of microalgae, prepared microalgal slurry was subjected into different pretreatment methods.

Harvesting

Effluents of SCP were collected at the end of each cycle, centrifuged at 4000g, 5460 rpm for 30 minutes. The recovery efficiency was calculated measuring optical densities of the effluents and final supernatants and found out to be over 90%.

Obtained slurries of each cycle were collected and mixed in a 1-L autoclave bottle. The bottle was refrigerated at 0^oC, prior to characterization and BMP

setup. A final volume of 1.2 L microalgal slurry was produced. Its characterization is given on Table 3-5.

Table 3-5: Characterization of Untreated Microalgal Slurry

Parameter	Untreated Microalgae
TS (mg/L)	33250 ± 70
VS (mg/L)	27950 ± 70
VS as %TS	84.1
TSS (mg/L)	32186 ± 702
VSS (mg/L)	26753 ± 685
VSS as %TSS	81.2
tCOD (mg/L)	42943 ± 285
TKN (mg/L)	3332 ± 79
TP (mg/L)	377 ± 14
sCOD (mg/L)	209 ± 1.1
TAN (mg/L)	285 ± 7.1
ortho-P (mg/L)	117 ± 5.0
COD/TKN	12.9

Pretreatment

In this study, the effects of three different pretreatment types, namely, heat, autoclave and thermochemical, on biogas production and its biomethane content were investigated. The procedures followed during pretreatment processes are presented in this section.

Heat pretreatment was applied to 200 mL of microalgal slurry. The slurry was poured into a 500 mL autoclave bottle, its pH was adjusted to neutral and the bottle was capped tight in order to avoid any material loss at high temperatures. The bottle had been placed in an oven for 120 minutes at 121^0C. After it was cooled down to room temperature, pH was measured and the bottle was refrigerated at 0^oC, prior to characterization and BMP setup.

Autoclave Pretreatment was applied to 200 mL of microalgal slurry. The slurry was poured into a 500 mL autoclave bottle, its pH was adjusted to neutral and the bottle was capped tight in order to avoid any material loss at high temperature and pressure values. The bottle had been autoclaved for 5 minutes at 121^oC a under 5 psi pressure.

Thermochemical pretreatment was applied to 200 mL of microalgal slurry. The slurry was poured into a 500 mL autoclave bottle, its pH was adjusted to alkaline

(12.0) by addition of 0.6 mL of 6N NaOH. The bottle was capped tight in order to avoid any material loss at high temperatures. The bottle had been placed in an oven for 120 minutes at 121 ^{0}C. After it was cooled down to room temperature, pH was measured, reduced to neutral and the bottle was refrigerated at 0^{o}C, prior to characterization and BMP setup. Table 3-6 summarizes pretreatment procedures followed.

Table 3-6 :Pretreatment Methods Summary

Pretreatment Type	**Temperature**	**Pressure**	**pH**	**Duration**
Heat	121 ^{0}C	Atmospheric	Neutral	120 min.
Autoclave	121 ^{0}C	5 psi	Neutral	5 min.
Thermochemical	121 ^{0}C	Atmospheric	Alkaline (12.0)	120 min.

Characterization of microalgal slurries subjected to different pretreatment methods were performed and results are given on Table 3-7.

Table 3-7: Characterization of Pretreated Microalgal Slurry

Parameter	Untreated Microalgae	Pretreated Microalgae		
		Heat	Autoclave	Thermochemical
TS (mg/L)	33250 ± 70	34200 ± 283	30340 ± 300	33700 ± 990
VS (mg/L)	27950 ± 70	28350 ± 495	27091 ± 236	27300 ± 707
VS as %TS	84.1	82.8	81	81.0
TSS (mg/L)	32186 ± 702	32875 ± 318	27966 ± 416	32933 ± 76
VSS (mg/L)	26753 ± 685	27275 ± 177	23200 ± 200	26350 ± 150
VSS as %TSS	81.2	83.0	83.0	80.0
tCOD (mg/L)	42943 ± 285	41768 ± 289	40659 ± 425	42721 ± 236
TKN (mg/L)	3332 ± 79	3360 ± 40	2970 ± 99	2968 ± 79
TP (mg/L)	377 ± 14	348± 1.0	347 ± 0.0	400 ± 4
sCOD (mg/L)	209 ± 1.1	615 ± 21	6630 ± 141	2300 ± 127
TAN (mg/L)	285 ± 7.1	355 ± 21	1020 ± 57	480 ± 28
ortho-P (mg/L)	117 ± 5.0	173 ± 3.5	279 ± 4.9	272 ± 7.0
COD/TKN	12.9	12.4	13.8	14.4

3.3.2.2.2. Co - Substrates

In this part of the study, anaerobic co-digestion of microalgae with two different co-substrates, namely, model kitchen waste (MKW) co-digestion and waste activated sludge (WAS) co-digestion were investigated. The substrates used for co-digestion are presented in this section.

Model Kitchen Waste (MKW)

MKW was prepared in laboratory (Marin et al., 2010). The constituents of synthetic MKW is given on Table 3-8. All constituents were mixed to achieve final weight of 1 kg. Then, 500 mL of water was added and the ingredients were blended until a slurry with maximum particle size of 0.5 cm was achieved. The slurry had a final volume of 2 L. The slurry was kept in a glass beaker covered with parafilm and refrigerated at 0^0C prior to characterization and BMP Assay. Characterization of MKW is given on Table 3-9.

Table 3-8: Composition of MKW

Component	Percentage (%w/w)
Dog food	20
Cooked rice	25
Cabbage	25
Oat	10
Egg plant	20
Total	100

Waste Activated Sludge (WAS)

WAS used in this study was obtained from secondary clarifier of Greater Municipality of Ankara Tatlar Domestic Wastewater Treatment Plant, located in Ankara, Turkey. Sludge was screened through a sieve with a pore size of 0.3 cm, in order to collect larger particles and stored in a glass autoclave bottle at 0^{o}C prior to its characterization and BMP setup. Characterization of WAS is given on Table 3-9.

Table 3-9: Characterization of WAS and MKW

Parameter	MKW	WAS
TS (mg/L)	131546 ± 1837	10625 ± 389
VS (mg/L)	127653 ± 1776	6600 ± 354
VS as %TS	97.0	59.0
TSS (mg/L)	--	10573 ± 214
VSS (mg/L)	--	6240 ± 131
VSS as %TSS	--	59
tCOD (mg/L)	165994 ± 8148	8128 ± 27
TKN (mg/L)	678 ± 25	792 ± 44
TP (mg/L)	447 ± 4	106 ± 0.9
sCOD (mg/L)	--	359 ± 1.0
TAN (mg/L)	--	127 ± 1.5
ortho-P (mg/L)	--	257 ± 1.8
COD/TKN	245	10.3

3.3.2.3.BMP Assay Configurations

Batch reactors were operated for the purpose of anaerobic degradability determination and biogas production potential determination of raw, pretreated and co-digested microalgal slurry harvested from photobioreactor effluents.

Reactors with 100 mL total volume and 71 mL effective volume were used in the experiments. If necessary, distilled water was used to complete reactor volume up to effective volume.

Test reactors were prepared with different COD values ranging between approximately 9000 mg/L and 34000 mg/L. Control reactors were run without any substrate but seed sludge. In order to investigate the effect of BM supplementation, test and control reactors with identical COD values were operated with and without BM supplementation.

After addition of all the constituents, pH of the reactors were set as 7.1 ± 0.2. Effective volumes of reactors were purged with nitrogen gas for 3 minutes and headspaces were purged for an additional minute. Reactors were capped with rubber septa and placed in a constant-temperature room at 35±1^{o}C for incubation with constant mixing at 125 rpm. Daily gas production was measured and gas compositions were periodically analyzed.

Eight untreated algae reactors were operated with four pairs of different initial COD values; namely, 9000, 14000, 19000 and 34000 mg/L approximately. Each pair consisted of reactors with and without BM. COD/TKN values of reactors ranging between 13.4 and 15.1 were lower than the optimum range of 350/7 to 1000/7 (See Section 2.5.1.1). Substrate –to- inoculum ratios (S/X) on total VS basis were ranging between 1.01 and 6.21. Constituents of raw microalgal slurry reactors are .given on Table 3-10.

Six pairs of reactors were operated for BMP assay of pretreated algae. Two COD values, approximately 19000 mg/L and 34000 mg/L were initially maintained in each reactor for each pretreatment type either with or without BM supplement. COD/TKN values of reactors ranging between 13.0 and 15.2 were lower than the optimum range of 25 to 35 (See Section 2.5.1.1) and it can be seen that pretreatment had minor effect on this ratio. S/X on total VS basis were ranging between 6.12 and 6.53. Constituents of pretreated microalgal slurry reactors are given on 3-11.

Investigation of biochemical methane potential of MKW and microalgal slurry co-digestions were carried out in four pair of reactors. In first pair, MKW was digested anaerobically as sole substrate, providing approximately 19000 mg/L COD in the reactor with anaerobic seed. In second pair, equal contribution of

MKW and Microalgal slurry in COD content of the reactors were achieved and reactors were run either with or without BM addition. In third pair, same approach was implemented for a lower initial COD value, approximately 14000 mg/L. Reactors M1a, M1b, M4a and M4b has COD/TKN values within the optimum range, whereas the other MKW co-digestion reactors had lower values. In fourth pair, ratio between COD contribution of MKW and microalgal slurry was kept as 7. S/X on total VS basis were ranging between 2.21 and 7.36. (See Table 3-10, Table 3-12).

Three pairs of batch reactors were operated for determination of biochemical methane potential of co-digested WAS and microalgal slurry. In first pair, sole substrate was WAS and COD value obtained initially in the reactors was approximately 9000 mg/L. In second pair, approximately 14000 mg/L COD was maintained as initial concentration, with equal contributions of WAS and microalgae on COD basis. Same approach was followed for third pair of reactors, except for the lower COD value obtained as 9000 mg/L approximately. Constituents of co-digested microalgal slurry reactors are given on Table 3-12. It can be seen that WAS had minor effect on improvement of COD/TKN value for an efficient AD process. S/X on total VS basis were ranging between 1.16 and 2.28 (See Table 3-10, Table 3-12).

Table 3-10: Components of BMP Assay Reactors with Untreated Microalgal Slurry

Reactor	Volume (ml)				COD(mg/L)			Ratio (g/g)		
Code	Seed	BM	Algae	Water	Seed	Algae	Total	COD:TKN	C:N	S/X
C1	16	0.0	0.0	55.0	4454	0	4454	18.4	6.5	0
C2	16	6.0	0.0	49.0	4454	0	4454	18.4	6.5	0
A1a	16	0.0	49.0	6.0	4454	29637	34091	13.4	4.6	6.21
A1b	16	6.0	49.0	0.0	4454	29637	34091	13.4	4.6	6.21
A2a	16	0.0	24.5	30.5	4454	14819	19272	13.9	4.8	3.10
A2b	16	6.0	24.5	24.5	4454	14819	19272	13.9	4.8	3.10
A3a	16	0.0	16.0	39.0	4454	9677	14131	14.2	4.9	2.03
A3b	16	6.0	16.0	33.0	4454	9677	14131	14.2	4.9	2.03
A4a	16	0.0	8.0	47.0	4454	4839	9292	15.1	5.2	1.01
A4b	16	6.0	8.0	41.0	4454	4839	9292	15.1	5.2	1.01

Table 3-11: Components of BMP Assay Reactors with Pretreated Microalgal Slurry

Reactor	Volume (ml)				COD (mg/L)			Ratio (g/g)	
Code	Seed	BM	Algae	Water	Seed	Algae	Total	COD:TKN	S/X
C1	16	0.0	0.0	55.0	4454	0	4454	18.4	0
C2	16	6.0	0.0	49.0	4454	0	4454	18.4	0
H1a	16	0.0	49.0	6.0	4454	29637	33279	13.0	6.53
H1b	16	6.0	49.0	0.0	4454	29637	33279	13.0	6.53
H2a	16	0.0	24.5	30.5	4454	14819	18866	13.5	3.26
H2b	16	6.0	24.5	24.5	4454	14819	18866	13.5	3.26
At1a	16	0.0	49.0	6.0	4454	29637	32514	14.2	6.24
At1b	16	6.0	49.0	0.0	4454	29637	32514	14.2	6.24
At2a	16	0.0	24.5	30.5	4454	14819	18484	14.6	3.12
At2b	16	6.0	24.5	24.5	4454	14819	18484	14.6	3.12
TC1a	16	0.0	49.0	6.0	4454	29637	33937	14.8	6.29
TC1b	16	6.0	49.0	0.0	4454	29637	33937	14.8	6.29
TC2a	16	0.0	24.5	30.5	4454	14819	19195	15.2	3.14
TC2b	16	6.0	24.5	24.5	4454	14819	19195	15.2	3.14

Table 3-12: Components of BMP Assay Reactors with Co-digested Microalgal Slurry

	Volume (ml)						COD (mg/L)					Ratio (g/g)		
Code	Seed	BM	Algae	WAS	MKW	Water	Seed	Algae	WAS	MKW	Total	COD: TKN	C:N	S/X
C1	16	0.0	0.0	0	0.0	55.0	4454	0	0	0	4454	18.4	6.5	0
C2	16	6.0	0.0	0	0.0	49.0	4454	0	0	0	4454	18.4	6.5	0
M1a	16	0.0	0.0	0	6.3	48.7	4454	0	0	14729	19183	63.6	23.3	3.78
M1b	16	6.0	0.0	0	6.3	42.7	4454	0	0	14729	19183	63.6	23.3	3.78
M2a	16	0.0	24.5	0	6.3	24.2	4454	14819	0	14729	34001	23.4	8.3	6.88
M2b	16	6.0	24.5	0	6.3	18.2	4454	14819	0	14729	34001	23.4	8.3	6.88
M3a	16	0.0	8.0	0	2.0	45.0	4454	4839	0	4676	13968	22.0	7.8	2.21
M3b	16	6.0	8.0	0	2.0	39.0	4454	4839	0	4676	13968	22.0	7.8	2.21
M4a	16	0.0	6.0	0	11.0	38.0	4454	3629	0	25717	33800	53.8	19.6	7.36
M4b	16	6.0	6.0	0	11.0	32.0	4454	3629	0	25717	33800	53.8	19.6	7.36
W1a	16	0.0	0.0	41	0.0	14.0	4454	0	4694	0	9147	13.1	4.9	1.27
W1b	16	6.0	0.0	41	0.0	8.0	4454	0	4694	0	9147	13.1	4.9	1.27
W2a	16	0.0	8.0	41	0.0	6.0	4454	4839	4694	0	13986	13.0	4.7	2.28
W2b	16	6.0	8.0	41	0.0	0.0	4454	4839	4694	0	13986	13.0	4.7	2.28
W3a	16	0.0	4.0	21	0.0	30.0	4454	2419	2404	0	9277	14.0	5.0	1.16
W3b	16	6.0	4.0	21	0.0	24.0	4454	2419	2404	0	9277	14.0	5.0	1.16

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1.Microalgal Biomass Production and Nutrient Removal

4.1.1. Batch Cultivation Photobioreactor Operation

Prior to microalgal biomass production and determination of its nutrient removal potential, unialgal culture of *Chlorella vulgaris* had been cultivated in 3NBBM + V medium for 56 days in batch mode. Culture growth was monitored via optical density data. Before cultivation, the appropriate wavelength at which optical density values are measured was determined by scanning the values between 600 and 800 nanometers. In this study, the highest absorbance value of a constant sample was read at 685 nm for *Chlorella vulgaris* obtained from CCAP. This wavelength was used to determine absorbance values throughout this study. Figure 4-1 shows the relationship between Wavelength and Absorbance values for *Chlorella vulgaris.*

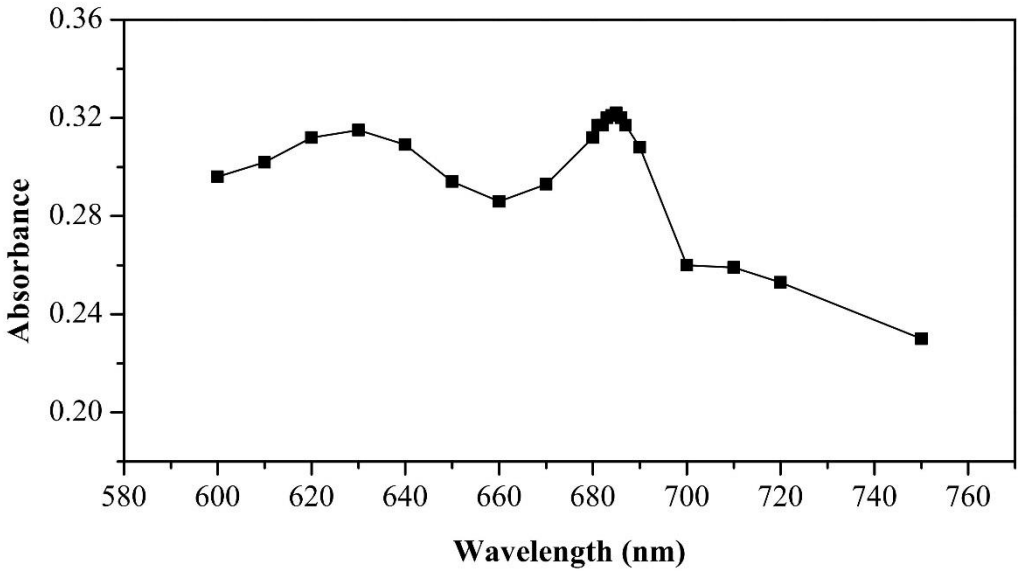


Figure 4-1: Wavelength vs. Absorbance curve for Chlorella vulgaris

In addition to optical density, pH values of the cultivation reactor were also measured in the BCP. Optical density, pH values at the end of day and night cycles as well as pH values after adjustment are presented on Figure 4-2.

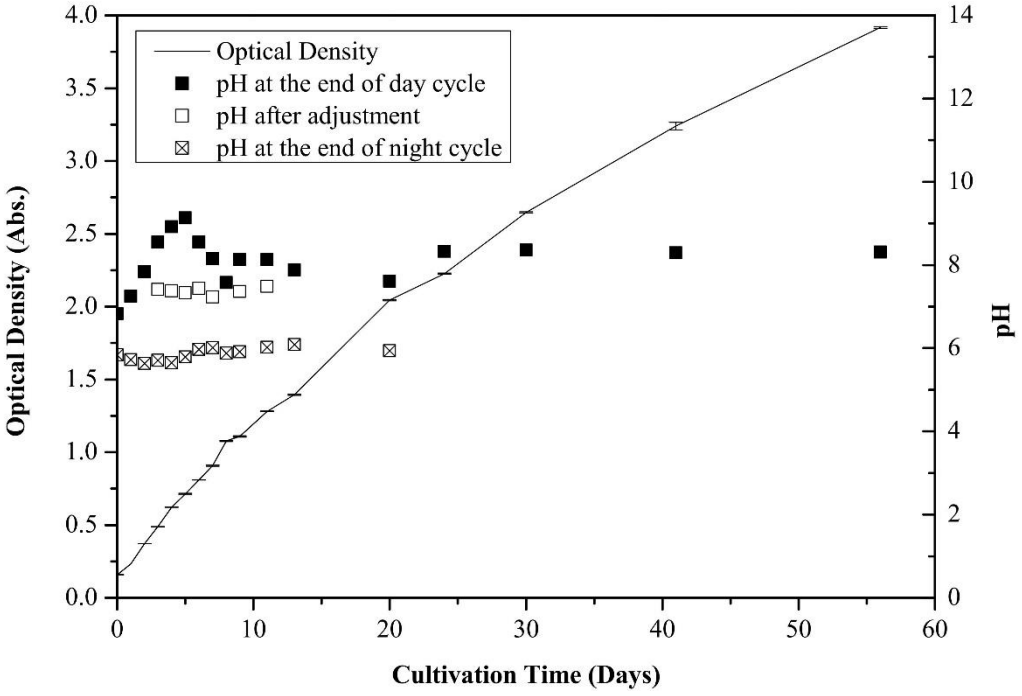


Figure 4-2: Time versus Optical Density and pH graph of BCP

As shown in Figure 4-2, there were no lag or accelerated growth phases during the cultivation of *Chlorella vulgaris*. Although consecutive growth phases were unclearly observed, it can be assumed that the exponential growth phase ended on day 12. Growth rate gradually declined during the cultivation period between days 12 and 56, which indicates that culture was in decreasing log growth phase. This decrease can be a result of shading effect in the BCP (See Section 2.4.4.6), as the optical density value exceeded 1.0. However, the stationary phase had not been reached, since mixing in the reactor was adequate to provide light-dark cycles. In addition, nutrients in the culture medium had also been sufficient even at day 56 (See Section 4.1.2).

The maximum net specific growth rate during batch cultivation was observed as 0.39 d^{-1}. This value is comparable to those reported by de Morais and Costa (2007), where specific growth rate of *Chlorella vulgaris* in basal medium fed with air varies between 0.12 and 0.25 $d^{-1}$for different reactor geometries. In the same study, maximum specific growth rate achieved was reported as 0.31 $d^{-1,}$ when CO_2 – enriched air was supplied to the photobioreactor. Li et al., (2013) cultivated *Chlorella vulgaris* in batch, semi-continuous and continuous modes in municipal wastewater treatment plant secondary effluents, which was a medium similar to the wastewater used in this study and specific growth rate was reported to be between 0.2 and 0.374 d^{-1}. Yet, Martinez and Orus (1991) and Filali et. al (2011) reported specific growth rates of 1.92 d^{-1} and 1.95 d^{-1} respectively. The common condition for the growth of *Chlorella vulgaris* in a basal medium was carbon-dioxide enriched air supplementation. It can be concluded, that carbon-dioxide feeding with air was relatively insufficient compared to nutrients in culture broth, in order to achieve a balanced microalgal growth at high rates.

Same conclusion can be made regarding productivity values. In this study, 24 mg/L.d productivity was achieved during exponential phase of *Chlorella Vulgaris* cultivation, which is same as the value reported by Scragg et. al. (2002) and within the specified range of 0.02 – 0.25 g/L.d for *Chlorella vulgaris* (Mata

et al., 2010). On the other hand, 104.76 mg/ L.d productivity was observed by Yoo et. al. (2010), when higher carbon-dioxide concentrations were used for cultivation of *Chlorella vulgaris*. Calculation of productivity and specific growth rate values are given in Appendix A.

Due to utilization of inorganic carbon, nitrate and phosphate species, pH increased during the day cycles up to values below 9.0, whereas during night cycle, pH dropped down to values below 5.6 due to carbon dioxide generated as a result of respiration (Larsdotter, 2006). Therefore, it was necessary to adjust the pH with HCl addition at the end of day cycles, beginning from third day of cultivation. It was aimed to lower the pH to a value which would not cause severe decrease during the night cycle, i.e., below 5.5. Thus, pH was fixed between 7 and 7.5 at each adjustment. After pH control was applied at five consecutive days, fluctuation between night and day values started to decrease. After day 10, minimum night cycle pH and maximum day cycle pH values were both in the optimal range for microalgal growth. Therefore, adjustment was stopped. High pH elevation and adjustment necessity were especially observed during accelerated growth phase, which suggests that the pH increase was due to accelerated metabolic activity of the culture. Afterwards, pH was sustained within the optimal range, by the help of balanced growth and night time respiration.

Prior to transfer of culture into semi-continuous cultivation photobioreactor, a calibration curve for the correlation of OD and TSS concentrations was prepared. For this purpose, enriched culture was diluted down to optical density values between 0.1 and 1 absorbance. This curve was used to estimate microalgal biomass concentration, since it was necessary for the feeding protocol. Figure 4-3 illustrates the calibration curve.

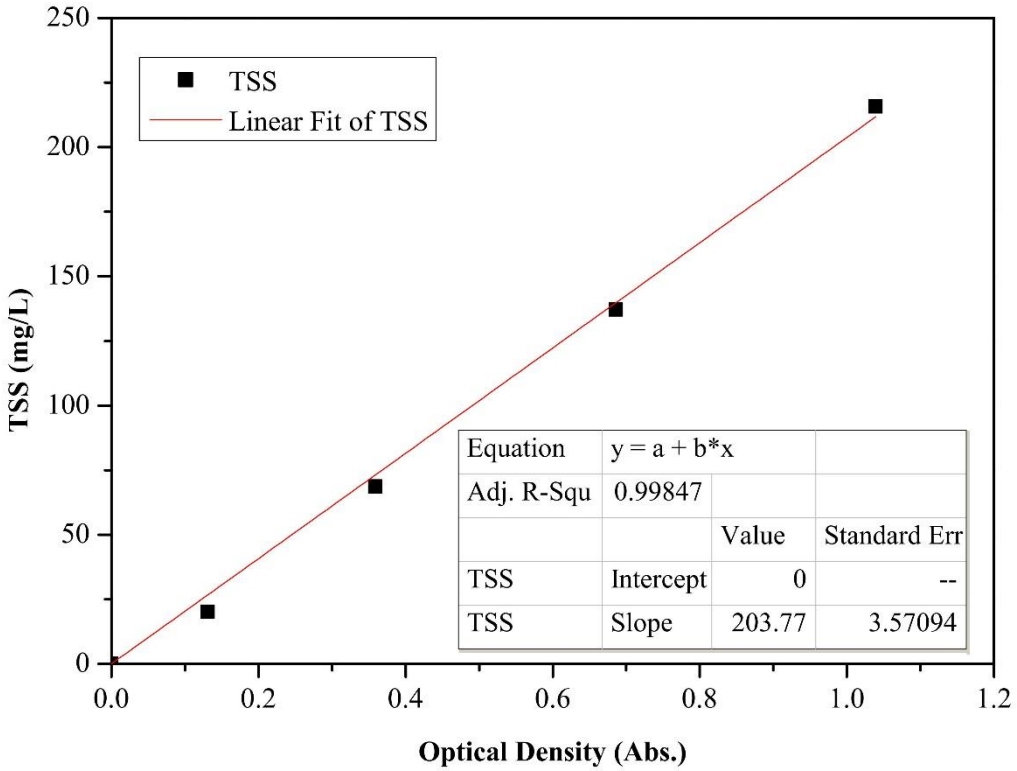


Figure 4-3: Optical Density versus TSS values for *Chlorella vulgaris*

Total suspended solids (TSS) values can be estimated using Equation 4-1.

$$[\text{TSS (mg/L)}] = 203.77\ [\text{OD}_{685}\ (\text{Abs.})] \quad (4\text{-}1)$$

The slope of the equation found was similar to that of reported by Chiu et. al. (2008) for *Chlorella* sp. which had a slope of 206, for conversion of absorbance values measured at 685 nm.

4.1.2. Semi- Continuous Cultivation Photobioreactor Operation

In order to produce microalgal biomass in high amounts for BMP studies and to determine nutrient removal potential of the culture, semi- continuous cultivation reactor had been operated for 21 days.

As it is required by the feeding protocol (See Section 3.3.1.3.2), constant ratio was maintained between total inorganic nitrogen species, i.e. sum of TAN and NO_3^--N, and TSS_{algae}, which is estimated by optical density values in SCP. It was aimed to fill the SCP in a short period of time and eliminate the effects of nutrients entered the system with 0.5 L *Chlorella vulgaris* inoculum broth, which

was high in nutrient concentrations. Characteristics of batch cultivation broth is given on Table 4-1. For this reason, during the first four cycles with fed- batch operation, N/*TSSalgae* ratio was 0.18, 0.22, 0.18 and 0.18 respectively. As shown in Figure 4-4c when semi- continuous cultivation was started, N/TSS_{algae} value was fixed at 0.13.

Table 4-1: Culture Broth Constituents after 56 Days of Cultivation

Culture Broth Constituents	
NO_3^--N (mg/L)	19.6 ± 0.94
PO_4^{3-}-P (mg/L)	44.2 ± 1.1
sCOD (mg/L)	149.5 ± 0.7
OD (Abs.)	3.92 ± 0.006

Since N/TSS_{algae} values were taken into account for determination of the wastewater amount to be fed in SCP at the beginning of each cycle, daily volume of added wastewater varied in relation to the optical density reached at the end of the previous cycle. Without altering the ratio, daily wastewater volume and effective reactor volume were changed. Although wastewater volume changed between 8.5 L and 14.5 L, effective reactor volume was kept constant at 25L (See Figure 4-4a,b.). Theoretically, HRT of the SCP varied between 1.8 and 2.9 days, depending on the daily feeding and wasting volumes, according to the feeding protocol.

During the operation of SCP, five different batches of primary clarifier effluents were used (See Table 3-3). Inorganic N/P ratio of the wastewater fed to reactor was therefore variable. All wastewaters except first two, obtained on January 1st and February ^{1st} were phosphorus- limited, as N/P values were higher than the Redfield Ratio of 7.2. However, feeding protocol was not changed regarding phosphorus limitation. Nitrogen concentrations were monitored as feedback mechanism throughout the study, since nitrogen- limited conditions favor lipid accumulation and low biomass productivity, whereas nitrogen abundance promotes biomass production (Ras et at., 2011).

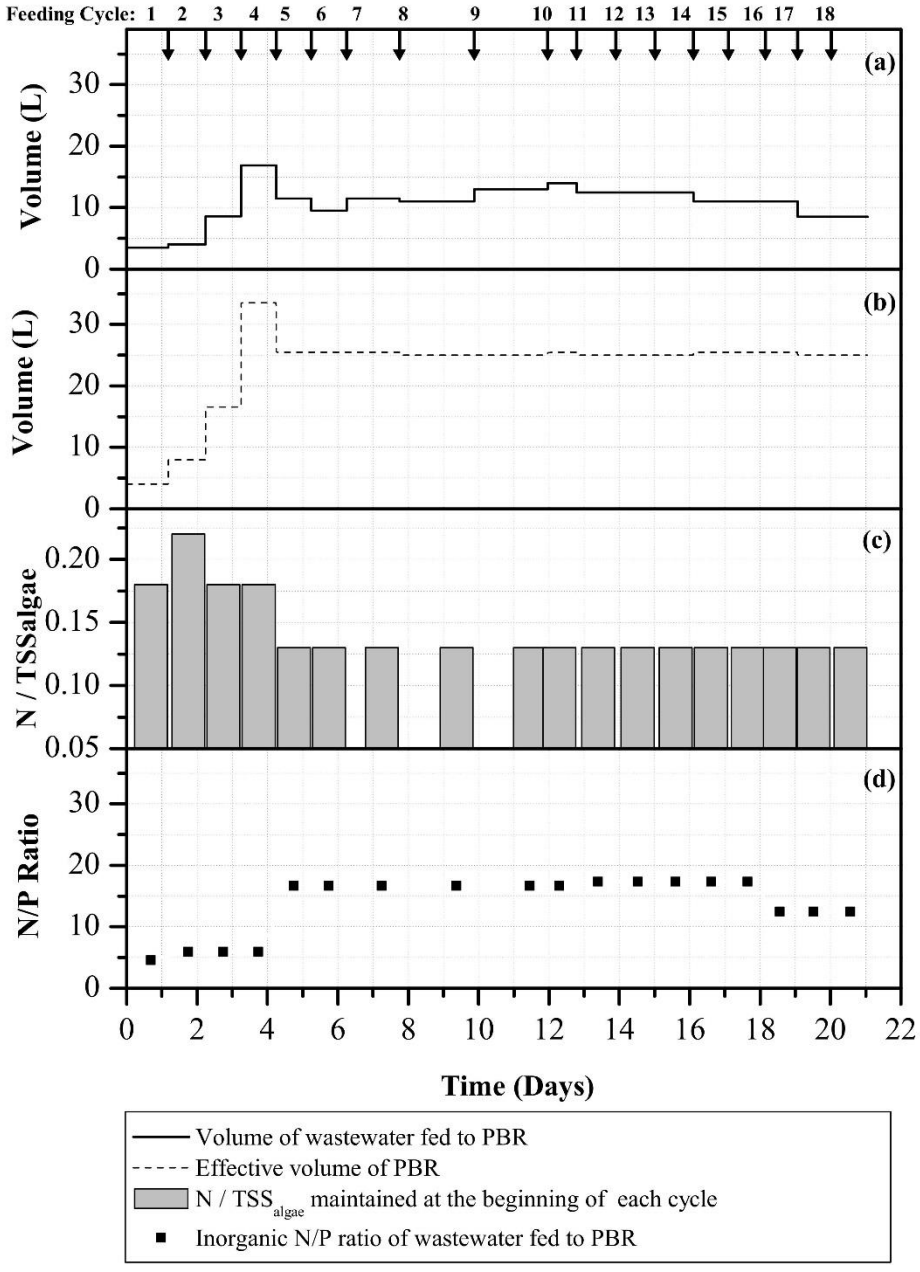


Figure 4-4: Semi Continuous Cultivation Operating Parameter Details: (a) Volume of wastewater fed to SCP at each cycle; (b) Effective volume of SCP at each cycle; (c) N/TSSalgae ratio maintained at each cycle; (d) Inorganic N/P ratio of the wastewater fed to SCP.

4.1.2.1.SCP Kinetics

4.1.2.1.1. Variations in Culture Concentration

During semi-continuous cultivation of *Chlorella vulgaris* in wastewater, biomass production was monitored via optical density measurements. Initial optical density in the reactor was 0.7 during the first cycle and decreased to 0.5 by the second cycle. Then, initial optical densities per cycle gradually increased up to 0.7 at the beginning of Cycle 10. Starting from Cycle 11, nutrient concentrations of the feed wastewater decreased. As a result, lower optical density value corresponded to the constant N/TSS_{algae} ratio. Until Cycle 10, the final optical density of each cycle had a tendency to increase compared to the previous cycle.

As shown in Figure 4-5a, optical density at the end of first cycle was 0.903, whereas it was 1.2 at the end of Cycle 10. The exceptional optical density values of 1.472 and 1.38 were observed when wasting and feeding was not done until all inorganic nitrogen species in the SCP were consumed. Maximum optical density value was observed on day 10 as 1.472, at the end of Cycle 8, whereas the minimum final optical density of 0.755 was observed at Cycle 16, which corresponds to 18^{th} day of cultivation. After Cycle 10, final optical density values at the end of cycles started to drop until Cycle 17. This decrease is probably a result of unbalanced inorganic N/P ratio in the reactor, which is over 10 (Wang et al., 2010) and lower nutrient concentrations of the incoming wastewater. After Cycle 16, final optical densities were relatively constant; nutrient input started to increase and N/P ratio of the influent wastewater was 12.4, which is relatively closer to the optimum range of 6.8 – 10 for microalgae (Wang et al., 2010).

Productivity of the culture depends highly upon the duration of cycle. For instance, although the highest optical density was observed at the end of Cycle 8, productivity value is not among the highest productivity values. In addition,

the highest productivity was reached at Cycle 10, when the highest initial optical density was observed (See Figure 4-5b.).

At the beginning of Cycle 1 and at the end of Cycle 18, algae were identified under microscope (Paxinos and Mitchell, 2000). It was found that there were no algal species other than *Chlorella vulgaris* in both examinations. However, in Cycle 1, protozoa such as ciliates and flagellates were observed, less than 1:10,000 cells. At the end of Cycle 18, *Chlorella vulgaris* was still the only microalga, although minor amounts of diatoms (less than 1:1000 cells) and oocysts (less than 1:10,000 cells) were detected. It can be concluded that high nutrient concentrations of wastewater were selective for *Chlorella* sp. (Brennan & Owende, 2010). Micrographs are presented in Appendix B.

4.1.2.1.2. Variations in Nutrient Concentrations

Apart from initial optical density values of each cycle, nutrient concentrations also varied due to the feeding protocol used. Concentrations of nutrients in different batches of wastewaters also affected the initial nutrient concentrations at the beginning of each cycle. For example, average initial TAN concentration in SCP until Cycle 11 was 16.6 mg/L whereas after Cycle 11, the average initial TAN concentration was 10.0 mg/L. This decrease mainly occurred due to lower – strength wastewater feeding to the SCP. However, higher the optical density value observed, higher nutrient concentration at the beginning of the next cycle was achieved. For instance, maximum initial TAN concentration of 17.9 mg/L was observed at the beginning of the Cycle 9, after the optical density value reached its maximum (1.472 Abs.) at the previous Cycle, 8.

It can be seen from Figure 4-5c, that the general trend of TAN concentration in reactor was decreasing on a cyclic basis, down to values near zero. This cyclic changes in TAN concentrations show that TAN was effectively consumed by microorganisms or converted into different nitrogen species through other fate mechanisms such as nitrification. However, nitrate concentrations in the SCP did not show the same trend and a temporal trend cannot be observed (See Figure

4-5d). During fed-batch cycles, an overall decrease in NO_3^--N concentrations can be observed, especially when TAN was consumed in the SCP, since TAN is preferentially utilized first (Becker, 2008). It can clearly be seen that NO_3^--N concentrations below 0.1 mg/L were observed only when TAN was depleted at the end of Cycles 4, 8 and 9. On the other hand, when TAN is consumed, nitrate concentrations were not necessarily zero, such as at the end of Cycles 3 and 12. This was probably observed because nitrates were used as secondary nitrogen source and were being consumed. Another reason for the unique trend of NO_3^--N concentrations during cycles could be nitrification (Tchobanoglous et al., 2003).

Ortho- phosphate concentration in the reactor had a regular cyclic trend and initial PO_4^{3-}-P concentrations per cycle in the SCP were gradually decreasing by time. It can be seen from Figure 4-5e, that nutrient- rich culture broth interfered PO_4^{3-}-P concentrations in the wastewater during fed- batch mode. When wastewater was added at the end of each cycle, PO_4^{3-}-P concentrations decreased due to dilution. Therefore, PO_4^{3-}-P uptake by microalgae was unclear. Beginning from semi-continuous operation at Cycle 5, cyclic decreases in PO_4^{3-}-P concentrations could be observed. Since PO_4^{3-}-P concentrations in wastewaters fed into SCP varied and were lower than previous feed, the general initial PO_4^{3-}-P concentration was in a decreasing trend by time.

Although N/P ratio of wastewaters were higher than optimum range for microalgae, phosphorus content of culture broth decreased the ratio below 10 during fed- batch operation. In addition, residual phosphorus from previous cycles sustained N/P ratio near 10, when the reactor was operated in semi-continuous mode. This effect can be inferred from the tendency of N/P ratio to increase in SCP, after PO_4^{3-}-P fed to the system at each cycle started to be utilized at higher extent beginning from Cycle 10, relative to previous Cycles 1-9. Although N/P ratio of the fed wastewater was constant, since phosphorus removal was relatively higher, N/P ratio in the reactor started to increase especially after Cycle 16 (See Figure 4-5f).

The ratio between removed inorganic soluble nitrogen and ortho-P was expected to approximately equal 7.0; average N/P ratio of *Chlorella vulgaris* is on mass basis. However, as shown in Figure 4-5f, inorganic N/P removed per cycle were higher than the expected value. This fact indicates that there were fate mechanisms of nitrogen other than direct utilization by microalgae(Griffiths, 2010). One of these possible mechanisms is denitrification; however, considering that dissolved oxygen values were always higher than 2 mg/L, anoxic conditions never occurred and continuous mixing avoided possible oxygen gradient in the SCP. Another possible mechanism for nitrogen removal is ammonia stripping, which is unlikely to occur in the SCP due to pH control, since elevated pH values above 9.5, at which ammonia could form and volatilize, was not observed. One possible explanation of high N/P ratio of removed nutrients is luxury uptake of nitrogen, for further uses in case of starvation (Larsdotter, 2006).

4.1.2.1.3. Variations in Physical Parameters

In SCP temperature, DO and pH were measured, since they are the most important parameters affecting microalgae cultivation systems (Becker, 2008).

Temperature values were within the optimum range over 95% of measurements. Temperature variation in SCP was low, as the system was operated with continuous illumination using fluorescent lamps as sole light source. There were no day and night cycles and variations due to weather changes were insignificant. Temperature values in SCP medium were measured twice in each cycle and recorded between 24.8 and 29.6 ^{o}C. However, at the beginning of Cycle 2, 7 and 10, temperature were recorded as 18.1, 17.6 and 21.3, due to low temperatures of wastewaters fed to the reactor.

DO values were also significant in the photobioreactor system, since elevated concentrations may cause photosynthetic inhibition (Becker, 2008), whereas lower values than 2mg/L may cause denitrification, in turn, nitrogen loss to the atmosphere (Tchobanoglous et al., 2003). SCP was continuously aerated in order

to provide sufficient carbon dioxide for microalgae and to strip excessive oxygen in the culture medium. Therefore, DO values varied between 6.47 and 8.63 mg/L in the SCP.

pH was another important factor influencing physiological state of microalgae and was an unstable parameter, compared to temperature and DO. As shown in Figure 4-5g, pH was in a tendency to increase. At the beginning of cycles, pH was set to 6.0 ± 0.5 and adjusted to the same range during the cycles, in order to avoid elevation of pH beyond 9.3 and to avoid nitrogen stripping. The arrows on Figure 4-5g indicate adjustment times. The increase in pH could be a result of nitrate, phosphate and/or carbon dioxide utilization. Probably, effect of nitrate utilization was insignificant in pH elevation, since TAN is the primary source of nitrogen for microalgae and were absent only in short time periods, due to feeding protocol. On the other hand, comparison of culture productivity and specific growth rate with that of the studies where carbon dioxide enriched air was provided indicate carbon dioxide deficiency, as well as the tendency of pH elevation. Therefore, low carbon dioxide levels in the SCP is most probably the reason for this increasing trend. In addition, when the cycle durations were extended during Cycles 8 and 9, pH elevation was stopped at the end of cycles and pH values were measured as 6.42 and 6.23, without any pH adjustment. The stability of pH could be related to nutrient depletion and in turn, decrease in photosynthetic activity, since pH changes in microalgal systems are highly dependent on nutrient uptake (Larsdotter, 2006). This fact could enable carbon dioxide accumulation in the medium as buffer.

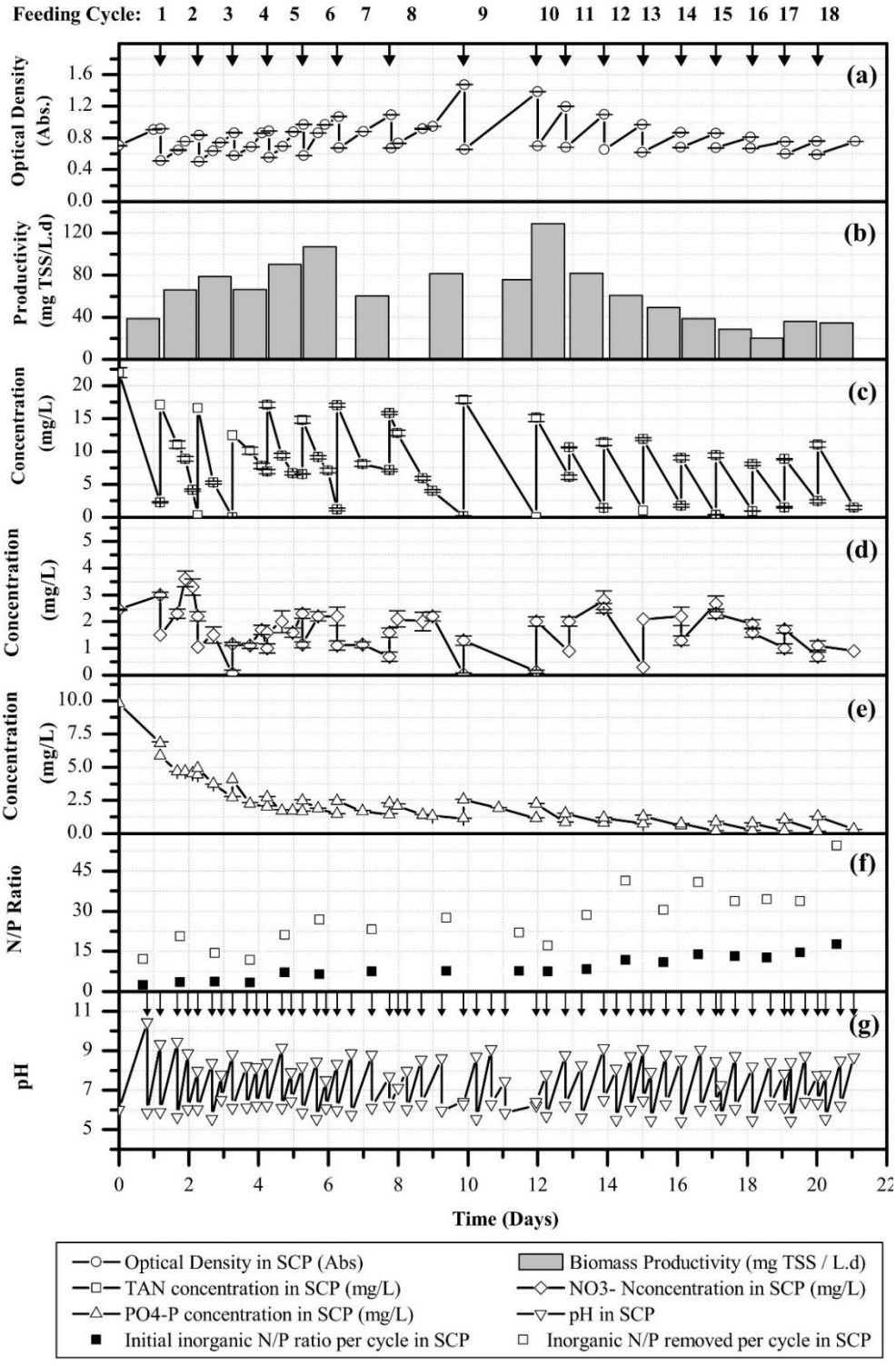


Figure 4-5: SCP Kinetics: (a) OD variations in SCP vy time; (b)Culture productivity observed at each cycle; (c) TAN concentration variations in SCP by time; (d) NO3—N concentration variations in SCP by time; (e) PO43 + - P concentration variations in SCP by time; (f) Inorganic N/P ratio in the SCP and inorganic N/P ratio of the removed nutrients at each cycle; (g) pH adjustments and variations in SCP by time.

4.1.2.2.Nutrient Removal in SCP

In this study, nutrient removal potential of *Chlorella vulgaris* in primary clarifier effluents of municipal wastewater treatment plant was investigated. Loss of TAN cannot be considered as treatment, since TAN is converted into NO_3^--N in the SCP. Thus, nitrogen removal efficiencies were calculated by taking into account the difference between sum of TAN and NO_3^--N concentrations in influent wastewater and effluent from each cycle. Ortho-P removal efficiencies were also calculated, considering influent concentration in wastewater and effluent of each cycle.

As shown in Figure 4-6a, nitrogen removal efficiencies were above 80%, except for cycles 4, 5, 7 and 10 as 75.1%, 71.5%, 74.7% and 77.4%, respectively. However, the removal efficiencies in these four cycles were still acceptable in terms of feeding protocol. In order to completely remove nitrogen fed to the SCP in each cycle, N/TSS_{algae} ratio was reduced from 0.18 to 0.13. Then, cycle duration was increased. Maximum N removal efficiency of 99.6% was achieved at the end of Cycle 9. When wastewater strength was decreased, removal efficiencies were over 90%, with the maximum value of 92.5 % at Cycle 18.

Phosphorus removal efficiencies were unclear for the first four cycles, due to the interference of culture broth (3N BBM + V) with wastewater phosphorus concentrations. This effect disappeared, as the final phosphorus concentrations at the end of the cycles were below than the concentrations present in wastewater fed. However, the wastewater phosphorus concentrations in primary clarifier effluents of treatment plant started to decrease as well. Therefore, remaining phosphorus from previous cycles interfered with phosphorus removal efficiency calculations, until the phosphorus content in the reactor became lower than the concentration in feeding wastewater, which corresponds to Cycle 13. As shown in Figure 4-6b, phosphorus removal efficiency in SCP was over 80% afterwards. Maximum phosphorus removal efficiency of 91.2% was observed at the end of Cycle 17.

Nutrient removal efficiencies observed in this study are in consistency with relevant studies. For example, ammonium and phosphorus removal efficiencies of high rate algal ponds were reported as 89% and 49% respectively (Green et al., 1995). Li et al., (2013) conducted batch, modified semi- continuous and continuous cultivation experiments of *Chlorella vulgaris* in municipal effluent. It was found that ammonia-N, total nitrogen and total phosphorus can effectively be removed by 98.0%, 90.3 – 93.6% and 89.9 – 91.8% respectively.

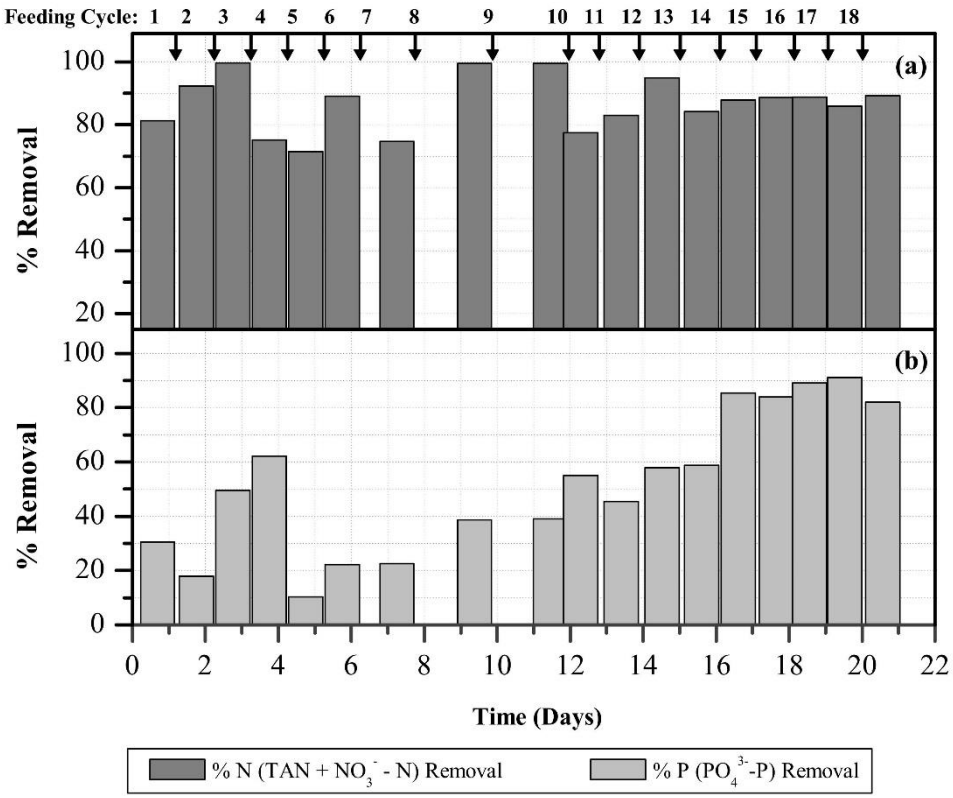


Figure 4-6: Nutrient Removal Efficiencies in SCP: (a) Inorganic nitrogen removal efficiency at each cycle; (b) Inorganic phosphorus removal efficiency at each cycle.

4.2.BMP Assay Results

In this part of the study, biochemical methane production potentials of raw, pretreated and co-digested microalgal slurry were investigated. For this purpose, 36 batch reactors were operated for 66 days. All reactors were terminated when the variation in daily volume of gas produced in each measurement were less than 15% for a two-week period.

In this section, reactor performances are evaluated in three sections; (i) AD of raw microalgal slurry; (ii) AD of pretreated microalgal slurry; (iii) AD of microalgal slurry with co-substrates.

4.2.1. AD of Raw Microalgal Slurry

Eight batch reactors were operated for BMP test of raw microalgae for 66 days. Reactors were evaluated in terms of biogas production potential, biogas and methane yields and COD treatment efficiencies.

4.2.1.1.Biogas Production

Biogas production was examined in each reactor with raw microalgal slurry; COD content and BM had direct influence on quantity and quality of biogas produced. Figure 4-7 shows cumulative biogas production data of reactors with raw microalgal slurry. As can be seen from Figure 4-7, BMP test reactors had different COD concentrations, namely, 9000, 14000, 19000 and 34000 mg/L, with or without BM. Cumulative gas production values of each reactor differed significantly, as a result of different COD concentrations and BM supplementations provided to the reactors. It can also be seen that the majority of biogas produced within first 25 days in each reactor. In addition, by increasing COD, cumulative biogas production increased. This increase was mostly evident beginning from first days of AD, which is an outcome in consistency with literature. Sung and Dague (1995) stated that high F/M values lead to high biogas production.

Maximum biogas production was achieved in reactor A1a, in which initial COD concentration was 34000 mg/L and had no BM. In this reactor, total biogas production was 519 mL, no lag phase was observed and exponential biogas production was observed until Day 30. Afterwards, biogas production rate decreased. In reactor A1b, where same COD concentration of 34000 mg/L and BM were initially provided, lower cumulative gas production value of 387 mL, 25.3% lower than Reactor A1a, was recorded. It can be concluded that BM had a negative effect on gas production; however, it can also be seen from Figure 4-7, that BM addition had a positive effect until Day 19, until the end of exponential biogas production period of A1b. That is, biogas production rate is higher in Reactor A1b.

For reactors with 19000 mg/L COD concentration, namely, Reactor A2a and Reactor A2b. It can be seen that exponential biogas production period in Reactor A2b, which was supplemented with BM, showed a steeper slope, however, ended at Day 18. On the other hand, in Reactor A1a, in which BM was absent, this period was extended until Day 27, although the slope was smaller. In this reactor, biogas production of 267 mL was observed, whereas in Reactor A2b, this value was 212 mL, 20.5% lower than reported for A2a.

Reactors A3a and A3b were started with initial COD concentrations of 14000 mg/L. Reactor A3a had no BM, whereas Reactor A3b was supplemented with BM. It can be seen that Reactor A3a produced 184 mL biogas and 29% less biogas generation, that is, 130 mL was observed in A3b. In this set of reactors, the largest percent difference between reactors with and without BM addition was encountered. Moreover, the biogas production rate in both reactors were equal, where to exponential production period in Reactor A3b was much shorter and lasted at Day 19, whereas this period was observed for A3a until Day 30.

In reactors started with 9000 mg/L initial COD, namely, Reactor A4a and Reactor A4b, lowest volume of biogas were produced, when compared to other reactors with or without BM addition. Until Day 30, there were no difference between these reactors in terms of biogas production. Afterwards, in Reactor A4a, which was missing BM, biogas production was further observed, whereas there was no significant change in cumulative biogas production data of A4b, which was supplemented with BM. IN A4a, total of 99 mL biogas was produced, whereas 74 mL biogas was generated in A4b, which is 25.1% less than observed in A4a.

It was observed that BM had a negative effect on biogas production. This can be a result of higher pH values achieved in reactors with BM supplementation. Due to higher pH, ammonia inhibition may have occurred in higher extent. Similarly, anaerobic digestion of other substrates with high ammonia content such as broiler and cattle manures (Güngör-Demirci and Demirer, 2004), resulted in inverse effect of BM.

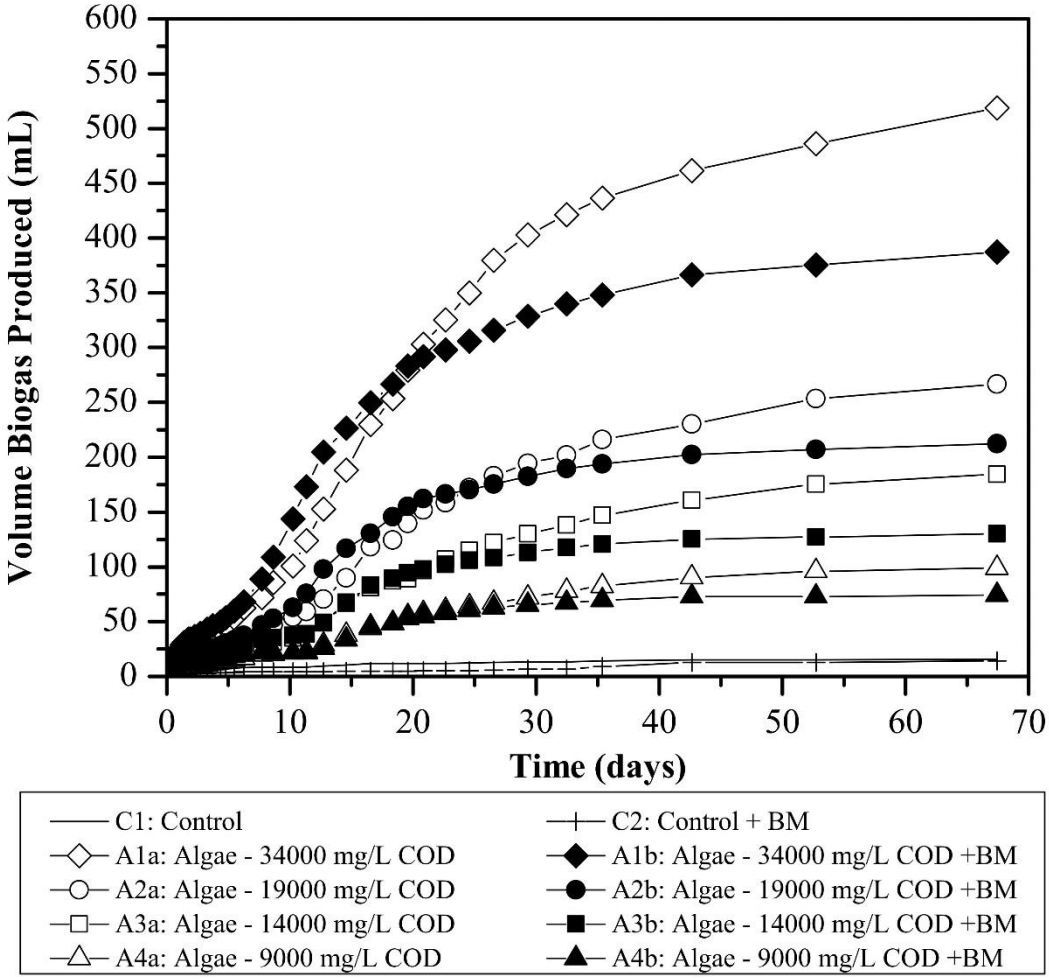


Figure 4-7: Cumulative biogas production data of microalgal slurry reactors

As initial COD concentrations among the reactors differed, biogas yields on VS basis were calculated in order to compare biogas production efficiencies of reactors. Table 4-2 provides the calculated biogas yields of untreated microalgal slurry reactors.

Table 4-2: Biogas Yields of Reactors fed with microalgal slurry

	A1a	**A1b**	**A2a**	**A2b**	**A3a**	**A3b**	**A4a**	**A4b**
VS added (g)	1.37	1.37	0.68	0.68	0.45	0.45	0.22	0.22
Biogas Yield (mL/g VS added)	379	283	390	310	412	290	442	331

As shown on Table 4-2, initial COD concentration was inversely proportional with biogas yield in reactors with no BM supplementation. This fact may be a

result of substrate inhibition occurred in reactors with higher VS addition. In fact, final VFA concentrations observed in reactors A1a and A2a above 300mg/L (Speece, 2008) also address inhibition (See Section 4.2.1.3). A similar correlation between substrate addition and biogas yield was reported by Alzate et al. (2012); as substrate to inoculum ratio increased, the biogas yields decreased during AD process of microalgae. Maximum biogas production yield was observed in Reactor A4a, which had the lowest initial COD content of 9000 mg/L and contained no BM. BM also had an inverse effect on biogas production yield in each set of reactors with same COD values. Although there are variations in biogas yields in each reactor, results are in consistency with values recorded between 287 mL/g VS added and 587 mL/g VS added in literature for AD process of microalgae (See Section 2.5.4.). Biogas yields of reactors on COD basis are given on Appendix C.

4.2.1.2. Methane Contents

In order to determine methane production yields of reactors, methane contents were determined on percentage basis. Measurements were performed at Day 14, 21, 28, 35 and 56; started after the headspaces of reactors were washed with produced biogas; that is, when produced biogas volume exceeded three-folds volume of headspace. Figure 4-8 depicts methane contents of microalgal slurry reactors.

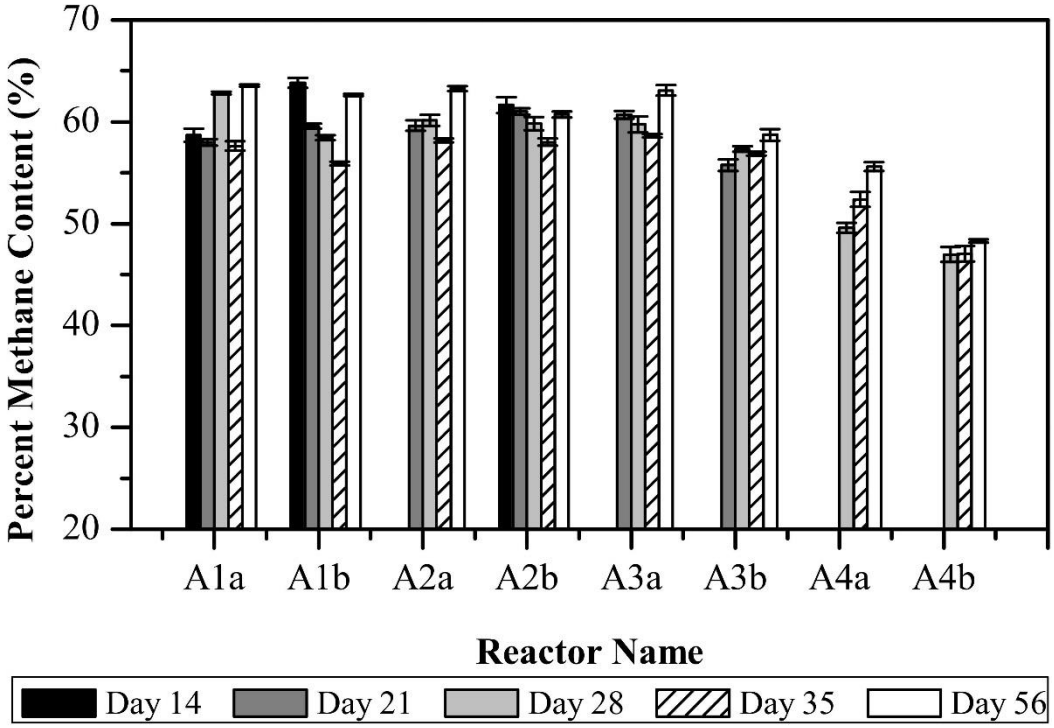


Figure 4-8: Methane contents of reactors fed with microalgal slurry

As can be seen from Figure 4-8, methane contents of reactors were between 46% and 64%. Methane content variations in reactors with 34000 COD, namely, Reactor A1a and Reactor A1b, show distinct trends, comparing the methane contents observed at each measurement day. That is, the decreasing trend in methane content of Reactor A1b was not observed in Reactor A1a. This can be related to BM supplement, which is the only difference between these reactors. After 58.7 ± 0.66 % and 57.97 ± 0.33 % methane contents at Day 14 and Day 21 respectively, this value was increased to 62.82 ± 0.12 % at Day 28. Then, 57.63 ± 0.44 % methane was observed in the reactor at Day 35. However, methane content in produced biogas increased up to 63.57 ± 0.12 at Day 56. On the other hand, in Reactor A1b, highest methane content was achieved at Day 14, with a value of 63.4 ± 0.51 %. In consecutive measurements, methane content decreased down to 59.58 ± 0.24 %, 58.46 ± 0.25 and 55.92 ± 0.18 % at Day 21, Day 28, and Day 35 respectively. At Day 56, methane value was measured as 62.65 ±0.13. The decrease in methane percentage within consecutive three

weeks reveals inhibition of methanogenic activity in Reactor A1b, due to a byproduct such as ammonia. Since BM enhances AD process especially if there is imbalance in COD/N/P ratios such as in untreated microalgal slurry reactors, methane percentage at Day 14 was higher in Reactor A1b than in Reactor A1a. This fact may have caused rapid assimilation of proteins and other complex nitrogenous species into ammonia at a higher rate in Reactor A1b (See Table 4-4), which can be related to decreasing methane content. On the other hand, the ammonia production rate in A1a was slower, therefore acclimation of microorganisms to new ammonia levels were possible. Therefore, in Reactor A1a, methane content was lower in first two measurements, however, increased at Day 28, showing that the inhibitory effect of ammonia on methanogenic activity decreased, in turn, ammonia production rate in the system increased and lower methane content was observed at Day 35. Since exponential gas production period lasted 30 days, it can also be speculated that substrate assimilation rate slowed down after day 30 (See Figure 4-7), as well as ammonia production rate. Therefore, methanogenic activity was then influenced less by ammonia production, elevating methane content in the headspace of the reactor up to 63.57 ± 0.12 at Day 56.

Same trend was observed in reactors with 19000 mg/L COD. At Day 14, three-fold headspace volume biogas had not been produced in Reactor A2a, therefore methane content measurement was not performed. On the other hand, in Reactor A2b, higher amount of biogas was produced due to BM supplement until Day 14 and highest methane content of 61.65 ± 0.78 % was seen, although a decreasing trend in methane content was evident until Day 56 when $60.73 \pm 0.28\%$ methane was achieved. In Reactor A2a, methane contents were rather fluctuating, with a highest value reached at Day 56 as $63.27 \pm 0.24\%$.

Methane content trends in reactors with 14000 mg/L COD was the opposite of the ones observed in reactors with higher COD concentrations. That is, the decreasing methane content trend was observed in Reactor A3a, rather than in Reactor A3b with BM. This result also proves that there had been ammonia

inhibition in reactors with higher S/X value, since final ammonia concentrations were below toxic levels in Reactors A3a and Reactor A3b, measured as 345 ± 49 and 215 ± 7 respectively (See Table 4-5).

In Reactors A4a and A4b, the lowest methane contents were observed. This was probably because of the fact that biogas production in these reactors were the lowest due to low COD values. Maximum methane content in these reactors were recorded at Day 56, as 55.59 ± 0.43 % in Reactor A4a and 48.31 ± 0.16 % in A4b.

Although methane contents of the reactors were varying among different COD values and BM presence, all values are within the range of 61% and 73% found in different studies (See Section 2.5.4.), except for Reactors A4a and A4b. In these reactors, low methane content may be due to the fact that low gas production was observed in these reactors; true methane content could not be measured.

Methane contents of the rectors were used for the calculation of observed methane volume produced in the reactors. For this purpose, methane content of the biogas was assumed to be constant until the first measurement, and equal to that of reported value at Day 14. Afterwards, methane content was assumed to be constant and equal to that of any measurement, beginning from the median of time between previous measurement until the median of time between the next measurement. After day 56, it was assumed that methane content in produced biogas was equal to that of Day 56, until the end of BMP test. Results of observed methane production calculations were used to calculate the percent ratio between observed and theoretical methane production, which are given on Table 4-3 with methane yields of reactors.

Table 4-3: Observed/Theoretical methane production and methane yield values of microalgal slurry reactors

	A1a	A1b	A2a	A2b	A3a	A3b	A4a	A4b
Observed /Theoretical** CH_4 produced (v/v * 100)	41.0	29.6	36.8	32.6	39.4	22.6	30.9	18.2
Methane Yield (ml CH4 / g VS added)	249	180	223	198	239	137	188	110

** Theoretical CH_4 volume was calculated considering that maximum of 0.395 L CH_4 can be produced per g COD added (Speece, 2008).

As shown on Table 4-3, percent ratio of observed and theoretical methane production values were linearly proportional to methane yields in reactors without BM addition. However, same relation was not observed for methane yields. BM had negative effect on both percent ratio of observed and theoretical methane production values and methane yields. The closest methane production to theoretical value was observed in Reactor A1a, with the high COD and no BM addition, which was equal to 41.0%. Compared to literature, this value is higher than that of reported by Williams (2012) as 15% for AD of *Chlorella vulgaris* as the sole substrate. Maximum methane yield of 249 ml CH_4/ g VS added was also observed in this reactor. This value is higher than that of those reported in the same study as 193 ml CH_4/ g VS added. In another study, Ras et. al. (2011) found that maximum of 240 ml CH_4/ g VS added could be produced by AD of *Chlorella vulgaris* grown in wastewater. Methane yields of reactors on COD basis are given on Appendix C.

4.2.1.3. tCOD, TKN, TP, sCOD, NH_4^+-N, PO_4^{3-}-P and pH Variations and Final VFA Concenrations

Observed tCOD, TKN, TP values and removal efficiencies of the reactors are presented on Table 4-4. Total COD removal efficiencies observed in Reactors A1a, A1b, A2a, A2b, A3a, A3b, A4a and A4b were ranging between 32% – 48.7 %. Although low efficiencies observed indicate inadequate digestion, these values are comparable to those reported in literature for digestion of algae, except for Reactor A4a, with a COD conversion efficiency of 18.5%. Similarly, Ras et al., (2011) reported COD removal of 33% to 51% in AD process of

Chlorella vulgaris. Percent ratio of observed methane production to theoretical value were in accordance with COD removal efficiencies, which indicate effective conversion of removed COD into biomethane (See Table 4-3). It can be concluded that low observed methane production values relative to theoretical methane production value is due to low removal efficiency of algae, rather than low conversion efficiency into methane. It was reported by Ras et al., (2011), that 50% of microalgae could be anaerobically digested. In another study conducted by Vergarafernandez et al. (2008), it was found that only 17% of COD can be removed by AD of marine macroalga *M. pyrifera.* On the other hand, Jegede, (2012) achieved 75- 85% COD removal using microalgae and cyanobacteria as substrates; however, after autoclave pretreatment of substrates prior to AD process.

Ammonification in the reactors were calculated using Equation 4-2.

$$Ammonification\ (\%) = \frac{(NH_4^+ - N)_{final} - (NH_4^+ - N)_{initial}}{TKN_{initial} - (NH_4^+ - N)_{initial}} \qquad (4\text{-}2)$$

In the reactors, ammonification ranged between 8.9% and 27.7% (See Table 4-4). These values are also similar to those reported by Ras et al. (2011) as 19% - 68%. However, microalgal AD results in lower ammonification than other nitrogen-rich substrates, such as poultry manure with nitrogen ammonification values ranging between 63 to 89% (Field et al., 1985). For TKN and NH_4^+-N values, negative removal data can be attributed to bioconversion of proteins into amino acids and to ammonia (Demirer and Chen, 2005). It can be seen from Table 4-5 that final ammonia concentrations in the reactors were above 200mg/L, which is a level with potential of inhibition. This observation is an explanation of low biogas yields observed in the reactors, especially with BM supplementation, which leads to higher concentrations of free ammonia due to higher pH values. Excessive ammonia formation in these reactors may have inhibited destruction of organic compounds and methanogenesis, caused accumulation of VFA (Krylova et al., 1997).

It can be seen from Table 4-4 that TP removal efficiencies in microalgal slurry reactors were between 0.9 and 4.7%. This is an expected result, since anaerobic digesters are known to reduce minor amounts of nutrients (Lusk, 1998).However, N:P value in all reactors were near or within the optimum range at the beginning and end of BMP assay (See Section 2.5.1.1 and Section 3.3.2.3).

Negative removal efficiencies of sCOD observed in Reactors A1a and A1b indicate hydrolyzed substrates could not be utilized efficiently, which may be a result of combined effect of substrate and ammonia inhibition in Reactor A1a, since both TAN and VFA concentrations in this reactor are very high. On the other hand, Inhibitory conditions observed in Reactor A1b can be related to ammonia toxicity only, since VFA accumulation was not significant. Biogas yield results shown on Table 4-2 also confirm that inhibition in Reactors A1a and A1b were in higher extent. It can be seen from Table 4-5, that correlation between final sCOD and final VFA values were observed not only in Reactors A1a and A1b, but in all reactors.

It can also be stated that BM had a negative effect on AD process of microalgae, considering lower tCOD removal efficiencies, which is a result not only of its negative effect on methanogens, but also causing less efficient hydrolysis and acidification. In other words, it can be inferred that lower VFA and sCOD values observed in reactors supplemented with BM is not due to enhanced methanogenic activity, but because of poor solubilization of tCOD.

Table 4-4: Initial and final tCOD, TKN, TP, COD:N and N:P values in untreated microalgal slurry reactors

	tCOD (mg/L)			TKN (mg/L)				TP (mg/L)			COD:N (g:g)		N:P (g:g)	
	Initial	**Final**	**Removal (%)**	**Initial**	**Final**	**Removal (%)**	**Ammonification (%)**	**Initial**	**Final**	**Removal (%)**	**Initial**	**Final**	**Initial**	**Final**
A1a	34091	21457 ± 0	37.1	2541	2450 ± 20	3.6	12.3	339	334 ± 12	1.5	13.4	8.8	7.5	7.3
A1b	34091	24899 ± 880	27.0	2541	2380 ± 119	6.3	8.9	339	332 ± 3	2.1	13.4	10.5	7.5	7.2
A2a	19272	12196 ± 72	36.7	1391	1316 ± 40	5.4	16.2	209	205 ± 4	1.9	13.9	9.3	6.7	6.4
A2b	19272	13259 ± 144	31.2	1391	1225 ± 10	12.0	10.2	209	200.± 7	4.2	13.9	10.8	6.7	6.1
A3a	14131	7247 ± 294	48.7	993	854 ± 20	14.0	27.7	164	156 ± 9	4.7	14.2	8.5	6.1	5.5
A3b	14131	8785 ± 174	37.8	993	1036 ± 0	-4.4	13.2	164	162 ± 6	1.1	14.2	8.5	6.1	6.4
A4a	9292	6316 ± 357	32.0	617	609 ± 0	1.3	14.6	121	120 ± 7	0.9	15.1	10.4	5.1	5.1
A4b	9292	7571 ± 57	18.5	617	585 ± 25	5.3	15.5	121	119 ± 4	1.5	15.1	13.0	5.1	4.9

Table 4-5: Initial and final pH, sCOD, TAN and PO_4^{3-}- P values in microalgal slurry reactors

	pH		sCOD (mg/L)			TAN (mg/L)			PO_4^{3-}- P (mg/L)			Final VFA
	Initial	**Final**	**Initial**	**Final**	**Removal (%)**	**Initial**	**Final**	**Removal (%)**	**Initial**	**Final**	**Removal (%)**	**(mg HAc/L)**
A1a	7.21	7.49	199.6	970 ± 14	-386.0	230	515 ± 49	-124.3	96	103 ± 5	-6.6	623.8
A1b	7.13	7.67	199.6	245 ± 7	-22.7	230	435 ± 21	-89.5	96	84 ± 0.7	13.2	91.9
A2a	6.95	7.22	127.4	105 ± 7.1	17.6	131	335 ± 9	-155.3	56	81 ± 0	-45.6	402.4
A2b	7.31	7.67	127.4	11.5 ± 0.7	91.0	131	260 ± 28	-98.1	56	47 ± 2.1	16.4	79.5
A3a	7.04	7.00	102.3	74 ± 4.2	27.7	97	345 ± 49	-255.2	42	99 ± 0	-138.1	194.4
A3b	7.22	7.71	102.3	13.5 ± 0.7	86.8	97	215 ± 7	-121.6	42	49 ± 0	-17.9	37.4
A4a	6.89	6.89	78.7	19 ± 1.4	75.9	65	146 ± 2	-123.8	28	61 ± 0.7	-115.6	96.0
A4b	7.32	7.76	78.7	10.5 ± 0.7	86.7	65	151 ± 0	-131.5	28	55 ± 0	-94.4	11.6

4.2.2. AD of Pretreated Microalgal Slurry

In order to investigate effect of pretreatment on AD of microalgae, batch anaerobic reactors with initial COD values of either 19000 ± 500 mg/L or 34000 ± 1500 had been operated. Three pretreatment methods, namely heat (H) autoclave (At) and thermochemical (Tc) pretreatments were applied to microalgal slurry. Twelve batch reactors were operated for BMP test of pretreated microalgae for 66 days. Reactors were evaluated in terms of biogas production potential, biogas and methane yields and treatment efficiencies.

4.2.2.1.Effect of Pretreatment on substrate characteristics

Efficiencies of different pretreatment methods were evaluated by comparison of COD solubilization values. Solubilization of COD was calculated using Equation 4-3 (Davidsson and La Cour- Jansen, 2006). Comparison of COD solubilization values are given on Table 4-6.

$$\%\, Solubilization = \frac{sCOD_{pretreated} - sCOD_{untreated}}{tCOD_{unterated}} \times 100 \quad (4\text{-}3)$$

Table 4-6: COD solubilization efficiencies of different pretreatment methods on microalgal slurry

Pretreatment Type	sCOD after pretreatment (mg/L)	Solubilization (%)
Heat	615 ± 21	1
Autoclave	6630 ± 141	15
Thermochemical	2300 ± 127	5

It can be seen from Table 4-6 that the COD solubilization efficiencies of heat, autoclave and thermochemical pretreatment were 1%, 15% and 5%, respectively, regarding 42943 ± 285 mg/L tCOD value of untreated microalgae (See Table 3-5). Autoclave pretreatment appears to be the most effective pretreatment method in terms of COD solubilization. Although pretreatment studies of microalgae for enhanced biomethane production are rather limited, these values are comparable with the literature in terms of solubilization efficiencies of WAS varying between 10.8 and 51.8% after various pretreatment methods such as heat and alkali (Kim et al., 2003).

Pretreatment solubilization effects were also expressed as sVS/VS percent increase calculated by Equation 4-4. (Passos et al., 2013).

$$VDS\ increase\ (\%) = \frac{\left(\frac{VDS}{VS}\right)_{pretreated} - \left(\frac{VDS}{VS}\right)_{untreated}}{\left(\frac{VDS}{VS}\right)_{untreated}} \times 100 \qquad (4\text{-}4)$$

Results of the calculation given on Table 4-7 reveal that VDS/VS increase values were not directly proportional to COD solubilization values for three pretreatment types, considering VSS and VS concentrations of 27950 ± 70 and 26753 ± 685 of untreated microalgal slurry. The highest VDS/VS increase value was observed in autoclave pretreated microalgal slurry as 1868%. This value was followed by heat- pretreated slurry as 420%. Lowest VDS/VS value was observed in thermochemically pretreated microalgal slurry as 276%. These results can be compared to the outcomes of the study conducted by Passos et al. (2013), revealing that microwave pretreatment of microalgae collected from wastewater treatment pond achieved 280 – 800% VDS/VS increase, depending on target specific energy, temperature and output power. It can be stated that thermochemical pretreatment caused lower VDS/VS increase than microwave pretreatment, whereas heat pretreatment is comparable to and autoclave pretreatment is higher than the range given by Passos et al. (2013).

Table 4-7: VDS/VS ratio and percent increase values of pretreatment types

Microalgal Slurry Type	VDS/VS ratio	VDS/VS increase (%)
Untreated	0.007	-
Heat Pretreated	0.038	420
Autoclave Pretreated	0.144	1868
Thermochemically Pretreated	0.027	276

As shown on Table 3-7 , total solids value of the pretreated microalgal slurry using this method caused decrease in TS and TKN concentrations. It can be inferred that autoclave pretreatment caused loss of dissolved species such as ammonia, since VS concentration was rather constant after pretreatment. Same observation was valid for TKN values of thermochemically-pretreated microalgae as well. These are expected results after the slurry was heated,

favoring ammonia loss (APHA, 2004). Moreover, this result is favorable to minimize ammonia toxicity in AD process.

4.2.2.2.Biogas Production

In all reactors, gas production was observed; however, gas production varied among reactors with different COD and depending on BM presence. Figure 4-9 and Figure 4-10 depict cumulative biogas production data of batch reactors with initial COD values of 19000 ± 500 mg/L and 34000 ± 1500 mg/L respectively, containing untreated or pretreated microalgae as substrates.

Compared to total biogas production values of 519 and 387 mL in A1a and A1b, reactors containing untreated microalgal slurry with 34000 ± 1500 mg/L initial COD , it can be seen from Figure 4-9 that heat and autoclave pretreatment were effective in terms of biogas production volume. Conversely, thermochemical pretreatment lowered cumulative biogas volume produced in reactors. Among reactors with 34000 ± 1500 mg/L initial COD, minimum cumulative biogas production was observed as 406 mL in Reactor TC1b containing thermochemically pretreated algae and BM; whereas maximum cumulative biogas production was observed as 637 mL in Reactor At1b, containing autoclave- pretreated algae and BM. In all reactors, biogas production rate increased after day 10 and slowed down by Day 25.

Heat pretreatment increased biogas production value to 595 mL in the absence of BM, which corresponds to an increase of 14.8%. Among reactors A1b and H1b, which contained BM supplementation, the percent increase in biogas production due to pretreatment was 48.3%. However, final biogas production volume was 575mL – 20 mL less than the value observed in H1a. It can be stated that pretreatment compensated inverse effect of BM.

Autoclave pretreatment revealed the highest results in terms of biogas production in both presence and absence of BM in reactors with 34000 ± 1500 mg/L COD. Autoclave pretreatment increased volume of biogas produced by 21.7% in At1a and 64.3% in At1b, compared to A1a and A1b, up to values 631

and 637 ml respectively. In this reactor pair, BM supplementation had a positive effect, slightly increasing biogas production compared to the reactor without BM.

Unlike heat and autoclave pretreatment, thermochemical pretreatment reduced biogas volume in Tc1a, causing cumulative biogas volume of 469 ml, 9.6% lower than in A1a. Biogas production in Tc1b was similar to that of A1b, with a value of 406 ml. In both pairs, BM supplementation resulted in less biogas production. Lower AD efficiency observed in reactors with thermochemical pretreatment where NaOH was used as pH increasing agent was also reported by Chen and Oswald (1998), compared to sole heat pretreatment.

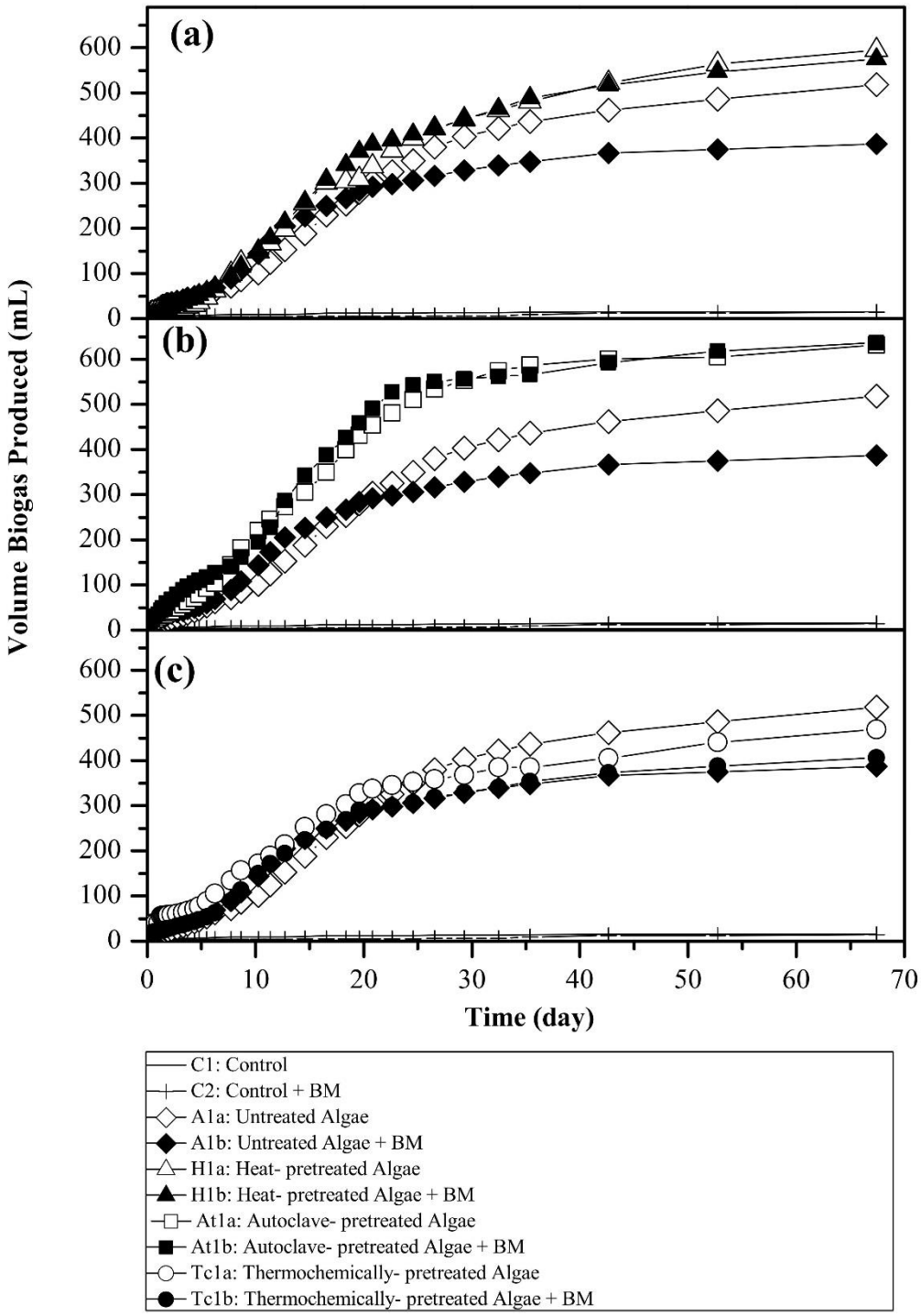


Figure 4-9: Cumulative biogas production data of pretreated microalgae reactors with 34000 ± 1500 mg/L COD: (a) Untreated Algae and Heat-pretreated Algae; (b) Untreated Algae and Autoclave- pretreated Algae; (c) Untreated Algae and Thermochemically- pretreated Algae.

Compared to total biogas production values of 267 and 212 mL in A1a and A1b, reactors containing untreated microalgal slurry with 19000 ± 500 mg/L initial COD , it can be seen from Figure 4-10 that heat and autoclave pretreatment were effective in terms of biogas production volume. On the other hand, thermochemical pretreatment lowered cumulative biogas volume produced in reactor without BM supplementation and its effect on BM supplemented reactor was insignificant. Among reactors with 19000 ± 500 mg/L initial COD, minimum cumulative biogas production was observed as 211 mL in Reactor TC2a containing thermochemically pretreated algae and no BM; whereas maximum cumulative biogas production was observed as 403 mL in Reactor H2a, containing heat- pretreated algae and no BM. In all reactors, biogas production rate increased after day 10 and slowed down by Day 25.

Heat pretreatment revealed the highest biogas production volume of 403 mL in the absence of BM, among pretreated microalgae reactors with 19000 ± 500 mg/L initial COD. This volume corresponds to an increase of 49.4% compared to A2a. Among reactors A2b and H2b, which contained BM supplementation, the increase in biogas production due to pretreatment was 86% and final biogas production volume was 395 mL, slightly lower than observed in H2a. It can be stated that pretreatment compensated inverse effect of BM.

Autoclave pretreatment increased volume of biogas produced by 46.3% in At2a and 57.9% in At2b, compared to A2a and A2b, up to values 395 and 335 ml respectively. In this reactor pair, BM supplementation had a negative effect in both pretreated and untreated microalgal slurry (See Section 4.2.1.1and Section 4.2.1.2).

Unlike heat and autoclave pretreatment, thermochemical pretreatment reduced biogas volume in Tc1a, causing cumulative biogas volume of 211 ml, 21.9% lower than in observed in A2a. Biogas production in Tc2b was 7.9% higher than that of A2b, with a value of 229 ml. However, this result is insignificant, since

highest biogas production among untreated and thermochemically pretreated microalgal slurry reactors was observed in A2a as 267 ml.

It can be seen from Figure 4-9 and Figure 4-10 that BM had negative or insignificant effect on cumulative gas production, regardless of initial COD concentrations and pretreatment method. This result can be related to ammonia inhibition in reactors with BM supplementation, which increases buffer capacity and pH (See Table 4-11); in turn, increased free ammonia concentration (See Section 2.5.1.1.)

Considering that ammonia concentrations reached at the end of AD process in pretreated microalgal slurry reactors were higher than observed in untreated microalgal slurry reactors, whereas the biogas production methane yields were also higher, it can be concluded that difficulty of cell disintegration negatively affected AD of untreated microalgae more than elevated ammonia concentrations in the reactors. This was an expected result, since hydrolysis of microalgal cell wall has been known to be problematic (See Section 2.5.4.1.).

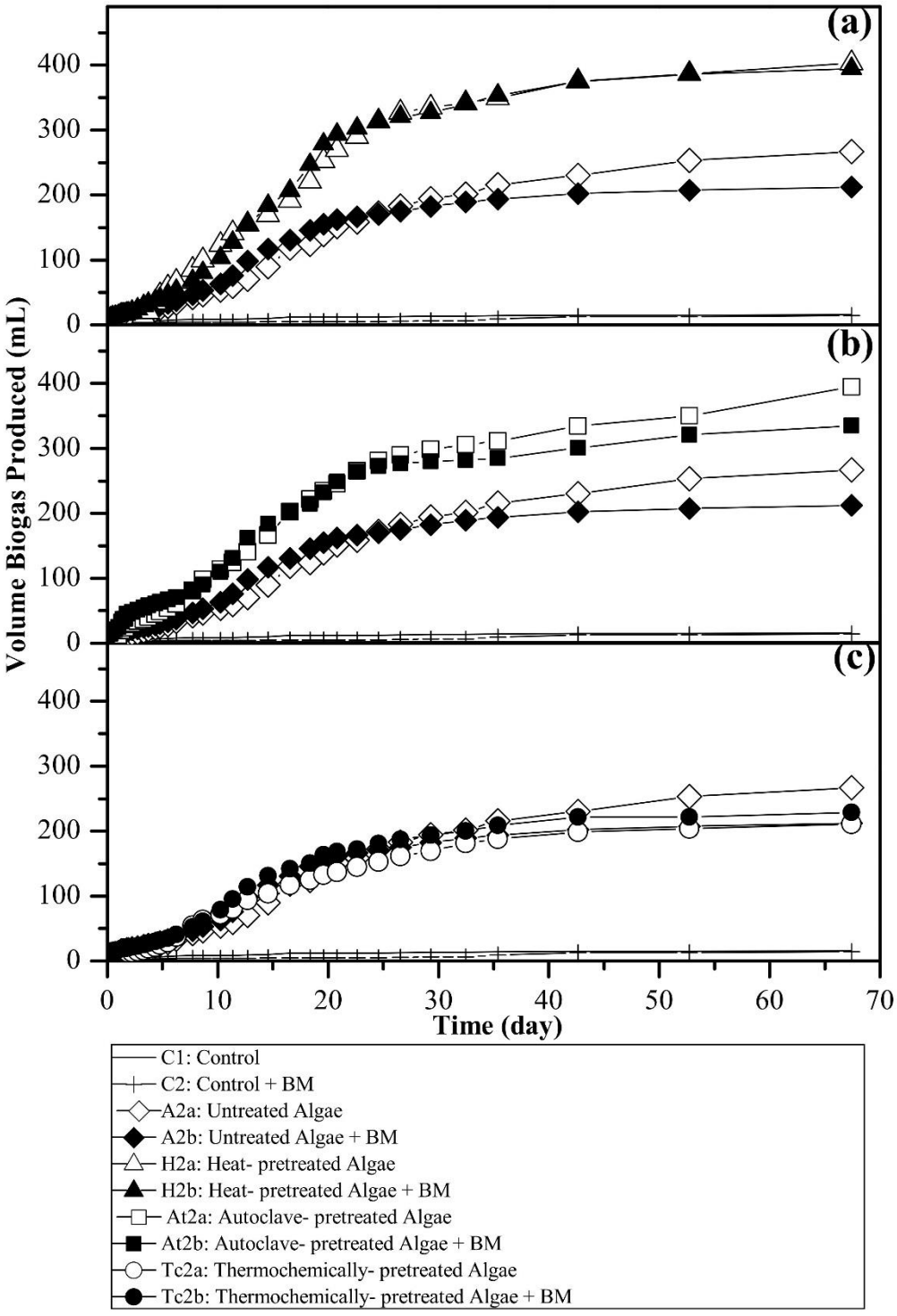


Figure 4-10: Cumulative biogas production data of pretreated microalgae reactors with 19000 ± 500 mg/L COD: (a) Untreated Algae and Heat-pretreated Algae; (b) Untreated Algae and Autoclave- pretreated Algae; (c) Untreated Algae and Thermochemically- pretreated Algae.

Biogas production yields on VS basis are given on Table 4-8 for pretreated microalgal slurry reactors. It can be seen that highest biogas yield was observed in Reactor At2a as 595 mL/g VS and lowest biogas yield was observed in Tc1b as 303 mL/g VS. All values lie between the range of 287 - 587 mL/g VS added, reported in literature (See Section 2.5.4.). It was also observed that biogas yields of heat pretreated and autoclave pretreated microalgae were higher than those of untreated microalgae reactors, whereas reactors without BM supplementation resulted in lower biogas yields in thermochemically pretreated microalgae, compared to untreated microalgae reactors. In addition, biogas yield of reactors with 19000 ± 500 mg/L were found to be higher than of reactors with 34000 ± 1500 mg/L for heat and autoclave pretreated microalgal slurry reactors. Same result was evident for untreated microalgae reactors as well. This result is reasonable, since degradation of microalgae is difficult and relative conversion of substrate into biogas was lower in highly loaded reactors, although cumulative gas production values were higher. Moreover, extent of substrate inhibition may be higher in highly- loaded systems (Angenent et al., 2002). Biogas yields of pretreatment reactors on COD basis are given on Appendix C.

Table 4-8: Biogas Yields of Reactors fed with pretreated microalgal slurry

	H1a	**H1b**	**H2a**	**H2b**	**At1a**	**At1b**	**At2a**	**At2b**	**TC1a**	**TC1b**	**TC2a**	**TC2b**
VS added (g)	1.39	1.39	0.69	0.69	1.33	1.33	0.66	0.66	1.34	1.34	0.67	0.67
Biogas Yield (mL/g VS added)	428	414	580	568	476	479	595	505	350	303	315	342

4.2.2.3.Methane contents of reactors with pretreated microalgal slurry

In order to determine methane production yields of reactors, methane contents were determined on percentage basis. Measurements were performed at Day 7, 14, 21, 28, 35 and 56; started after the headspaces of reactors were washed with produced biogas; that is, when produced biogas volume exceeded three-folds volume of headspace. Figure 4-11 depicts methane contents of pretreated microalgal slurry reactors.

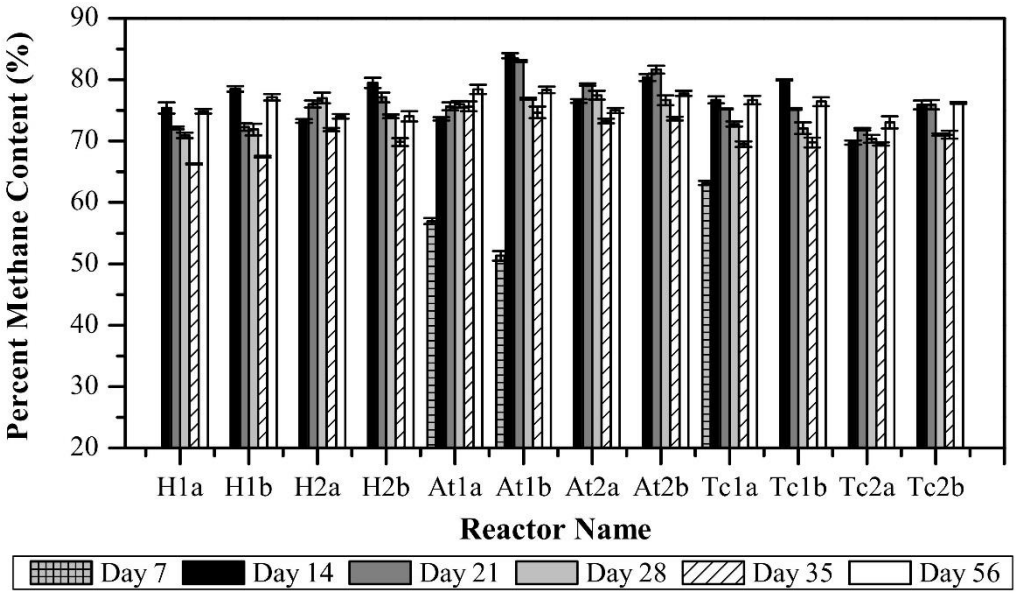


Figure 4-11: Methane contents of reactors fed with pretreated microalgal slurry

As can be seen from Figure 4-11, methane contents of reactors varied between 51.3 ± 0.79 % and 83.9 ± 0.43. When compared to the range reported in literature as 61% to 73% for anaerobic digestion of microalgae, observed range in this study for reactors with pretreated microalgal slurry had a broader range. However, it can be seen that methane contents were between 63.2 and 79.9 % among 90% of the measurements. Lower values of 56.9 ± 0.51 and 51.3 ± 0.79, 63 were observed in first methane content measurements of At1a and At1b at Day 7. Higher values above 79.9% were observed in reactors At1b and At2b as 83.9 ± 0.43 and 80.4 ± 0.58 at Days 14 and as 83.0 ± 0.13 and 81.7 ± 0.65 at Day 21. These values, however, are also feasible in AD process (See Section 2.5.). Variations in methane contents show that methanogenic bacteria activity did not have a constant rate and can be a result of physical or chemical changes in the reactors such as pH, ammonia and VFA concentrations in different time increments.

Methane contents of the reactors were used for the calculation of observed methane volume produced in the reactors. Results of observed methane production calculations were used to calculate the percent ratio between

observed and theoretical methane production, which are given on with methane yields of reactors.

Table 4-9: Observed/Theoretical methane production and methane yield values of pretreated microalgal slurry reactors

	H1a	H1b	H2a	H2b	At1a	At1b	At2a	At2b	TC1a	TC1b	TC2a	TC2b
Observed /Theoretical CH_4 **produced (v/v * 100)**	48.1	48.0	65.0	67.4	56.1	60.0	67.2	58.7	40.3	32.5	26.0	35.9
Methane Yield (ml CH4 / g VS added)	291	290	393	408	332	356	398	348	258	208	195	230

** Theoretical CH_4 volume was calculated considering that maximum of 395 mL CH_4 can be produced per g COD added (Speece, 2008).

As shown in Table 4-9, maximum methane yield of 408 ml CH_4/ g VS was observed in Reactor H2b, which contained 19000 ± 500 mg/L initial COD and BM. This value is comparable and slightly higher than reported by Mussgnug et al. (2010) as 387 ml CH_4/ g VS. It can also be seen that negative effect of BM on methane yields in untreated microalgal slurry reactors was not evident in heat and autoclave- pretreated microalgal slurry reactors. Conversely, methane yields were equal in the presence and absence of BM in reactors with 34000 ± 1500 mg/L initial COD and heat-pretreated microalgae. In heat- pretreated microalgal slurry reactors with 19000 ± 500 mg/L initial COD, BM supplementation increased methane yield to 408 ml CH_4/ g VSadded, compared to BM-free reactor H2a, having methane yield value of 393 ml CH_4/ g VSadded. Methane yields of reactors on COD basis are given on Appendix C.

Similar result was observed in autoclave- pretreated microalgal slurry reactors with 34000 ± 1500 mg/L initial COD. BM addition increased methane yield from 356 to 398 ml CH_4/ g VS added. However, for lower COD values of 19000 ± 500 mg/L initial COD, BM addition resulted in lower methane yield, despite increased values after autoclave pretreatment, compared to untreated microalgal slurry reactors with the same initial COD concentrations.

It can also be seen from Table 4-3 and Table 4-9 that thermochemical pretreatment had relatively negative effect on methane yields. BM

supplementation caused negative effect on methane yield in Reactor TC1b, compared to Reactor TC1a at same initial COD concentrations of 34000 ± 1500 mg/L. In reactors with 19000 ± 500 mg/L initial COD, effect of BM was the opposite; BM supplementation increased methane yield slightly, compared to untreated microalgal slurry reactors.

Changes in methane yields after pretreatment relative to untreated microalgal slurry diffeed among pretreatment types. Figure 4-12 presents percent increases in methane yields of reactors with different initial COD values, namely, 34000 ± 1500 mg/L and 19000 ± 500 mg/L after different application of various pretreatment methods, relative to the maximum methane yield observed in their untreated microalgal slurry equivalents. That is, percent increase relative to A1a for pretreated microalgal slurry reactors with 34000 ± 1500 mg/L and percent increase relative to A2a for pretreated microalgal slurry reactors with 19000 ± 500 mg/L were calculated, since these reactors yielded higher methane contents compared to their BM supplemented equivalents.

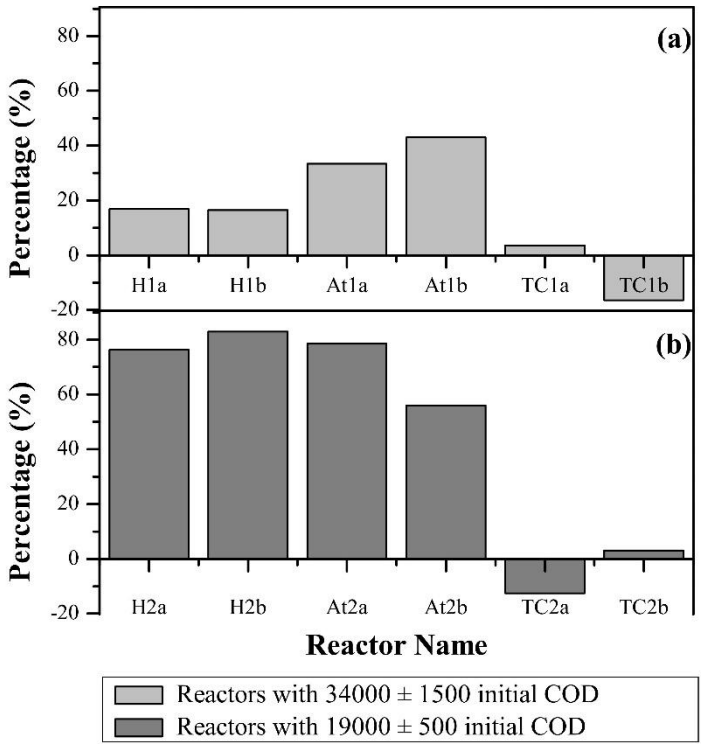
For reactors with 34000 ± 1500 mg/L initial COD, it can be seen that the highest percent increase was observed in Reactor At1b, as 43.0%, which is followed by Reactor At1a, with 33.3%. It can be stated that autoclave pretreatment was the most effective method for relatively higher COD values, in terms of methane yield. This pretreatment type was followed by heat pretreatment, with 16.9 and 16.4% increase in methane yields in reactors H1a and H1b respectively. The negative effect of thermochemical pretreatment on methane yield was observed in Reactor Tc1b as 16.5% relative decrease in methane yield, whereas the slightly positive effect of only 3.6% was achieved in Tc1a.

As shown on Figure 4-12b, reactors with 19000 ± 500 mg/L initial COD, it can be seen that the highest percent increase was observed in Reactor H2b, as 83.0%, which is followed by Reactor At2a, with 78.5%, H2a with 76.23% and At2b with 56.1%. It can be stated that heat pretreatment was the most effective method for relatively lower COD values, in terms of methane yield. This pretreatment type was followed by autoclave pretreatment. The negative effect of thermochemical

pretreatment on methane yield was evident in Reactor Tc2a as 12.6% relative decrease in methane yield, whereas the slightly positive effect of only 3.1% was achieved in Tc1a.

Relatively, reactors with lower COD values responded better to pretreated microalgal slurry utilization as substrate. This fact proves that hydrolysis enhancement by pretreatment methods enhances methanogenesis. Overall, performances of pretreated microalgal slurry in this study are comparable to the literature. Chen and Oswald (1998) found that heat pretreatment improves methane yield from AD of microalgae by 33%. In the same study, negative effect of high NaOH concentrations on biomethane production during thermochemical pretreatment was also revealed. In another study conducted by Alzate et al. (2012), thermal hydrolysis was found to be effective in biomethane production enhancement increasing yield up to 46-62%.

Pretreatment studies other than heat, autoclave and thermochemical were available for microalgae in the literature. For instance, Passos et al. (2013) found that microwave pretreatment of microalgae enhances biogas yield by 12-78%. High presuure thermal hydrolysis (HPTH) was also studied as a pretreatment method (Keymer et al., 2013), increasing methane yield up to 81%. In the same study, it was revealed that after lipid extaction, HPTH increased methane yield up to 110% over raw algae.



H1a: Heat-pretreated algae - 34000 mg/L COD
H1b: Heat-pretreated algae - 34000 mg/L COD + BM
H2a: Heat-pretreated algae - 19000 mg/L COD
H2b:Heat-pretreated algae - 19000 mg/L COD + BM
At1a: Autoclave- pretreated algae - 34000 mg/L COD
At1b: Autoclave- pretreated algae - 34000 mg/L COD +BM
At2a: Autoclave- pretreated algae - 19000 mg/L COD
At2b: Autoclave- pretreated algae - 19000 mg/L COD +BM
Tc1a: Thermochemically pretreated algae - 34000 mg/L COD
Tc1b: Thermochemically pretreated algae - 34000 mg/L COD +BM
Tc2a: Thermochemically pretreated algae - 19000 mg/L COD
Tc2b: Thermochemically pretreated algae - 19000 mg/L COD + BM

Figure 4-12: Percent yield increases in batch reactors fed with pretreated microalgal slurry: (a) Percent yield increase in reactors with 34000 ± 1500 mg/L initial COD relative to A1a; (b) Percent yield increase in reactors with 19000 ± 500 mg/L initial COD relative to A2a

4.2.2.4. tCOD, TKN, TP, sCOD, NH_4^+-N, PO_4^{3-}-P and pH Variations and Final VFA Concentrations

Observed tCOD, TKN, TP values and removal efficiencies of the reactors are given in Table 4-10. Total COD removal efficiencies observed in pretreated microalgal slurry reactors between 28.7% and 60.5%, having a broader range than that of reactors fed with raw microalgal slurry reported as 32% – 48.7 %. It can be stated that thermochemical pretreatment lowered COD removal efficiency of microalgae, whereas heat and autoclave pretreatment improved the efficiency. The highest COD removal efficiencies were observed in autoclave-pretreated microalgal slurry reactors. In these reactors, COD removal efficiencies were higher than that of reported by Ras et al. (2011) as 33% to 51%. However; Jegede, (2012) achieved 75- 85% COD removal using microalgae and cyanobacteria as substrates after autoclave pretreatment of substrates prior to AD process.

Percent ratio of observed methane production to theoretical value were in accordance with COD removal efficiencies, which indicate effective conversion of removed COD into biomethane (See Table 4-9).

In the reactors, ammonification ranged between 9.7% and 64.5% (See Table 4-11). These values are also similar to those reported by Ras et al., (2011) as 19% - 68%. For TKN and NH_4^+-N values, negative removal data can be attributed to bioconversion of proteins into amino acids and to ammonia (Demirer and Chen, 2005). It can be seen from Table 4-5 that final ammonia concentrations in the reactors were above 200 mg/L, which is a level with potential of inhibition. On the other hand, although final ammonia concentrations in pretreated microalgal slurry reactors were higher than those fed with raw microalgal slurry, especially heat and autoclave pretreatment positively affected methane yields, COD removal and ammonification. It can be concluded, that the main reason for low efficiencies in untreated microalgal slurry reactors was not ammonia toxicity, but difficulty in solubilization. Nevertheless, ammonia inhibition may be the cause of low biogas yields

observed in the reactors, especially with BM supplementation, which leads to higher concentrations of free ammonia due to higher pH values.

It can be seen from Table 4-10 that TP removal efficiencies in pretreated microalgal slurry reactors were between 0.3 and 4.5%. This is an a result similar to those observed in untreated microalgal slurry reactors and in consistency with the literature (Lusk, 1998). Although initial N:P values in some reactors were slightly higher than optimum range, N:P values at the end of the BMP assay were all within the optimum range .

Inhibitory conditions observed in AD process can be indicated by final VFA concentrations above 100 - 300 mg HAc/L (Speece, 2008). It can be seen from Table 4-11 that there has not been significant inhibition in methanogenic activities, considering VFA concentrations less than or equal to 191.8 mg/L mg HAc/L. It can also be seen that final sCOD concentrations were in correlation with final VFA concentrations.

Table 4-10: Initial and final tCOD, TKN, TP, COD:N and N:P values in untreated microalgal slurry reactors

	tCOD (mf/L)			TKN (mf/L)				TP (mf/L)			COD:N (g/g)		N:P (g/g)	
	Initial	Final	Removal (%)	Initial	Final	Removal (%)	Ammonification (%)	Initial	Final	Removal (%)	Initial	Final	Initial	Final
H1a	33279	18421 ± 283	44.6	2561	2296 ± 40	10.3	62.5	319	333 ± 2.9	2.0	13.0	8.0	8.0	7.3
H1b	33279	18927 ± 964	43.1	2561	2240 ± 119	12.5	31.2	319	330 ± 1.7	2.8	13.0	8.4	8.0	7.2
H2a	18866	9636 ± 0	48.9	1401	1246 ± 20	11.1	51.8	199	202 ± 8.6	3.4	13.5	7.7	7.0	6.5
H2b	18866	10324 ± 170	45.3	1401	1428 ± 0	-1.9	23.5	199	200 ± 9.0	4.2	13.5	7.2	7.0	7.5
At1a	32514	12955 ± 555	60.2	2291	2310 ± 59	-0.8	43.7	339	338 ± 4.9	0.3	14.2	5.6	8.5	6.8
At1b	32514	12854 ± 144	60.5	2291	2268 ± 40	1.0	64.5	339	336 ± 5.0	1.0	14.2	5.7	8.5	6.7
At2a	18484	7449 ± 116	59.7	1267	1246 ± 59	1.6	50.0	209	205 ± 10.7	1.7	14.6	6.0	7.3	6.1
At2b	18484	7844 ± 0	57.6	1267	1253 ± 30	1.1	39.2	209	203 ± 13.2	3.0	14.6	6.3	7.3	6.2
Tc1a	33937	23785 ± 144	29.9	2290	2450 ± 139	-7.0	15.1	355	329 ± 11.9	3.1	14.8	9.7	6.5	7.1
Tc1b	33937	24190 ± 143	28.7	2290	2492 ± 119	-8.8	9.7	355	333 ± 13.8	2.0	14.8	9.7	6.5	7.2
Tc2a	19195	13462 ± 420	29.9	1266	1281 ± 69	-1.2	17.7	217	200 ± 7.9	4.5	15.2	10.5	5.8	6.2
Tc2b	19195	13512 ± 212	29.6	1266	1274 ± 40	-0.6	9.9	217	202 ± 11.1	3.2	15.2	10.6	5.8	6.1

Table 4-11: Initial and final pH, sCOD, TAN, PO_4^{3-}-P and final VFA values in pretreated microalgal slurry reactors

	pH		sCOD (mf/L)			TAN (mf/L)			PO_4^{3-}-P (mf/L)			Final VFA
	Initial	Final	Initial	Final	Removal (%)	Initial	Final	Removal (%)	Initial	Final	Removal (%)	(mg HAc/L)
H1a	7.22	7.66	480	650 ± 42	-35.5	278	1540 ± 57	-177.1	135	117 ± 1.4	13.2	191.8
H1b	7.34	7.92	480	425 ± 35	11.4	278	890 ± 12	-220.3	135	46 ± 0.7	66.3	110.3
H2a	6.92	7.43	267	410 ± 14	-53.4	155	720 ± 14	-363.3	75	81 ± 0.7	-7.4	136.0
H2b	7.11	7.83	267	190 ± 14	28.9	155	455 ± 21	-192.8	75	54 ± 2.8	28.0	81.4
At1a	7.3	7.88	4631	1055 ± 7	77.2	737	1425 ± 41	-93.4	208	113 ± 0.0	45.7	109.3
At1b	7.33	8.06	4631	450 ± 57	90.3	737	1725 ± 17	-134.1	208	58 ± 1.4	72.1	57.5
At2a	7.31	7.5	2343	170 ± 14	92.7	385	815 ± 49	-111.8	112	90 ± 2.1	19.8	44.4
At2b	7.29	7.81	2343	125 ± 7	94.7	385	725 ± 21	-88.4	112	56 ± 3.5	50.2	19.6
Tc1a	6.9	7.44	1642	385 ± 21	76.6	364	680 ± 14	-86.7	203	122 ± 4.2	39.8	92.9
Tc1b	7.1	7.89	1642	565 ± 35	65.6	364	570 ± 17	-56.5	203	49 ± 0.7	76.1	49.8
Tc2a	7.14	7.12	849	211 ± 13	75.1	199	390 ± 28	-96.4	109	91 ± 0.7	16.9	60.6
Tc2b	7.32	7.74	849	280 ± 14	67.0	199	305 ± 11	-53.6	109	56 ± 1.4	48.6	56.8

4.2.3. Anaerobic Co-digestion of Microalgal Slurry

In order to investigate effect of co-digestion on AD of microalgae, batch anaerobic reactors with initial COD values of 9000 ± 300 mg/L, 14000 ± 50 mg/L, 19000 ± 500 mg/L and 34000 ± 1500 mg/L had been operated. Two co-substrates, namely, MKW and WAS, were mixed with microalgal slurry in several proportions or COD values, and batch reactors were fed. MKW and WAS were used as sole substrates as well, in order to determine their biomethane potentials and compare with those of co-digested reactors (See Table 3-12). A total of 14 batch reactors, eight of which contained MKW (Reactors M1a, M1b, M2a, M2b, M3a, M3b, M4a and M4b) and remaining six containing WAS (Reactors W1a, W1b, W2a, W2b, W3a and W3b) were operated for BMP test of co-digestion for 66 days. Reactors were evaluated in terms of biogas production potential, biogas and methane yields and treatment efficiencies.

4.2.3.1.Biogas Production

Depending on initial COD values and presence of BM, gas production values varied among co-digestion reactors. Four co-digestion reactors with 34000 ± 1500 mg/L initial COD, namely, reactors M2a, M2b, M4a and M4b, M2a and M2b were initially fed with 1/1 (g COD added/ g COD added) ratio of microalgal slurry to MKW whereas M4a and M4b were initially fed with 1/7 (g COD added/ g COD added) ratio of microalgal slurry to MKW. M2a and M4a contained no BM, whereas M2b and M4b were supplemented with BM. Compared to total biogas production values of reactors containing untreated microalgal slurry with 34000 ± 1500 mg/L initial COD recorded as 519 and 387 mL in A1a and A1b, it can be seen from Figure 4-13 that M2a and M4a were not effective in terms of biogas production volume. In Reactor M2a, biogas production stopped after a rapid production period at Day 2 with the final value of 344 ml. In M4a, gas production was observed until day 2 and was insignificant until Day 18. Afterwards, gas production rate increased, however, reached a final value of 334 ml. These low cumulative biogas volumes indicate presence of inhibition in these reactors. Considering relatively high COD/N values of these reactors,

inhibition could be caused by VFA accumulation (Zhao and Ruan, 2013). Conversely, among all reactors with 34000 ± 1500 mg/L, including the ones fed with pretreated microalgal slurry initial COD, maximum cumulative biogas production was observed as 1089 mL in Reactor M2b.This reactor is followed by 748 ml total gas production value on M4b. In these reactors, BM addition provided buffer against acidification of reactors due to high VFA concentrations. In both reactors, biogas was produced rapidly for the first four days. Then, gas production became insignificant until Day 20 in Reactor M2b and until Day 15 in reactor M4b and exponential biogas production was observed in both reactors afterwards. It can be stated that readily biodegradable organics were first consumed in the reactors and hydrolysis and consecutive biogas production continued later, especially due to difficult hydrolization of microalgal biomass (Zhao and Ruan, 2013).

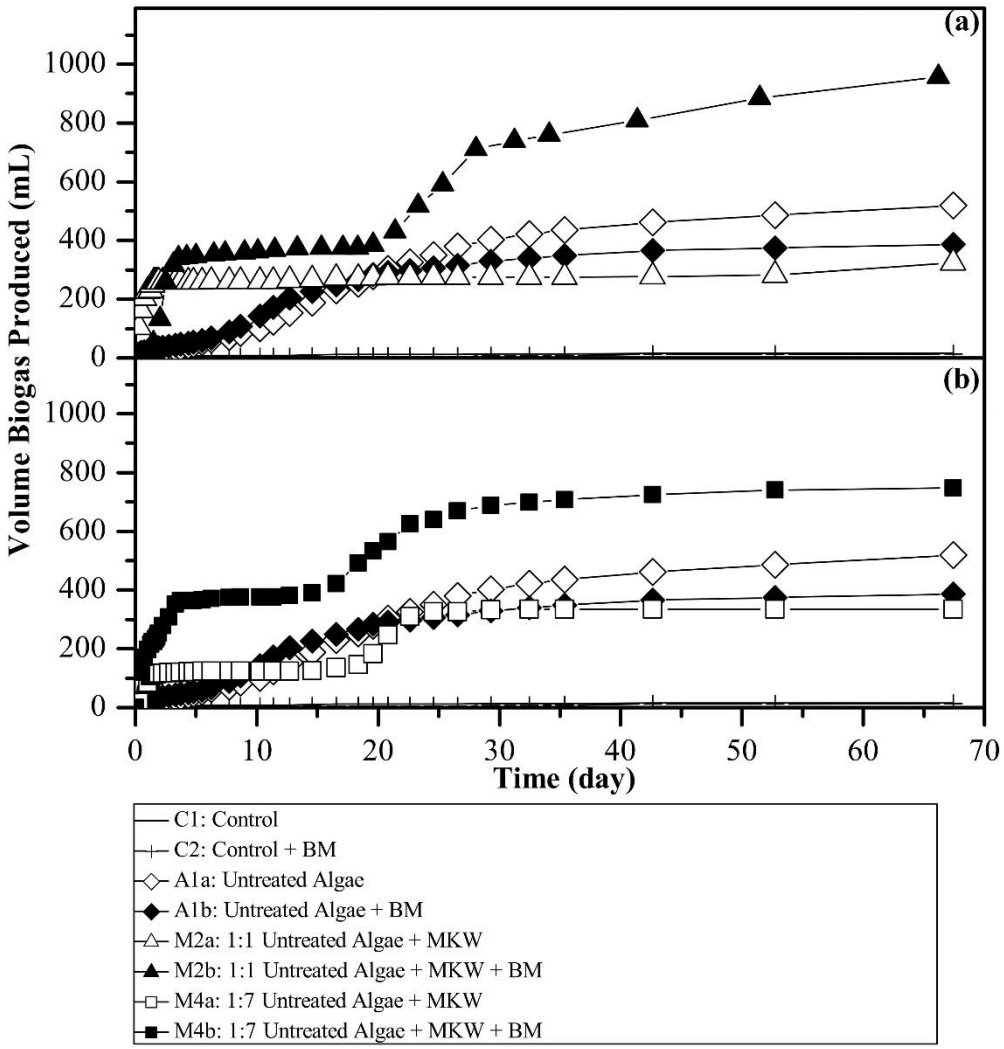


Figure 4-13: Cumulative biogas production data of co-digestion reactors with 34000 ± 1500 mg/L COD: (a) Untreated Algae and 1/1 (CODadded/CODadded) Co-digestion of Untreated Algae and MKW ; (b) Untreated Algae and 1/7 (CODadded/CODadded) Co-digestion of Untreated Algae and MKW.

In order to determine biogas potential of MKW as sole substrate, two reactors with initial COD concentrations of 19000 ± 500 mg/L, namely, M1a and M1b were operated. M1a contained no BM, whereas BM was added to M1b. Figure 4-14 demonstrates cumulative biogas production values of these reactors, compared to A2a and A2b, microalgal slurry reactors with the same initial COD concentrations. It can be seen that 621 ml biogas was produced in M1b, at the

end of 66 days operation. Rapid biogas production was examined during the first 48 hours. Then, biogas production rate slowed down until Day 10 and another exponential biogas production period was observed until Day 20. Afterwards, biogas production was still evident, however, in a slower rate. On the other hand, in M1a, 244 ml biogas was produced within the first 48 hours and then production stopped permanently. This result indicates complete inhibition. Comparing biogas production values of M1a and M1b, it can be stated that the inhibition was observed due to the absence of BM, which provides nutrients and alkalinity. This fact can be proven by the final VFA concentrations in these reactors, 5130 and 107 mg HAc/L, respectively (See Section 4.2.3.3).

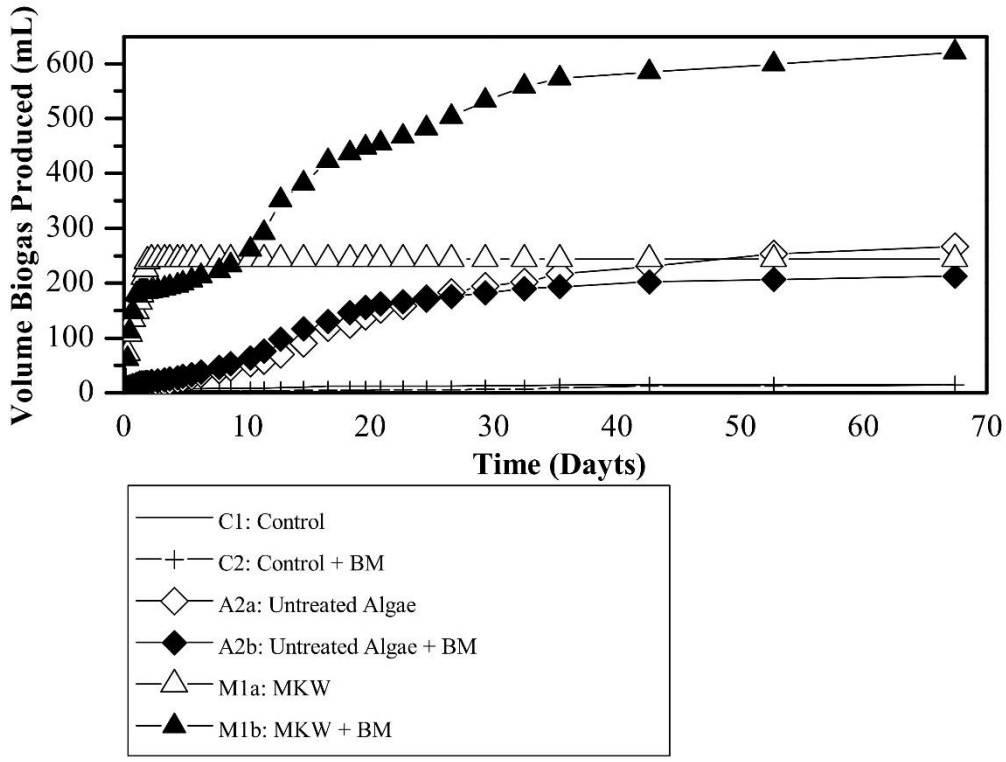


Figure 4-14: Cumulative biogas production data of untreated algae and MKW reactors with 19000 ± 500 mg/L COD.

Apart from untreated microalgal slurry reactors, four co-digestion reactors with 9000 ± 300 mg/L, namely, W1a, W1b, W3a and W3b were operated. W1a and W1b contained WAS as sole substrate, whereas W3a and W3b were fed with 1/1 (g COD added/ g COD added) ratio of microalgal slurry to WAS. Reactors W1a

and W3a had no BM. Reactors W1b and W3b were supplemented with BM. As shown in Figure 4-15, compared to total biogas production values of reactors containing untreated microalgal slurry with 9000 ± 300 mg/L initial COD recorded as 99 and 74 mL in A4a and A4b. Reactor W3a was not significantly more effective in terms of biogas production volume, considering the final value of 102 ml. Nevertheless, its BM supplemented equivalent, Reactor W3b produced 117 ml cumulative biogas. However, final cumulative biogas production values in both reactors were lower than that of reactors W1a and W1b, resulted in 140 and 143 ml total biogas production from WAS as the sole substrate.

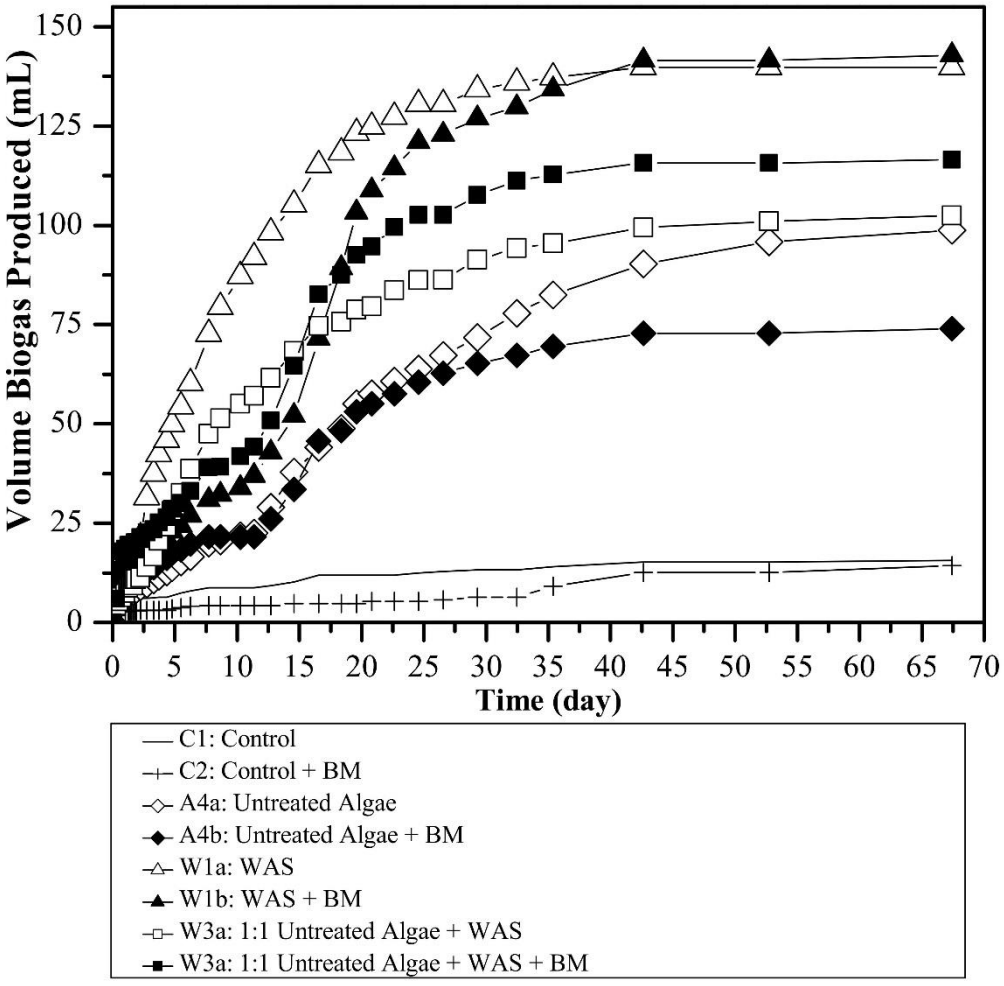


Figure 4-15: Cumulative biogas production data of untreated microalgae, WAS and co-digestion reactors with 9000 ± 300 mg/L COD.

In order to compare co-digestion effects of MKW and WAS, four reactors with initial COD values of 14000 ± 50 mg/L, namely, reactors M3a, M3b, W2a and W2b were initially fed with 1/1 (g CODadded/ g CODadded) ratio of microalgal slurry to MKW or WAS. MKW was used as co-substrate in M3a and M3b, whereas WAS was used as co-substrate in W2a and W2b. M3b and W2b were supplemented with BM; M3a and W2a were free of BM. As shown on Figure 4-16, BM addition had slightly lowering effect on total biogas production of in both MKW and WAS co-digestion reactors. However, It can be seen that BM addition shortened lag phase of biogas production in MKW co-digestion reactors. It can therefore be stated that VFA accumulation was firstly evident in reactor M3a; thus, acclimation period was necessary for anaerobic flora. On the other hand, owing to balanced pH values and trace element concentrations provided by BM, acclimation period was not observed in Reactor M3b. In WAS co-digestion reactors, final cumulative biogas volumes were close to that of A4a; nevertheless, slightly higher biogas production value of 222 mL was observed in W2a.

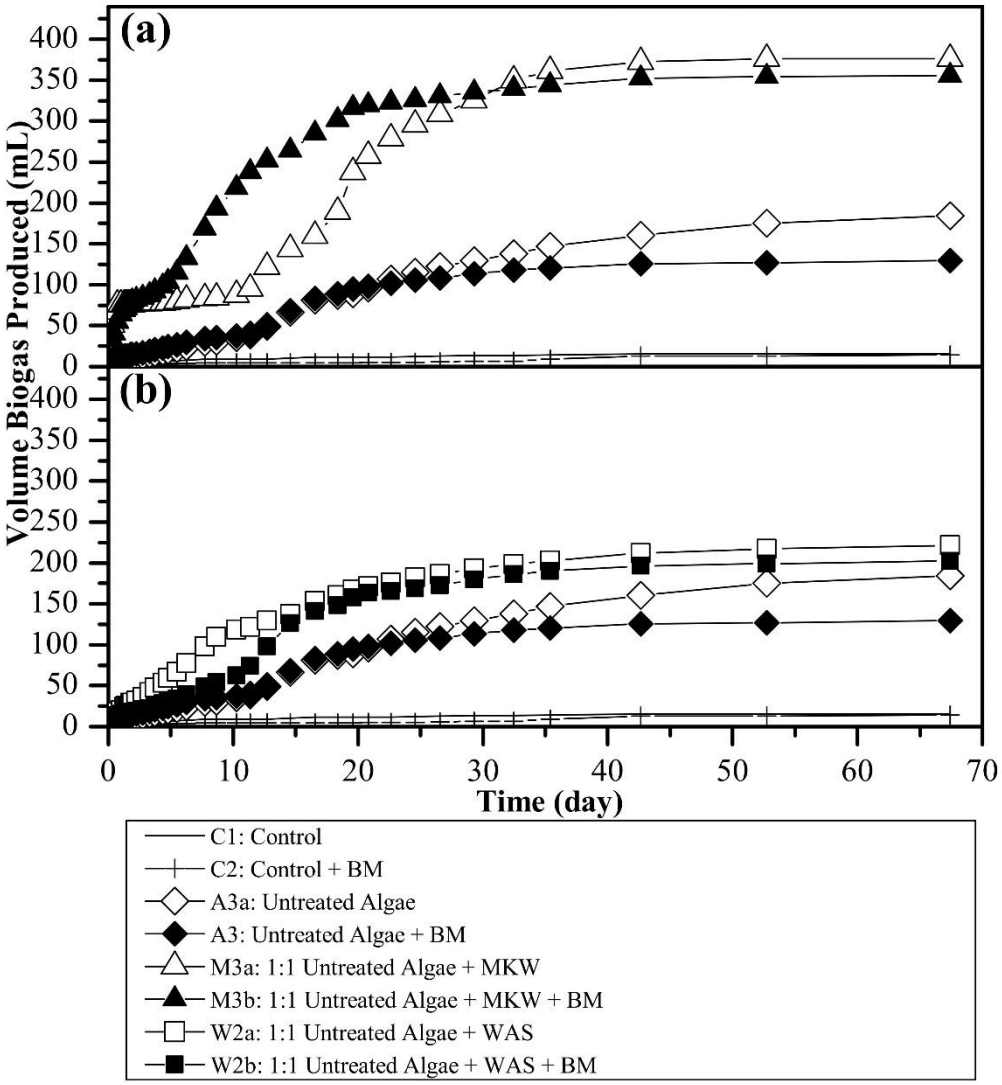


Figure 4-16: Cumulative biogas production data of co-digestion reactors with 14000 ± 50 mg/L COD: (a) Untreated Algae and 1/1 (CODadded/CODadded) Co-digestion of Untreated Algae and MKW ; (b) Untreated Algae and 1/1 (CODadded/CODadded) Co-digestion of Untreated Algae and WAS.

The comparison about biogas production efficiencies of co-digestion reactors with different COD concentrations and biogas production yields on VS basis were calculated and are given in Table 4-12. It can be seen that the highest biogas yield was achieved in Reactor M3a as 785 ml / d VS added. Biogas yield in this co-digestion reactor was higher than that of MKW and untreated microalgal slurry, fed as sole substrates. Minimum biogas yield was also achieved in a MKW co-digestion reactor, Reactor M4a. It can also be seen that BM-free MKW reactors, namely, Reactors M1a, M2a and M4a failed in biogas production, compared to their BM- supplemented equivalents, M1b, M2b and M4b. However, same situation was not observed in M3a. This fact could be a result of lower C/N and COD/N ratios achieved in this reactor (See Table 3-12), in turn, less VFA accumulation (See Section 4.2.3.3.)

As shown in Table 4-12, among WAS co-digestion reactors, highest biogas yield was observed in W1b, in which no microalgal slurry existed. Moreover, in co-digestion reactors W2b and W3a, lowest biogas yields of 409 ml were observed. Biogas yields of co-digestion reactors on COD basis are given on Appendix C.

Table 4-12: Biogas Yields of Co-digestion Study Reactors

	M1a	**M1b**	**M2a**	**M2b**	**M3a**	**M3b**	**M4a**	**M4b**	**W1a**	**W1b**	**W2a**	**W2b**	**W3a**	**W3b**
VS added (g)	0.8	0.8	1.5	1.5	0.5	0.5	1.6	1.6	0.3	0.3	0.5	0.5	0.3	0.3
Biogas Yield (mL/gVSadded)	304	772	231	732	785	742	213	476	517	528	449	409	409	465

4.2.3.2.Methane Contents

In order to determine methane production yields of reactors, methane contents were determined on percentage basis. Measurements were performed at Day 7, 14, 21, 28, 35 and 56; started after the headspaces of reactors were washed with the produced biogas; that is, when produced biogas volume exceeded three-folds volume of the headspace. Figure 4-17 depicts methane contents of pretreated microalgal slurry reactors.

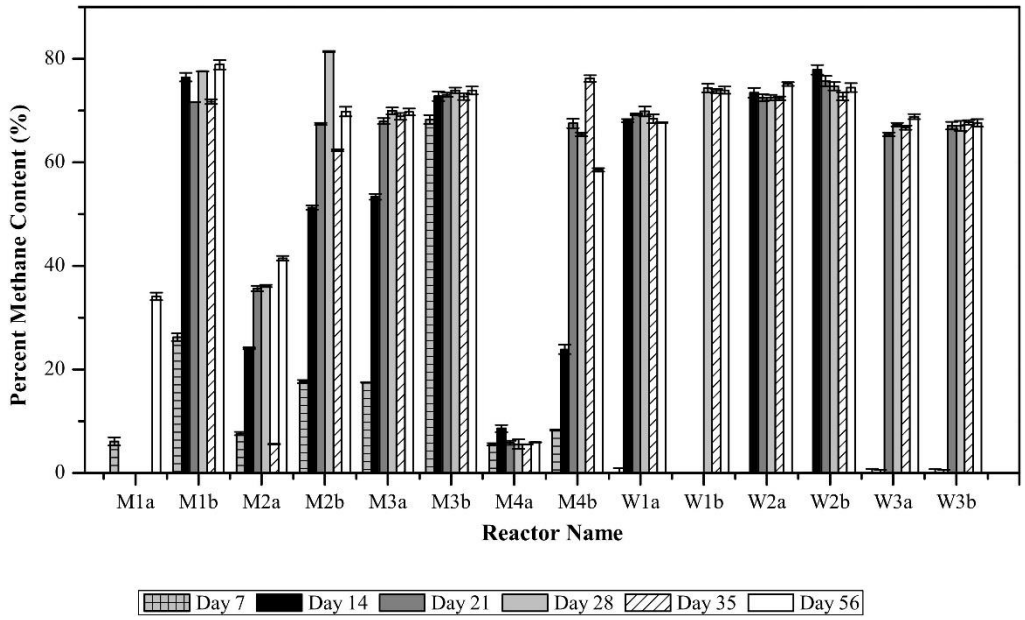


Figure 4-17: Methane contents of co-digestion study reactors.

As can be seen from Figure 4-17, methane contents of reactors varied between 5.6 ± 0.2 % and 77.9 ± 0.9, although methane contents of produced biogas were not constant in any reactor. In many MKW co-digestion reactors, typical methane content over 60% was not reached in reactors M2a and M4a in which MKW was used as substrate. Therefore, it can be stated that there was inhibition on methanogenic activity. Increase in methane contents of other reactors up to values above 60% were achieved through increase between consecutive measurements. Thereafter, methane contents were more or less constant, except for reactors M2b and M4b, where biogas production rates were relatively higher.

On average, abovementioned increase was not evident in reactors in which WAS was used as sole substrate or co-substrate. This observation was mainly due to the fact that first measurements were performed not as early as the ones for MKW reactors, since three-fold gas production of headspace volume was achieved later. Thus, increasing trend in methanogenic activity was not monitored. Among WAS reactors, methane contents were rather stable and within the range of 67 ± 1.0 and 75.1 ± 0.3 %.

In literature, methane content of KW varied between 75.1 and 87.9% (Li et al., 2011). In this study, similar methane contents were achieved. Consistency with literature in terms of biogas contents of WAS AD process was also present. In a study conducted by Kim et al., (2003), methane content of untreated WAS was found to be 41%; whereas Lin et al., (1997) revealed methane content of 72%. Williams (2012) investigated effect of co-digestion of *Chlorella vulgaris* and wastewater sludge. In this study, methane contents of reactors were reported as 72% and relatively constant as well.

Methane contents of the rectors were used for the calculation of observed methane volume produced in the reactors. Results of observed methane production calculations were used to calculate the percent ratio between observed and theoretical methane production, which are given on with methane yields of reactors.

Table 4-13: Observed/Theoretical methane production and methane yield values of co-digestion study reactors

	M1a	**M1b**	**M2a**	**M2b**	**M3a**	**M3b**	**M4a**	**M4b**	**W1a**	**W1b**	**W2a**	**W2b**	**W3a**	**W3b**
Observed/Theoretical** CH_4 produced(v/v*100)	3.6	85.8	6.2	66.6	76.2	93.7	2.4	31.9	51.9	49.8	45.7	50.2	29.5	37.7
Methane Yield (ml CH_4 / g VS added)	18	441	34	371	424	523	12	167	253	242	247	272	159	204

** Theoretical CH_4 volume was calculated considering that maximum of 0.395 L CH_4 can be produced per g COD added (Speece, 2008).

It can be seen from Table 4-13 that the highest methane yield was recorded in Reactor M3b, containing 14000 ± 50 mg/L initial COD and BM, as 523 ml CH_4 / g VS added. This value is higher than that of reported by Marin et al. (2010) as 300 ml/VS added for sole utilization of kitchen waste as a substrate for AD process. In this study, sole anaerobic digestion of MKW was found to reach methane yield of 441 ml CH_4 / g VS added. This could be a result of variations among kitchen waste composition. In another study, 50:50 co- digestion of algae and municipal solid waste yielded 212 ml CH_4 / g VS added (Gunaseelan, 1997). Methane yields of co-digestion reactors on COD basis are given on Appendix C.

Among reactors fed with WAS, the highest methane yield was observed in Reactor W2b, containing 14000 ± 50 mg/L initial COD and BM, as 272 ml CH_4 / g VS added, which is in consistency with the literature. A study conducted by Williams (2012) revealed maximum methane yield of 227 ml CH_4 / g VS added as a result of co-digestion of *Chlorella vulgaris* and wastewater sludge, with 2200 mg/L initial COD introduction.

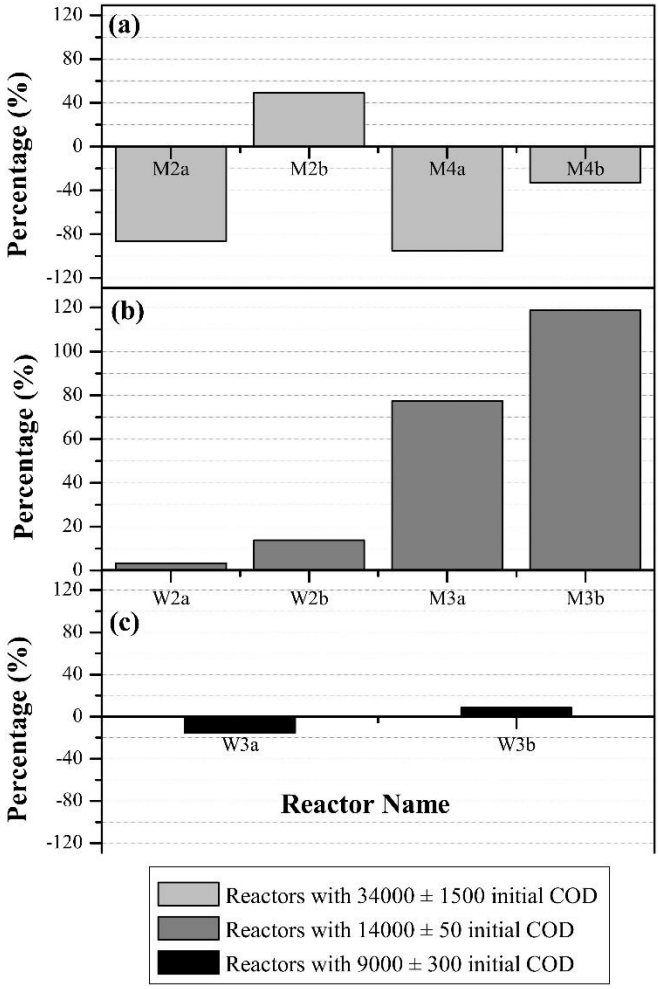
Changes in co-digestion reactors relative to untreated microalgal slurry reactors differed among co-digestion types and initial COD concentrations. Figure 4-18 depicts percent increases in methane yields of co-digestion reactors with different initial COD values. Percent increase values were calculated relative to the maximum methane yield observed in their untreated microalgal slurry equivalents. That is, percent increase relative to A1a for co-digestion reactors with 34000 ± 1500 mg/L, percent increase relative to A3a for co- digestion reactors with 14000 ± 50 mg/L and percent increase relative to A4a for co-digestion reactors with 9000 ± 30 mg/L were calculated, since these reactors yielded higher methane contents compared to their BM supplemented equivalents.

For co-digestion reactors with 34000 ± 1500 mg/L initial COD, it can be seen that the only positive increase was observed in Reactor M2b, as 49.0. The least negative effect was observed in Reactor M4b as -32.9 % and followed by M2a as -86.4% and M2b as -95.2% respectively. These results indicate alkalinity deficiency in reactors with high COD/N ratios. By increasing COD/N ratio, inhibition effects due to acidic conditions became more evident.

As shown on Figure 4-18, the reactors with 14000 ± 50 mg/L initial COD, it can be seen that the highest percent increase was observed in Reactor M3b, as 118.8%, which is followed by Reactor M3a, with a value of 77.4%, W2b with 13.8% and W2a with 3.4%. It can be stated that co-digestion with MKW provides a better environment for methanogenesis compared to WAS. This fact may be mainly due to initially balanced substrate composition, when MKW was used as co-substrate (Zhao and Ruan, 2013).

Reactors with 9000 ± 300 mg/L initial COD, it can be seen that the only positive percent increase was observed in Reactor W3b, as 8.5%, whereas W3a yielded 15.4% lower methane than that of Reactor A4a. It can be concluded that, despite lower gas production values observed in WAS co-digestion reactors with initial COD of 9000 ± 300, higher methane contents achieved in co-digestion reactors compensate the difference.

When compared to 442 ml CH_4 / g VS added methane yield of MKW as sole substrate, co-digestion reactor, M3b resulted in 19% increase in methane yield, with the value of 523 ml CH_4 / g VS added (See Table 4-13). Although biogas yield of co-digestion reactors in which WAS was used as co-substrate was lower than that of reactors where Was was used as sole substrate(See Table 4-9), co-digestion increased methane yield from 253 ml CH_4 / g VS added to 272 ml CH_4 / g VS added, which accounts for 8.3% increase (See Table 4-13).



M1a: MKW - 19000 mg/L COD
M1b: MKW - 19000 mg/L COD + BM
M2a: MKW + Algae 1/1 co-digestion - 34000 mg/L COD
M2b: MKW + Algae 1/1 co-digestion - 34000 mg/L COD + BM
M3a: MKW + Algae co-digestion - 14000 mg/L COD
M3b: MKW + Algae co-digestion - 14000 mg/L COD +BM
M4a: MKW + Algae 7/1 codigestion - 34000 mg/L COD
M4b: MKW + Algae 7/1 codigestion - 34000 mg/L COD + BM
W1a: WAS - 9000 mg/L COD
W1b: WAS - 9000 mg/L COD + BM
W2a: WAS + Algae 1/1 co-digestion 14000 mg/L COD
W2b: WAS + Algae 1/1 co-digestion 14000 mg/L COD + BM
W3a: WAS + Algae 1/1 co-digestion 9000 mg/L COD
W3b: WAS + Algae 1/1 co-digestion 9000 mg/L COD + BM

Figure 4-18: Percent yield increases in batch co-digestion reactors (a) Percent yield increase in reactors with 34000 ± 1500 mg/L initial COD relative to A1a; (b) Percent yield increase in reactors with 14000 ± 50 mg/L initial COD relative to A3a; (c) Percent yield increase in reactors with 9000 ± 300 mg/L initial COD relative to A4a

4.2.3.3. tCOD, TKN, TP, sCOD, NH_4^+-N, PO_4^{3-}-P and pH Variations and Final VFA Concentrations

Observed tCOD, TKN, TP values and removal efficiencies of the reactors are given on Table 4-14.Total COD removal efficiencies observed in pretreated microalgal slurry reactors between 0.3% and 74.1% for MKW reactors and 22.5% and 30.5% for WAS reactors. Compared to untreated microalgal slurry reactors, MKW reactors achieved higher COD removal efficiencies, except for the MKW reactors in which substrate inhibition occurred. However, WAS reactors resulted in less COD removal when compared to untreated microalgal slurry reactors having 32% – 48.7 % COD removal. The highest COD removal efficiency was achieved in Reactor M1b, containing 19000 ± 500 mg/L initial COD, which was supplemented with MKW as sole substrate. In these reactors, COD removal efficiencies were higher than that of reported by Ras et al., (2011) as 33% to 51% for AD of microalgae as sole substrate.

Percent ratio of observed methane production to theoretical value were in accordance with COD removal efficiencies, which indicate effective conversion of removed COD into biomethane (See Table 4-9 and Table 4-14).

In the reactors, ammonification ranged between 14.9% and 39.8% (Table 4-14). These values are similar to but slightly lower than those reported by Ras et al., (2011) as 19% - 68% for AD of raw microalgae. On the other hand, Bujoczek et al., (2000) reported 62.6– 80.3% of ammonification as a result of high solid anaerobic digestion study on chicken manure. Lowest ammonification was observed in reactor W1a as 14.9%, which was supplemented with WAS only. This value is similar to that reported by Epstein et al., (1978) as 14% - 25% for sewage sludge. For TKN and NH_4^+-N values, negative removal data can be attributed to bioconversion of proteins into amino acids and to ammonia (Demirer and Chen, 2005). It can be seen from

Table 4-15 that most of the final ammonia concentrations in the reactors were above 200mg/L, which is a level with potential of inhibition. On the other hand, although ammonia concentrations were above 200 mg/L in WAS reactors, VFA

concentrations were lower than 300 mg/L, which is a limit above which inhibition is evident (Speece, 2008). Therefore, it can be concluded, that the main reason for low efficiencies in WAS reactors was not ammonia toxicity, but difficulty in solubilization. In MKW reactors, high VFA concentrations were not correlated to final ammonia concentrations either. Therefore, substrate inhibition may be the main cause of inhibition in these reactors, due to high COD/TKN ratio of MKW (See Table 3-12).

It can be seen from Table 4-14 that TP removal efficiencies in co-digested microalgal slurry reactors were between 0.1 and 4.4%. This is a result similar to those observed in untreated microalgal slurry reactors and in consistency with the literature (Lusk, 1998). Although initial N:P values in some reactors were slightly lower than optimum range of 5/1 and 7/1 (See Section 2.4.4.1), N:P values at the end of the BMP assay were all within the optimum range, except for M1a, M1b, M4a and M4b. (See Table 4-14)

Inhibitory conditions observed in AD process can be indicated by final VFA concentrations above 100 - 300 mg HAc/L (Speece, 2008). It can be seen from

Table 4-15 that there has not been significant inhibition in methanogenic activities of WAS reactors, considering VFA concentrations less than or equal to 83 mg HAc/L On the other hand, inhibition of methanogenic activity was evident in all MKW reactors without BM supplementation and partially in MKW with BM supplementation. Considering low tCOD removal efficiencies, as well as sCOD accumulation, it can be stated that substrate inhibition prevented acetogens and methanogens from conversion of the VFAs produced in these reactors. Low final pH values in reactors M1a, M2a and M4a prove that the reason for inhibition is lack of alkalinity in these reactors. It can also be seen that final sCOD concentrations were in correlation with final VFA concentrations.

Table 4-14: Initial and final tCOD, TKN, TP, COD:N and N:P values in co-digestion study reactors

	tCOD (mg/L)			TKN (mg/L)				TP (mg/L)			COD:N (g:g)		N:P (g:g)	
	Initial	**Final**	**Removal (%)**	**Initial**	**Final**	**Removalv(%)**	**Ammonification (%)**	**Initial**	**Final**	**Removal (%)**	**Initial**	**Final**	**Initial**	**Final**
M1a	19183	19134 ± 436	0.3	302	356 ± 2	-17.8	32.1	118	118 ± 7.4	0.1	63.6	53.8	2.5	3.0
M1b	19183	4960 ± 85	74.1	302	291 ± 8	3.5	51.1	118	113 ± 7.2	4.4	63.6	17.0	2.5	2.6
M2a	34001	29150 ± 1481	14.3	1452	1631 ± 49	-12.4	16.9	249	248 ± 8.2	0.3	23.4	17.9	5.8	6.6
M2b	34001	15587 ± 421	54.2	1452	1659 ± 10	-14.3	15.6	249	239 ± 6.4	3.8	23.4	9.4	5.8	6.9
M3a	13968	6012 ± 87	57.0	636	644 ± 49	-1.2	26.9	134	128 ± 9.0	4.2	22.0	9.3	4.8	5.0
M3b	13968	7814 ± 57	65.0	636	651 ± 49	-2.3	25.5	134	128 ± 9.1	4.3	22.0	7.5	4.8	5.1
M4a	33800	33449 ± 288	1.0	628	760 ± 15	-20.9	10.6	180	177 ± 6.6	1.7	53.8	44.0	3.5	4.3
M4b	33800	12045 ± 746	64.4	628	742 ± 99	-18.1	39.8	180	174 ± 0.8	3.6	53.8	16.2	3.5	4.3
W1a	9147	7085 ± 175	22.5	699	732 ± 45	-4.6	14.9	140	135 ± 8.9	3.8	13.1	9.7	5.0	5.4
W1b	9147	6741 ± 265	26.3	699	721 ± 20	-3.1	21.6	140	139 ± 4.1	1.0	13.1	9.3	5.0	5.2
W2a	13986	9717 ± 233	30.5	1075	1064 ± 10	1.0	23.8	183	179 ± 1.8	2.1	13.0	9.1	5.9	5.9
W2b	13986	10040 ± 225	28.2	1075	1026 ± 5	4.6	17.6	183	178 ± 11.4	2.7	13.0	9.8	5.9	5.8
W3a	9277	6599 ± 58	28.9	664	641 ± 15	3.5	21.5	131	129 ± 4.8	1.5	14.0	10.3	5.0	4.9
W3b	9277	6883 ± 234	25.8	664	662 ± 5	0.3	13.0	131	126 ± 14.5	4.2	14.0	10.4	5.0	5.3

Table 4-15: Initial and final pH, sCOD, TAN, PO_4^{3-}-P and final VFA values in co-digestion study reactors

	pH		sCOD (mg/L)			TAN (mg/L)			PO4-P (mg/L)			Final VFA
	Initial	**Final**	**Initial**	**Final**	**Removal (%)**	**Initial**	**Final**	**Removal (%)**	**initial**	**Final**	**Removal (%)**	**(mg HAc/L)**
M1a	7	4.62	55	7155 ± 318	-12877.0	33	137 ± 8	-314.9	15	300 ± 1	-1883.6	5130
M1b	7.28	7.74	55	605 ± 35	-997.3	33	165 ± 6	-401.5	15	50 ± 0	-231.2	107
M2a	6.98	6.53	127	8345 ± 163	-6451.7	131	385 ± 15	-193.3	56	40 ± 2	29.0	1515
M2b	7.1	7.77	127	505 ± 21	-296.5	131	370 ± 0	-181.9	56	15 ± 1	73.9	922
M3a	7	4.61	79	575 ± 7	-630.4	65	221 ± 4	-239.2	28	208 ± 4	-634.0	535
M3b	7.27	7.85	79	38 ± 3	51.7	65	215 ± 7	-230.7	28	61 ± 1	-115.3	294
M4a	6.9	7.22	73	14700 ± 283	-20085.0	57	132 ± 3	-131.6	25	13 ± 1	48.1	4773
M4b	7.22	7.91	73	1425 ± 21	-1856.7	57	330 ± 14	-479.1	25	32 ± 4	-25.9	221
W1a	6.9	7.3	262	50 ± 0	80.9	107	200 ± 1	-87.2	164	75 ± 1	54.1	53
W1b	7.13	7.8	262	165 ± 21	37.1	107	240 ± 9	-124.2	164	49 ± 1	70.3	33
W2a	6.9	7.0	286	170 ± 14	40.6	139	360 ± 28	-159.1	177	76 ± 0	57.0	83
W2b	7.25	7.8	286	445 ± 7	-55.6	139	295 ± 35	-112.3	177	66 ± 1	62.9	4
W3a	7.2	7.5	173	465 ± 21	-168.6	87	206 ± 2	-137.3	98	83 ± 2	15.6	33
W3b	7.21	7.1	173	370 ± 14	-113.7	87	162 ± 3	-86.6	98	83 ± 1.	15.6	16

4.2.4. Overall Evaluation on Economic Feasibility of Anaerobic Digestion of Microalgae

Due to low biogas yields associated with AD process of microalgae, pretreatment or co-digestion methods can be viable for enhanced biogas production. While evaluating feasibility, apart from increases in methane yields, costs and energy requirements of AD processes should be taken into account in each case.

Regarding substrate inhibition observed at high COD concentrations of untreated microalgal slurry, it can be stated, that AD process at lower COD values is more viable. Harvesting cost can therefore be reduced, since it is not necessary to achieve concentrated microalgal slurry, when it is used as the sole substrate.

As a part of this study, three types of pretreatment methods, namely, heat, autoclave and thermochemical pretreatments were practiced. It was revealed that thermochemical pretreatment is not a feasible alternative with respect to biogas production, since this method negatively influenced AD process. Moreover, the necessity of chemical addition increases the cost of this pretreatment type for certain, compared to heat pretreatment.

Considering aforementioned results, cost evaluation of thermochemical pretreatment was omitted and energy requirements of heat and autoclave pretreatment methods are given on Table 4-16. Energy requirements of two methods were compared for 1200 L of microalgal slurry pretreatment. It was assumed that industrial scale autoclave (Priorclave, PS Series, London, UK) would be used for autoclave pretreatment, whereas an industrial scale furnace (Changzhou YuTong, DMH 3, Jiangsu, China) would be used for heat pretreatment.

Table 4-16: Properties of heat and autoclave pretreatment methods

Properties	Autoclave	Furnace
Inner volume (L)	100	1200
Pretreatment duration* (min)	10	120
Number of operating cycles (h-1)	6	0.5
Pretreatment capacity (L/h)	600	600
Heating input requirement (kW)	7	12
Heat maintenance input requirement (kW/cycle)	1	1.1
Total eat maintenance input requirement (kW/cycle)	13	14.2
*Includes pressurizing and de-pressurizing time, besides 5 min. of actual pretreatment duration.		

As shown on Table 4-16, after two hours of operation, 1200 L of pretreated microalgal slurry can be obtained from each method. Considering initial heating and maintenance heating requirements, autoclave pretreatment demands 13 kW total power, whereas heat pretreatment demands 14.2 kW. It is necessary to determine the increase in biogas yields, in turn, net power generation compared to AD process of untreated microalgal slurry. Table 4-17 summarizes net power generation calculations, considering that 12.3 m^3 biogas can be converted into 25 kW electricity and 47.5 kW heat using an industrial scale co-generation unit (TEDOM, Micro T30, Czech Republic).

Table 4-17: Co-generation estimates of pretreatment reactors

	H1a	H1b	H2a	H2b	At1a	At1b	At2a	At2b
Biogas yield (ml biogas/ g VS added)	428	414	580	568	476	479	595	505
Biogas yield ıncrease after pretreatment (ml biogas/ g VS added)*	49	35	190	178	97	100	205	115
g VS / 1200 L slurry	33540	33540	33540	33540	33540	33540	33540	33540
Extra biogas production potential after pretreatment (m^3/ 1200 L slurry)	1.64	1.17	6.37	5.97	3.25	3.35	6.88	3.86
Generated electricity (kw/ 1200l slurry)	3.3	2.4	13.0	12.1	6.6	6.8	14.0	7.8
Generated heat (kw/ 1200l slurry)	6.4	4.5	24.6	23.1	12.6	13.0	26.6	14.9
Total generation (kw/ 1200l slurry)	9.7	6.9	37.6	35.2	19.2	19.8	40.5	22.7
* Biogas yield increases were calculated relative to untreated microalgal slurry reactors								

It can be seen from Table 4-17 that electricity generation increase after pretreatment compensates the cost of pretreatment itself only for autoclave pretreatment at lower COD concentrations (19000 ± 500 mg/L) in the absence of BM addition, resulting in 14 kW electricity production after 13 kW elecrtricity consumption. It can be stated, that due to energy intensive nature of pretreatment types, electricity recovery is less than 10% in this respect. However, when heat

generation is also taken account, it can be seen that both pretreatment options were feasible, if the generated heat is properly managed.

Compared to energy intensive pretreatment processes, co-digestion options may be more beneficial in terms of AD process enhancement. However, it must be noted, that lower biogas yields were observed in reactors, in which WAS was used as co-substrates (See Section 4.2.3), relative to co-digestion process of microalgae and MKW.

It can be concluded, that co-digestion of microalgal slurry with MKW is the most feasible alternative among all pretreatment and co-digestion options investigated in this study. However, costs and energy requirements for transportation and sorting of kitchen wastes must be further investigated, in order to come up with a concrete decision.

CHAPTER 5

CONCLUSIONS

Utilization of algal cultures is an alternate method for biological wastewater treatment, which enables production of energy crops as a result of the process. In this respect, coupled nutrient removal and biogas production using microalgal and anaerobic microbial cultures is a sustainable approach, enhancing both environment and economy.

The objectives of this study were to investigate nutrient removal potential of green alga, *Chlorella vulgaris*, from primary effluents of municipal wastewater treatment plants and investigation of anaerobic digestibility of the produced microalgal sludge as a result of the treatment process. Laboratory- scale semi-continuous photobioreactor was operated for microalgal culture production in primary effluents of municipal wastewater treatment plants, as well as investigation of nitrogen and phosphorus removal efficiencies. BMP assays were conducted for determination of anaerobic digestibility of untreated microalgal slurry, evaluation of heat, autoclave and thermochemical pretreatment options for the improvement of anaerobic digestibility of microalgal slurry and investigation of waste activated sludge and model kitchen waste as alternative co-substrates for anaerobic digestion of microalgal slurry.

Results of batch cultivation of *Chlorella vulgaris* revealed that maximum specific net growth rate and productivity values of 0.39 d^{-1} and 24 mg /L.d could be achieved respectively.

It was found that *Chlorella vulgaris* cultures were capable of removing 99.6% nitrogen and 91.2% phosphorus from primary clarifier effluents of municipal wastewater treatment plant. Nitrogen in the forms of ammonia and nitrate and phosphorus in the form of ortho-phosphate were removed. It was revealed that ammonia nitrogen was used as the preliminary nitrogen source by microalgae.

The results of the BMP assay conducted with untreated microalgal slurry illustrated that maximum biogas yield of 442 mL biogas/ g VS added can be achieved when the reactor was started with initial COD value of 9000 ± 300 mg/L. This result was similar to the findings of other researches (See Section 2.5.4). Beyond this COD value, substrate inhibition was observed . It was also revealed that BM had a negative effect on cumulative gas production of untreated microalgae, increasing the free ammonia concentrations due to high pH.

The findings of BMP assay conducted for evaluation of pretreatment options indicated that heat pretreatment is superior at relatively lower COD values (19000 ± 500 mg/L), increasing methane yield by 83.0%, from 223 to 408 mL CH_4/ g VS added. Autoclave pretreatment was superior at relatively higher (34000 ± 1500 mg/L) COD values, resulting in 43.0% increase in methane yield from 249 to 356 mL CH_4/ g VS added (See Figure 4-12). On the other hand, thermochemical pretreatment resulted in lower biogas production and COD treatment values, compared to untreated microalgae. Overall evaluation of pretreatment methods, which involve cost estimations for heat and autoclave pretreatments, showed that both pretreatment types were feasible only if applied to large amounts of microalgal slurry, in order to compensate initial energy requirements for heating. Due to its lower initial heating energy requirement, autoclave pretreatment is found to be superior to heat pretreatment for both high and low initial COD values, when economic feasibility of both methods were compared (See Section 4.2.4).

The results obtained from BMP assay conducted for co-digestion of microalgal slurry either with MKW or WAS indicated that co-digestion with MKW

increases methane yield by 118.8% compared to AD of microalgal slurry and 19% compared to MKW as sole substrates. Maximum methane yield was observed as 523 mL CH_4/ g VS added in reactor with BM supplement, in which initial COD of 34000 ± 1500 mg/L was provided by 1:1 (g COD added/:g COD added) ratio of microalgal slurry to MKW. WAS co-digestion resulted in 19% increase in methane yield relative to AD of microalgal slurry and 8.3% increase relative to WAS only (See Figure 4-18). Maximum methane yield among WAS co-digestion reactors was achieved as 272 mL CH_4/ g VS added in reactor with BM supplementation, which initially contained 9000 ± 300 mg/L COD, providing 1:1 (g COD added/:g COD added) ratio of microalgal slurry to WAS.

Based on the results obtained in this study, it is postulated that anaerobic digestion of microalgae is a viable option for the recovery of the bioenergy from wastewater by conversion of solar energy into chemical energy. However, implementation and operation costs, as well as energy requirements involved in pretreatment applications must be taken into account. Conversely, availability of waste activated sludge and kitchen waste in large amounts is advantageous for enhancing anaerobic digestion process of microalgae with lower costs. In this respect, considering that the highest biomethane yield among all reactors in this study was observed in microalgae and MKW co-digestion reactor, it can be concluded that there is high potential of biogas production from microalgae, especially when the process is enhanced by utilization of MKW as the co-substrate.

REFERENCES

Alvarez, J. A., Otero, L., & Lema, J. M. (2010). A methodology for optimising feed composition for anaerobic co-digestion of agro-industrial wastes. *Bioresource Technology*, *101*(4), 1153–8. doi:10.1016/j.biortech.2009.09.061

Alzate, M. E., Muñoz, R., Rogalla, F., Fdz-Polanco, F., & Pérez-Elvira, S. I. (2012). Biochemical methane potential of microalgae: influence of substrate to inoculum ratio, biomass concentration and pretreatment. *Bioresource Technology*, *123*, 488–94. doi:10.1016/j.biortech.2012.06.113

Anderson, L. A. (1995). On the hydrogen and oxygen content of marine phytoplankton. *Deep Sea Research Part I: Oceanographic Research Papers*, *42*(9), 1675–1680. doi:10.1016/0967-0637(95)00072-E

Angenent, L. T., Sung, S., & Raskin, L. (2002). Methanogenic population dynamics during startup of a full-scale anaerobic sequencing batch reactor treating swine waste. *Water Research*, *36*(18), 4648–54.

Ashman, M., & Puri, G. (2009). *Essential Soil Science: A Clear and Concise Introduction to Soil Science* (p. 208). John Wiley & Sons.

Barbosa, M. J., Janssen, M., Ham, N., Tramper, J., & Wijffels, R. H. (2003). Microalgae cultivation in air-lift reactors: modeling biomass yield and growth rate as a function of mixing frequency. *Biotechnology and Bioengineering*, *82*(2), 170–9. doi:10.1002/bit.10563

Becker, E. W. (2008). *Microalgae: Biotechnology and microbiology*. Cambridge: Cambridge University Press.

Berhe, A. A., Carpenter, E., Codispoti, L., Izac, A.-M., Lemoalle, J., Luizao, F., … Ward, B. (2005). Nutrient Cycling. In R. M. Hassan, R. Scholes, & A. Neville (Eds.), *Ecosystems and HumanWell-being: Current State and Trends* (pp. 333–351). Washington D.C.: Island Press.

Bilanovic, D., Andargatchew, A., Kroeger, T., & Shelef, G. (2009). Freshwater and marine microalgae sequestering of CO2 at different C and N concentrations – Response surface methodology analysis. *Energy*

Conversion and Management, *50*(2), 262–267. doi:10.1016/j.enconman.2008.09.024

Bitog, J. P., Lee, I.-B., Lee, C.-G., Kim, K.-S., Hwang, H.-S., Hong, S.-W., … Mostafa, E. (2011). Application of computational fluid dynamics for modeling and designing photobioreactors for microalgae production: A review. *Computers and Electronics in Agriculture*, *76*(2), 131–147. doi:10.1016/j.compag.2011.01.015

Brennan, L., & Owende, P. (2010). Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, *14*(2), 557–577. doi:10.1016/j.rser.2009.10.009

Bujoczek, G., Oleszkiewicz, J., Sparling, R., & Cenkowski, S. (2000). High Solid Anaerobic Digestion of Chicken Manure. *Journal of Agricultural Engineering Research*, *76*(1), 51–60. doi:10.1006/jaer.2000.0529

Callaghan, F. J., Wase, D. A. J., Thayanithy, K., & Forster, C. F. (2002). Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure. *Biomass and Bioenergy*, *27*, 71–77. doi:S0961-9534(01)00057-5

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*, *8*(3), 559–568. doi:10.1890/1051-0761(1998)008[0559:NPOSWW]2.0.CO;2

Carrère, H., Dumas, C., Battimelli, A., Batstone, D. J., Delgenès, J. P., Steyer, J. P., & Ferrer, I. (2010). Pretreatment methods to improve sludge anaerobic degradability: a review. *Journal of Hazardous Materials*, *183*(1-3), 1–15. doi:10.1016/j.jhazmat.2010.06.129

Carucci, G., Carrasco, F., Trifoni, K., Majone, M., and Beccari, M. (2005). "Anaerobic digestion of food industry wastes: effect of co-digestion on methane yield." *J. Environ. Eng.*, 131(7), 1037–1045

Carstensen, J., Conley, D. J., Andersen, J. H., & Ærtebjerg, G. (2006). Coastal eutrophication and trend reversal: A Danish case study. *Limnology and Oceanography*, *51*(1, part 2), 398–408. doi:10.4319/lo.2006.51.1_part_2.0398

Carvalho, A. P., Meireles, L. a, & Malcata, F. X. (2006). Microalgal reactors: a review of enclosed system designs and performances. *Biotechnology Progress*, *22*(6), 1490–506. doi:10.1021/bp060065r

Chaumont, D. (1993). Biotechnology of algal biomass production: a review of systems for outdoor mass culture. *Journal of Applied Phycology*, *5*(6), 593–604. doi:10.1007/BF02184638

Chen, C.-Y., Yeh, K.-L., Aisyah, R., Lee, D.-J., & Chang, J.-S. (2011). Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. *Bioresource technology*, *102*(1), 71–81. doi:10.1016/j.biortech.2010.06.159

Chen, P. H. (1987). *Factors Influencing Methane Fermentation of Micro-Algae*. University of California, Berkley, CA, USA.

Chen, P. H., & Oswald, W. J. (1998). Thermochemıcal Treatment for Algal Fermentation. *Environment International*, *24*(8), 889–897.

Chenl, C. Y., & Durbin, E. G. (1994). Effects of pH on the growth and carbon uptake of marine phytoplankton. *Marine Ecology Progress Series*, *109*(1992), 83–94.

Chisti, Y. (2008). Biodiesel from microalgae beats bioethanol. *Trends in Botechnology*, *26*(3), 126–31. doi:10.1016/j.tibtech.2007.12.002

Chiu, S.-Y., Kao, C.-Y., Huang, T.-T., Lin, C.-J., Ong, S.-C., Chen, C.-D., … Lin, C.-S. (2011). Microalgal biomass production and on-site bioremediation of carbon dioxide, nitrogen oxide and sulfur dioxide from flue gas using Chlorella sp. cultures. *Bioresource Technology*, *102*(19), 9135–42. doi:10.1016/j.biortech.2011.06.091

Cormier, I. (2010). *A STELLA Model for Integrated Algal Biofuel Production and Wastewater Treatment*. University of South Florida,Tampa, FL, USA.

Correll, D. L. (1998). Role of Phosphorus in the Eutrophication of Receiving Waters: A Review, The. *Journal of Environment Quality*, *27*(2), 261. doi:10.2134/jeq1998.00472425002700020004x

Danquah, M.K., Ang, L., Uduman, N., Moheimani, N., Fordea, G.M., 2009. Dewatering of microalgal culture for biodiesel production: exploring polymer flocculation and tangential flow filtration. *J. Chem. Technol. Biot.* 84, 1078–1083.

Davidsson, A., & La Cour- Jansen, J. (2006). Pre-treatment of wastewater sludge before anaerobic digestion – Hygienisation , Ultrasonic treatment and Enzyme dosing. *Vatten*, *62*, 335–340.

De Godos, I., González, C., Becares, E., García-Encina, P. a, & Muñoz, R. (2009). Simultaneous nutrients and carbon removal during pretreated swine

slurry degradation in a tubular biofilm photobioreactor. *Applied Microbiology and Biotechnology*, *82*(1), 187–94. doi:10.1007/s00253-008-1825-3

De Morais, M. G., & Costa, J. A. V. (2007). Carbon dioxide fixation by Chlorella kessleri, C. vulgaris, Scenedesmus obliquus and Spirulina sp. cultivated in flasks and vertical tubular photobioreactors. *Biotechnology Letters*, *29*(9), 1349–52. doi:10.1007/s10529-007-9394-6

Demirer, G N, & Chen, S. (2008). Anaerobic biogasification of undiluted dairy manure in leaching bed reactors. *Waste Management*, *28*(1), 112–9. doi:10.1016/j.wasman.2006.11.005

Demirer, G.N., & Chen, S. (2005). Two-phase anaerobic digestion of unscreened dairy manure. *Process Biochemistry*, *40*(11), 3542–3549. doi:10.1016/j.procbio.2005.03.062

Edberg, A. (2010). *Growth of Chlorella Vulgaris at high carbon dioxide levels in Swedish light conditions: Laboratory testing and development of methods*. Umeå Institute of Technology, Umeå, Sweden.

Elser, J. J. (2012). Phosphorus: a limiting nutrient for humanity? *Current Opinion in Biotechnology*, *23*(6), 833–8. doi:10.1016/j.copbio.2012.03.001

EPA. (2002). *Wastewater Technology Fact Sheet: Facultative Lagoons* (p. 4). Washington D.C.

EPA. (2009). *An Urgent Call to Action - Report of the State-EPA Nutrient Innovations Task Group* (p. 35). Washington D.C.

Epstein, E., Keane, D. B., Meisinger, J. J., & Legg, J. (1978). Mineralization of Nitrogen from Sewage Sludge and Sludge Compost. *Journal of Environmental Quality*, *7*(2), 217–221.

Field, J. a., Reneau, R. B., Kroontje, W., & Caldwell, J. S. (1985). Nutrient recoveries from plug-flow anaerobic digestion of poultry manure. *Agricultural Wastes*, *13*(3), 207–216. doi:10.1016/0141-4607(85)90035-6

Filali, R., Tebbani, S., & Dumur, D. (2011). Growth modeling of the green microalga Chlorella vulgaris in an air-lift photobioreactor. In *18th IFAC World Congress August 28 - September 2* (pp. 10603–10608). Milano.

Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., … Vorosmarty, C. J. (2004). Nitrogen cycles : past , present , and future. *Biogeochemistry*, *70*, 153–226.

García-Casal, M. N., Ramírez, J., Leets, I., Pereira, A. C., & Quiroga, M. F. (2009). Antioxidant capacity, polyphenol content and iron bioavailability from algae (Ulva sp., Sargassum sp. and Porphyra sp.) in human subjects. *The British Journal of Nutrition*, *101*(1), 79–85. doi:10.1017/S0007114508994757

Girard, J. (2009). *Principles of Environmental Chemistry* (2nd ed., p. 230). Jones & Bartlett Learning.

Golueke, C. G., Oswald, W. J., & Gotaas, H. B. (1957). Anaerobic Digestion of Algae. *Applied Microbiology*, *5*(1), 47–55.

Golueke, C.G., Oswald, W., 1965. Harvesting and processing sewage-grown planktonic algae. *J. Water Pollut. Contr. Fed.* 37, 471–498.

Green, F. B., Lundquist, T. J., & Oswald, W. J. (1995). Energetics of Advanced Integrated Wastewater Pond Systems. *Water Science and Technology*, *31*(12), 9–20.

Griffiths, E. W. (2010). *Removal and Utilization of Wastewater Nutrients for Algae Biomass and Biofuels*. Utah State University, Logan, UT, USA.

Güngör-Demirci, G., & Demirer, G. N. (2004). Effect of initial COD concentration, nutrient addition, temperature and microbial acclimation on anaerobic treatability of broiler and cattle manure. *Bioresource Technology*, *93*(2), 109–17. doi:10.1016/j.biortech.2003.10.019

Harrison, W. G., & Cota, G. F. (1991). Primary production in polar waters: relation to nutrient availability. *Polar Research*, *10*(1), 87–104. doi:10.1111/j.1751-8369.1991.tb00637.x

Harun, R., Singh, M., Forde, G. M., & Danquah, M. K. (2010). Bioprocess engineering of microalgae to produce a variety of consumer products. *Renewable and Sustainable Energy Reviews*, *14*(3), 1037–1047. doi:10.1016/j.rser.2009.11.004

Harwood, J. L., & Guschina, I. a. (2009). The versatility of algae and their lipid metabolism. *Biochimie*, *91*(6), 679–84. doi:10.1016/j.biochi.2008.11.004

He, L., Subramanian, V. R., & Tang, Y. J. (2012). Experimental analysis and model-based optimization of microalgae growth in photo-bioreactors using flue gas. *Biomass and Bioenergy*, *41*(314), 131–138. doi:10.1016/j.biombioe.2012.02.025

Hirata, S., Hayashitani, M., Taya, M., & Stone, S. (1996). Carbon Dioxide Fixation in Batch Culture of Chlorella sp . Using a Photobioreactor with a

Sunlight-Collection Device. *Jourbal of Fermentation and Bioengineering*, *81*(5), 470–472.

Ho, S.-H., Chen, C.-Y., Lee, D.-J., & Chang, J.-S. (2011). Perspectives on microalgal CO_2-emission mitigation systems-,-a review. *Biotechnology Advances*, *29*(2), 189–98. doi:10.1016/j.biotechadv.2010.11.001

Holm-Nielsen, J. B., Al Seadi, T., & Oleskowicz-Popiel, P. (2009). The future of anaerobic digestion and biogas utilization. *Bioresource Technology*, *100*(22), 5478–84. doi:10.1016/j.biortech.2008.12.046

Hu, Q., Guterman, H., & Richmond, A. (1996). A flat inclined modular photobioreactor for outdoor mass cultivation of photoautotrophs. *Biotechnology and Bioengineering*, *51*(1), 51–60. doi:10.1002/(SICI)1097-0290(19960705)51:1<51::AID-BIT6>3.0.CO;2-#

Hulatt, C. J., & Thomas, D. N. (2011). Productivity, carbon dioxide uptake and net energy return of microalgal bubble column photobioreactors. *Bioresource Technology*, *102*(10), 5775–87. doi:10.1016/j.biortech.2011.02.025

IPCC. (2007). *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (p. 996). United Kingdom and New York, NY, USA.

Janssen, M., de Winter, M., Tramper, J., Mur, L. R., Snel, J., & Wijffels, R. H. (2000). Efficiency of light utilization of Chlamydomonas reinhardtii under medium-duration light/dark cycles. *Journal of Biotechnology*, *78*(2), 123–37.

Jegede, A. O. (2012). Anaerobic digestion of cyanobacteria and chlorella to produce methane for biofuel. *International Journal of Agricultural and Biological Engineering*, *5*(3), 1–8. doi:10.3965/j.ijabe.20120503.00

Jeyanayagam, S. (2005). True Confessions of the Biological Nutrient Removal Process. *Florida Water Resources Journal*, 37–46.

Keymer, P., Ruffell, I., Pratt, S., & Lant, P. (2013). High pressure thermal hydrolysis as pre-treatment to increase the methane yield during anaerobic digestion of microalgae. *Bioresource Technology*, *131*, 128–33. doi:10.1016/j.biortech.2012.12.125

Kim, J., Park, C., Kim, T.-H., Lee, M., Kim, S., Kim, S.-W., & Lee, J. (2003). Effects of Various Pretreatments for Enhanced Anaerobic Digestion with

Waste Activated Sludge. *Journal of Bioscience and Bioengineering*, *95*(3), 271–5.

Kladitis, G., Diamantis, N., & Grigoropoulou, H. (1999). Amoonia and Phosphorus Removal in Municipal Wastewater Treatment Plant with Extended Aeration. *Global Nest the International Journal*, *1*(1), 47–53.

Krylova, N. I., Khabiboulline, R. E., Naumova, R. P., & Nagel, M. A. (1997). The Influence of Ammonium and Methods for Removal during the Anaerobic Treatment of Poultry Manure. *Journal of Chemical Technology and Biotechnology*, *70*, 99–105.

Kumar, K., Dasgupta, C. N., Nayak, B., Lindblad, P., & Das, D. (2011). Development of suitable photobioreactors for CO2 sequestration addressing global warming using green algae and cyanobacteria. *Bioresource Technology*, *102*(8), 4945–53. doi:10.1016/j.biortech.2011.01.054

Larsdotter, K. (2006). Wastewater treatment With microalgae – a literature review. *Vatten*, *62*, 31–38.

Li, Changling, Yang, H., Li, Y., Cheng, L., Zhang, M., Zhang, L., & Wang, W. (2013). Novel bioconversions of municipal effluent and CO_2 into protein riched Chlorella vulgaris biomass. *Bioresource Technology*, *132*, 171–7. doi:10.1016/j.biortech.2012.12.017

Li, Chenxi, Champagne, P., & Anderson, B. C. (2011). Evaluating and modeling biogas production from municipal fat, oil, and grease and synthetic kitchen waste in anaerobic co-digestions. *Bioresource technology*, *102*(20), 9471–80. doi:10.1016/j.biortech.2011.07.103

Li, R., Chen, S., & Li, X. (2009). Anaerobic Co-digestion of Kitchen Waste and Cattle Manure for Methane Production. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, *31*(20), 1848–1856. doi:10.1080/15567030802606038

Lin, i J. a, Chang, C.-N., & Chang, S.-C. (1997). Enhancement of Anaerobic Digestion of Waste Activated Sludge by Alkaline Solubilization. *Bioresource Technology*, *62*, 85–90.

Liu, Y. (2010). *Green algae as a substrate for biogas production - cultivation and biogas potentials*. Linköping University, Linköping, Sweden.

Luo, H.-P., & Al-Dahhan, M. H. (2004). Analyzing and modeling of photobioreactors by combining first principles of physiology and

hydrodynamics. *Biotechnology and Bioengineering*, *85*(4), 382–93. doi:10.1002/bit.10831

Lusk, P. (1998). *Methane Recovery from Animal Manures The Current Opportunities Casebook* (p. 150). National Renewable Energy Laboratory, NREL/SR., 580-25145 Washington, DC.

Marin, J., Kennedy, K. J., & Eskicioglu, C. (2010). Effect of microwave irradiation on anaerobic degradability of model kitchen waste. *Waste Managementanagement*, *30*(10), 1772–9. doi:10.1016/j.wasman.2010.01.033

Mata, T. M., Martins, A. a., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, *14*(1), 217–232. doi:10.1016/j.rser.2009.07.020

Mata-Alvarez, J., Mace, S., & Llabes, P. (2000). Anaerobic digestion of organic solid wastes . An overview of research achievements and perspectives. *Bioresource Technology*, *74*, 3–16.

McCarty, P. L. (1964a). Anaerobic Waste Treatment Fundamentals Part Two: Environmental Requirements and Control. *Public Works*, *95*(10), 123–126.

McCarty, P.L. (1964b). Anaerobic Waste Treatment Fundamentals Part Three: Toxic Materials and their Control. *Public Works*, 95 (11), 91-94.

Miron, A. S., Camacho, F. G., Gomez, A. C., Grima, E. M., & Chisti, Y. (2000). Bubble-Column and Airlift Photobioreactors for Algal Culture. *AIChE Journal*, *46*(9), 1872– 1887.

Misi, S. N., & Forster, C. F. (2001). Batch co-digestion of two-component mixtures of agro-wastes. *Institution of Chemical Engineers*, *79*(2), 365 – 371.

Molina, E., Fernández, J., Acién, F. G., & Chisti, Y. (2001). Tubular photobioreactor design for algal cultures. *Journal of Biotechnology*, *92*(2), 113–31.

Morse, G., Brett, S., Guy, J., & Lester, J. (1998). Review: Phosphorus removal and recovery technologies. *The Science of the Total Environment*, *212*(1), 69–81. doi:10.1016/S0048-9697(97)00332-X

Moss, B., Jeppesen, E., Søndergaard, M., Lauridsen, T. L., & Liu, Z. (2012). Nitrogen, macrophytes, shallow lakes and nutrient limitation: resolution of a current controversy? *Hydrobiologia*, *710*(1), 3–21. doi:10.1007/s10750-012-1033-0

Mshandete, A., Kivaisi, A., Rubindamayugi, M., and Mattiasson, B. (2004). "Anaerobic batch co-digestion of sisal pulp and fish wastes." *Bioresour. Technol.*, 95, 19–24.

Muñoz, R., & Guieysse, B. (2006). Algal-bacterial processes for the treatment of hazardous contaminants: a review. *Water Research*, *40*(15), 2799–815. doi:10.1016/j.watres.2006.06.011

Mussgnug, J. H., Klassen, V., Schlüter, A., & Kruse, O. (2010). Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. *Journal of Biotechnology*, *150*(1), 51–6. doi:10.1016/j.jbiotec.2010.07.030

Nallathambi Gunaseelan, V. (1997). Anaerobic digestion of biomass for methane production: A review. *Biomass and Bioenergy*, *13*(1-2), 83–114. doi:10.1016/S0961-9534(97)00020-2

Neenan, B., Feinberg, D., Hill, A., Mclntosh, R., & Terry, K. (1986). *Finels from Microalcme : Technobgy, Status, Potential, and Research Requierements* (p. 172). Golden, Colorado.

Nielsen, H. B., & Angelidaki, I. (2008). Strategies for optimizing recovery of the biogas process following ammonia inhibition. *Bioresource Technology*, *99*(17), 7995–8001. doi:10.1016/j.biortech.2008.03.049

Nixon, S. W. (1995). Coastal marine eutrophication-A definition, social causes, and future concerns. *Ophelia*, *41*, 199–219.

Olaizola, M. (2003). Commercial development of microalgal biotechnology: from the test tube to the marketplace. *Biomolecular Engineering*, *20*(4-6), 459–466. doi:10.1016/S1389-0344(03)00076-5

Olsen, R. A. (1975). Rate of Dissolution of Phosphate from Minerals and Soils. *Soil Science Society of America*, *39*, 634–639. doi:10.2136/sssaj1975.03615995003900040020x

Oncel, S., & Sukan, F. V. (2008). Comparison of two different pneumatically mixed column photobioreactors for the cultivation of Artrospira platensis (Spirulina platensis). *Bioresource Technology*, *99*(11), 4755–60. doi:10.1016/j.biortech.2007.09.068

Oswald, W. J., & Gotaas, H. B. (1957). Photosynthesis in Sewage Treatment. *American Society of Civil Engineers Transactions*, (2849), 73–105.

Parawira, W., Murto, M., Zvauya, R., & Mattiasson, B. (2004). Anaerobic batch digestion of solid potato waste alone and in combination with sugar beet

leaves. *Renewable Energy*, *29*(11), 1811–1823. doi:10.1016/j.renene.2004.02.005

Parkin, B. G. F., & Owen, W. F. (1987). Fundamentals of anaerobic digestion of wastewater sludges. *Journal of Environmental Engineering*, *112*(5), 867–920.

Passos, F., Solé, M., García, J., & Ferrer, I. (2013). Biogas production from microalgae grown in wastewater: Effect of microwave pretreatment. *Applied Energy*, *108*, 168–175. doi:10.1016/j.apenergy.2013.02.042

Paxinos, R., & Mitchell, J. G. (2000). A rapid Utermöhl method for estimating algal numbers. *Journal of Plankton Research*, *22*(12), 2255–2262.

Pecegueiro do Amaral, M. F. (2012). *Evaluation of Algae Concentration in Manure Based Media*. University of Kentucky, Lexington, KY.

Perner-Nochta, I., Lucumi, a., & Posten, C. (2007). Photoautotrophic Cell and Tissue Culture in a Tubular Photobioreactor. *Engineering in Life Sciences*, *7*(2), 127–135. doi:10.1002/elsc.200620178

Posten, C. (2009). Design principles of photo-bioreactors for cultivation of microalgae. *Engineering in Life Sciences*, *9*(3), 165–177. doi:10.1002/elsc.200900003

Powell, E. E., Mapiour, M. L., Evitts, R. W., & Hill, G. a. (2009). Growth kinetics of Chlorella vulgaris and its use as a cathodic half cell. *Bioresource Technology*, *100*(1), 269–74. doi:10.1016/j.biortech.2008.05.032

Powell, N., Shilton, A. N., Pratt, S., & Chisti, Y. (2008). Factors influencing luxury uptake of phosphorus by microalgae in waste stabilization ponds. *Environmental Science & Technology*, *42*(16), 5958–62.

Pulz, O. (2001). Photobioreactors: production systems for phototrophic microorganisms. *Applied Microbiology and Biotechnology*, *57*(3), 287–293. doi:10.1007/s002530100702

Ras, M., Lardon, L., Bruno, S., Bernet, N., & Steyer, J.-P. (2011). Experimental study on a coupled process of production and anaerobic digestion of Chlorella vulgaris. *Bioresource Technology*, *102*(1), 200–6. doi:10.1016/j.biortech.2010.06.146

Raven, J. A., & Geider, R. J. (1988). Temperature and algal growth. *New Phycology*, (110), 441–461.

Ristenson, L. (2011). *Algal Biofilm Production and Harvesting System for Wastewater Treatment with Biofuels By-Products*. Utah State University, Logan, UT, USA.

Roels, J. A. (1983). *Energetics and Kinetics Ion Biotechnology*. Amsterdam: Elsevier Biomedical Press.

Ruiz-Marin, A., Mendoza-Espinosa, L. G., & Stephenson, T. (2010). Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresource Technology*, *101*(1), 58–64. doi:10.1016/j.biortech.2009.02.076

Sasi, D. (2009). *Biokinetic Bhavbior of Chlorella Vulgaris in a Continuously Stirred Bioreactor and a Circulating Loop Photobioreactor*. University of Saskatchewan, Saskatoon, Canada.

Scragg, A. H., Illman, A. M., Carden, A., & Shales, S. W. (2002). Growth of microalgae with increased calorific values in a tubular bioreactor. *Biomass Bioenergy*, (23), 67–73.

Smith, F. W., & Thompson, J. F. (1971). Regulation of Nitrate Reductase in Chlorella vulgaris. *Plant Psiology*, *48*, 224–227.

Song, D., Hou, L., & Shi, D. (2008). Exploitation and utilization of rich lipids-microalgae, as new lipids feedstock for biodiesel production. *Chinese Journal of Biotechnology*, *24*(3), 341–8.

Speece, R. E. (2008). *Anaerobic Biotechnology and Odor/Corrosion Control for Municipalities and Industries.* Nashville, USA.: Archea Press.

Sperling, M., Andreoli, C. V., & Von, M. (2007). Sludge Treatment and Disposal. In *Biological Wastewater Treatment* (p. 10). London: IWA Publishing.

Spierling, R. E. (2011). *Anaerobic Co-digestion of Microalgae With Food Waste and Wastewater Sludge*. California Polytechnic State University, San Luis Obispo, CA, USA.

Spolaore, P., Joannis-Cassan, C., Duran, E., & Isambert, A. (2006). Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, *101*(2), 87–96. doi:10.1263/jbb.101.87

Stewart, C., & Hessami, M.-A. (2005). A study of methods of carbon dioxide capture and sequestration–the sustainability of a photosynthetic bioreactor approach. *Energy Conversion and Management*, *46*(3), 403–420. doi:10.1016/j.enconman.2004.03.009

Su, Z., Kang, R., Shi, S., Cong, W., & Cai, Z. (2010). An effective device for gas-liquid oxygen removal in enclosed microalgae culture. *Applied Biochemistry and Biotechnology*, *160*(2), 428–37. doi:10.1007/s12010-008-8353-6

Sun, Y., & Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*, *83*(1), 1–11.

Sundarajan, R., Jayanthi, S., and Elango, R. (1997). "Anaerobic digestion of organic fractions of municipal solid waste and domestic sewage of Coimbatore." *Indian J. Environ. Health.*, 39 (3), 193-196.

Sung, S., & Dague, R. R. (1995). Laboratory studies on the anaerobic sequencing batch reactor. *Water Environment Research*, *67*(3), 294–301.

Tchobanoglous, G., Burton, F. L., & Stensel, H. D. (2003). *Wastewater Engineering: Treatment, Disposal and Reuse*. (Metcalf and Eddy, Ed.) (Fourth Edi.). New York: McGraw - Hill Inc.

Tredici, M., & Zittelli, G. (1998). Efficiency of sunlight utilization: tubular versus flat photobioreactors. *Biotechnology and Bioengineering*, *57*(2), 187–97.

Tsukahara, K., & Sawayama, S. (2005). Liquid Fuel Production Using Microalgae. *Journal of the Japan Petroleum Institute*, *48*(5), 251–259.

Uduman, N., Qi, Y., Danquah, M.K., Forde, G.M., Hoadley, A., 2010. Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. *J. Renew. Sust. Energ.* 2, 012701.

U.S. EPA. (1993). *Nitrogen Control* (p. 311). Washington D.C.

Ugwu, C. U., Aoyagi, H., & Uchiyama, H. (2008). Photobioreactors for mass cultivation of algae. *Bioresource Technology*, *99*(10), 4021–8. doi:10.1016/j.biortech.2007.01.046

Umetsu, K., Yamazaki, S., Kishimoto, T., Takahashi, J., Shibata, Y., Zhang, C., … Komiyama, M. (2006). Anaerobic co-digestion of dairy manure and sugar beets. *International Congress Series*, *1293*, 307–310. doi:10.1016/j.ics.2006.03.025

UNEP. (2006). *Challenges to International Waters: Regional Assessments in a Global Perspective*. Nairobi. Retrieved from http://www.unep.org/dewa/giwa/publications/finalreport/giwa_final_report.pdf

Vandevivere, P., De Baere, L., & Verstraete, W. (2003). Types of anaerobic digesters for solid wastes. In J. Mata-Alvarez (Ed.), *Biomethanization of the Organic Fraction of Municipal Solid Wastes* (pp. 1–31). London: IWA Publishing.

Vergarafernandez, A., Vargas, G., Alarcon, N., & Velasco, A. (2008). Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. *Biomass and Bioenergy*, *32*(4), 338–344. doi:10.1016/j.biombioe.2007.10.005

Verma, S. (2002). *Anaerobic digestion of biodegradable organics in municipal solid wastes*. Columbia University, New York, NY, USA. Retrieved from http://www.seas.columbia.edu/earth/vermathesis.pdf

Vunjak-Novakovic, G., Kim, Y., Wu, X., Berzin, I., & Merchuk, J. C. (2005). Air-Lift Bioreactors for Algal Growth on Flue Gas: Mathematical Modeling and Pilot-Plant Studies. *Industrial & Engineering Chemistry Research*, *44*(16), 6154–6163. doi:10.1021/ie049099z

Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., … Ruan, R. (2010). Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant. *Applied Biochemistry and Biotechnology*, *162*(4), 1174–86. doi:10.1007/s12010-009-8866-7

Wang, L., Wang, Y., Chen, P., & Ruan, R. (2010). Semi-continuous cultivation of Chlorella vulgaris for treating undigested and digested dairy manures. *Applied Biochemistry and Biotechnology*, *162*(8), 2324–32. doi:10.1007/s12010-010-9005-1

Wang, X. J., Xia, S. Q., Chen, L., Zhao, J. F., Renault, N. J., & Chovelon, J. M. (2006). Nutrients removal from municipal wastewater by chemical precipitation in a moving bed biofilm reactor. *Process Biochemistry*, *41*(4), 824–828. doi:10.1016/j.procbio.2005.10.015

Watanabe, Y., Joel, N., & Hall, D. O. (1995). Photosynthetic Performance of a Helical Tubular Photobioreactor Incorporating the Cya no bact er i u m Spirulina platensis. *Biotechnology and bioengineering*, *47*, 261–269. doi:0006-3592/95/020261-09

Watanabe, Y., & Saki, H. (1997). Development of a photobioreactor incorporating chlorella sp. For removal of CO2 in stack gas. *Energy Conversion and Manageement*, *38*(96), 499–503.

WHO. (1999). *Toxic Cyanobacteria in Water : A guide to their public health consequences , monitoring and management*. (I. Chorus & J. Bartram, Eds.) (p. 400). London: E & FN Spon.

Williams, C. L. (2012). *Methane production from anaerobic co-digestion of Chlorella vulgaris and wastewater sludge*. San Diego State University, San Diego, CA, USA.

Woertz, I., Fulton, L., & Lundquist, T. (2009). Nutrient Removal & Greenhouse Gas Abatement with CO2 Supplemented Algal High Rate Ponds. *Proceedings of the Water Environment Federation*, *2009*(7), 7924–7936. doi:10.2175/193864709793900177

Yen, H.-W., & Brune, D. E. (2007). Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresource Technology*, *98*(1), 130–4. doi:10.1016/j.biortech.2005.11.010

Yılmaz, V., & Demirer, G. N. (2008). Enhancing the Performance of Anaerobic Digestion of Dairy Manure through Phase-Separation. *Clean*, *36*(9), 760–766. doi:10.1002/clen.200800024

Yılmazel, Y. D. (2009). *Removal and Recovery of Nutrients as Struvite from Anaerobic Digestion / Co- digestion Residues of Poultry Manure*. Middle East Technical University, Ankara, Turkey.

Yoo, C., Jun, S.-Y., Lee, J.-Y., Ahn, C.-Y., & Oh, H.-M. (2010). Selection of microalgae for lipid production under high levels carbon dioxide. *Bioresource Technology*, *101*(1), S71–4. doi:10.1016/j.biortech.2009.03.030

Yun, Y.-S., Lee, S. B., Park, J. M., Lee, C.-I., & Yang, J.-W. (1997). Carbon Dioxide Fixation by Algal Cultivation Using Wastewater Nutrients. *Journal of Chemical Technology & Biotechnology*, *69*(4), 451–455. doi:10.1002/(SICI)1097-4660(199708)69:4<451::AID-JCTB733>3.0.CO;2-M

Zhao, M.-X., & Ruan, W.-Q. (2013). Biogas performance from co-digestion of Taihu algae and kitchen wastes. *Energy Conversion and Management*, *75*, 21–24. doi:10.1016/j.enconman.2013.05.037

Zhou, Y. (2010). *Improvıng Algal Bıofuel Productıon through Nutrıent Recyclıng and Characterızatıon of Photosynthetıc Anomalıes in Mutant Algae Specıes*. University of Illinois at Urbana-Champaign, Urbana, IL, USA.

APPENDIX A

CALCULATION OF MICROALGAL BIOMASS PRODUCTIVITY and MAXIMUM SPECIFIC NET GROWTH RATE

Microalgal productivity was calculated by curve-fitting on 12-day exponential growth period in batch cultivation of *Chlorella vulgaris.* As Equation 2.1 implies, slope of the linear fit corresponds to productivity value of the culture, in terms of optical density.

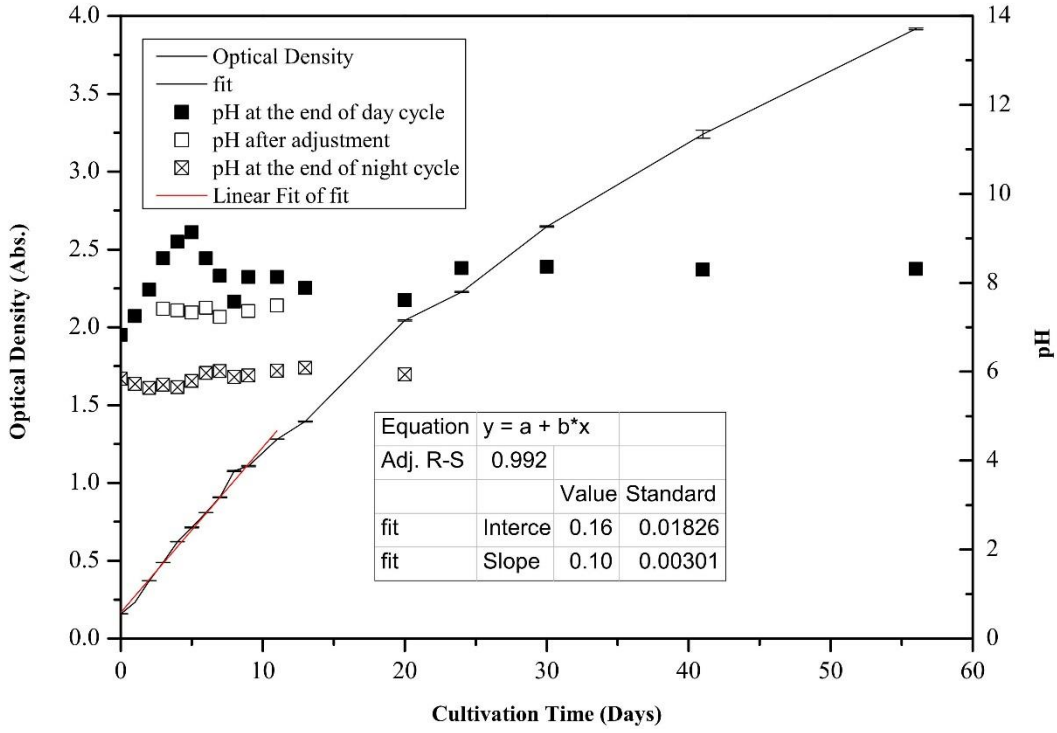


Figure A-1: Linear fit on exponential growth phase of batch culture.

As shown in Figure A-1, the slope of the linear curve is 0.10, which corresponds to productivity value of 0.1 Abs./L.d. When this value is substituted into Equation 4-1, productivity value on TSS basis is calculated as 20.4 mg/L.d.

Maximum specific net growth rate was calculated based on Equation 2-2. For this purpose, optical density values of exponential growth phase data was depicted on a semi-log paper, as shown in Figure A-2, in order to determine the linear trend within logarithmic values of this period.

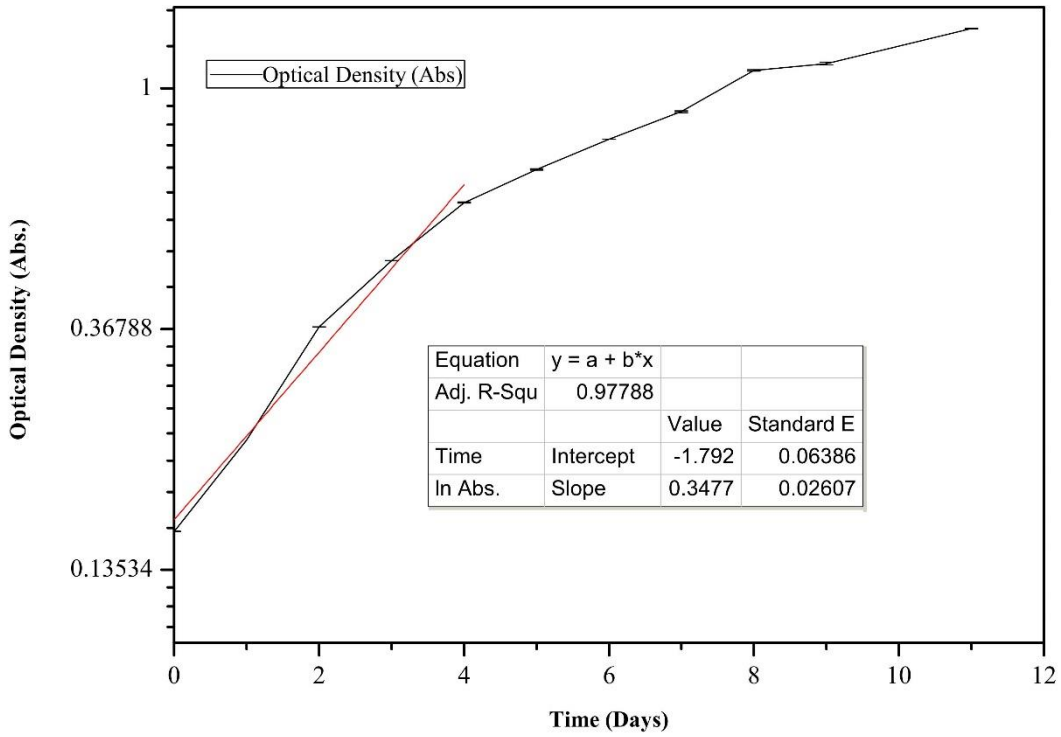


Figure A-2: Linear fit on exponential fit of batch culture on semi-ln scale

It can bee seen that data were linear until day 4. Thus, these data were used for specific net growth rate determination by linear curve fitting. The slope of the fit, 0.35, corresponds to maximum specific growth rate on d^{-1} basis.

APPENDIX B

PHOTOGRAPHS of MICROSCOPIC ANALYSES

B.1. Photographs of microscopic analysis conducted at day= 0, at the beginning of Cycle 1

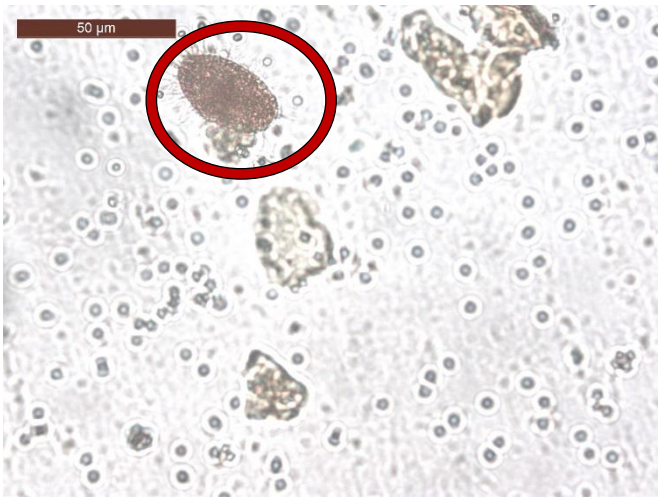


Figure B-1: Photograph of a cilliate in SCP, under inverted microscope

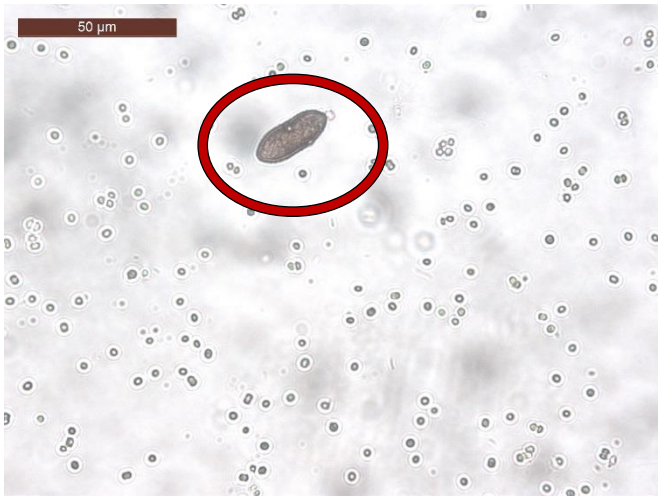


Figure B-2: Photograph of a cilliate in SCP, under inverted microscope

B.2. Photographs of microscopic analysis conducted at day= 21, at the end of Cycle 18

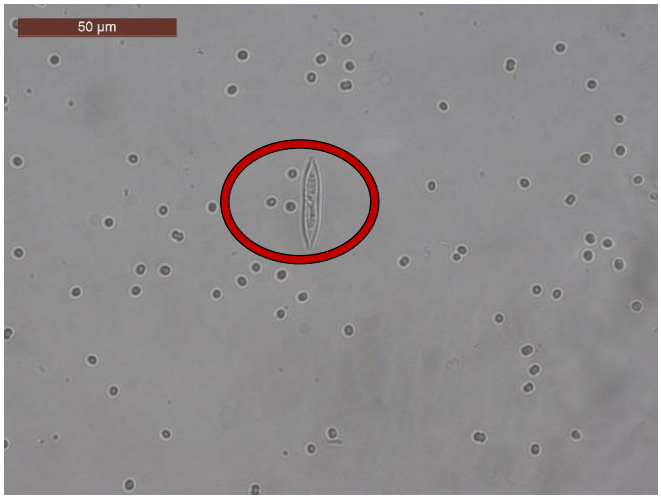


Figure B-3: Photograph of a diatom in SCP, under inverted microscope

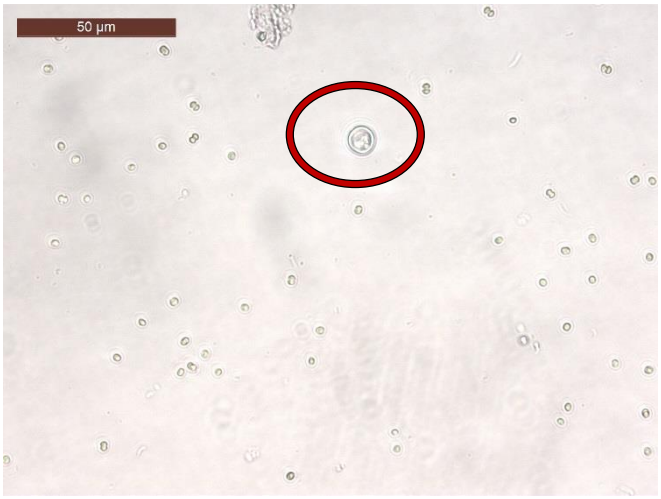


Figure B-4: Photograph of an oocyst in SCP, under inverted microscope

APPENDIX C

BIOGAS AND METHANE YIELDS OF REACTORS ON COD BASIS

Reactor Code	Biogas Yield (mL / g COD added)	Methane Yield (mL CH_4/g COD added)
A1a	246	162
A1b	184	117
A2a	254	145
A2b	202	129
A3a	268	155
A3b	189	89
A4a	288	122
A4b	215	72
H1a	291	190
H1b	281	190
H2a	394	257
H2b	386	266
At1a	317	222
At1b	319	237
At2a	396	265
At2b	336	232
TC1a	224	159
TC1b	194	128
TC2a	201	103
TC2b	219	142
M1a	233	14
M1b	593	339
M2a	164	24
M2b	519	263
M3a	557	301
M3b	526	370
M4a	160	9
M4b	359	126
W1a	420	205
W1b	429	197
W2a	328	181
W2b	299	198
W3a	299	116
W3b	340	149

Table C-1: Biogas and methane yields of reactors on COD basis