INTEGRATED CARBON DIOXIDE MITIGATION AND NUTRIENT REMOVAL FROM MUNICIPAL AND INDUSTRIAL WASTEWATER USING MICROALGAL SYSTEMS

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

BY

GECE CANSU KILIÇ

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

OCTOBER 2017

Approval of the thesis:

INTEGRATED CARBON DIOXIDE MITIGATION AND NUTRIENT REMOVAL FROM MUNICIPAL AND INDUSTRIAL WASTEWATER USING MICROALGAL SYSTEMS

submitted by **GECE CANSU KILIÇ** in partial fulfillment of the requirements for the degree of **Master of Science in Environmental Engineering Department, Middle East Technical University** by,

Prof. Dr. Gülbin Dural Ünver
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Kahraman Ünlü
Head of Department, **Environmental Engineering** _____

Assoc. Prof. Dr. Tuba Hande Bayramoğlu
Supervisor, **Environmental Engineering Dept., METU** _____

Prof. Dr. Sibel Uludağ Demirer
Co-Supervisor, **Environmental Engineering Dept., Karabük University** _____

Examining Committee Members:

Prof. Dr. İpek İmamoğlu
Environmental Engineering Dept., METU _____

Assoc. Prof. Dr. Tuba Hande Bayramoğlu
Environmental Engineering Dept., METU _____

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

Prof. Dr. Ayşegül Aksoy
Environmental Engineering Dept., METU _____

Assoc. Prof. Dr. Selim Sanin
Environmental Engineering Dept., Hacettepe University _____

Date: 11/10/2017

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 material and results that are not original to this work.

Name, Last Name: GECE CANSU KILIÇ

Signature:

ABSTRACT

INTEGRATED CARBON DIOXIDE MITIGATION AND NUTRIENT REMOVAL FROM MUNICIPAL AND INDUSTRIAL WASTEWATER USING MICROALGAL SYSTEMS

Kılıç, Gece Cansu
M.S., Department of Environmental Engineering
Supervisor: Assoc. Prof. Dr. Tuba H. Bayramoğlu
Co-supervisor: Prof. Dr. Sibel Uludağ Demirer

October 2017, 160 pages

The aim of this master thesis study is to investigate the nutrient removal efficiency from different kinds of wastewaters and the carbon dioxide mitigation in photobioreactors with unialgal culture, *Chlorella vulgaris*.

In the first part of the study, *Chlorella vulgaris* culture was cultivated in the Bold's basal medium in batch reactors to increase the biomass content and to observe the growth phases of alga. Then, two parallel photobioreactors (PBRs) were run to cultivate *Chlorella vulgaris* culture semi-continuously to determine the minimum control requirements on the system to achieve steady-state. pH control at every feeding procedure and temperature regulation requirements became evident.

Secondly, nutrient removal from municipal wastewater by *Chlorella vulgaris* was investigated. Three PBRs were operated at 2, 4 and 8 days of hydraulic retention time (HRT) in semi-continuous PBRs to determine the optimum HRT to achieve the

highest nutrient removal. At the 4 day of HRT, 98-100% total ammonium nitrogen (TAN) and 85-98% ortho-phosphate (PO_4-P) removal efficiencies were achieved, which was the highest removal among all other HRTs. Before and after the semi-continuous set of experiments, batch sets were run with unacclimated and acclimated algal culture. The highest biomass growth rates of the cultures were measured as 0.39 d^{-1} for unacclimated and 0.82 d^{-1} for acclimated culture showing that acclimation is important for system efficiency.

At the final part, it was aimed to treat coke factory wastewater, which was mixed with supernatant of primary sludge thickener (thickener supernatant) to provide phosphorus and dilution to the system by supplying 4% carbon dioxide (CO_2)-enriched air with *Chlorella vulgaris*. Mixing ratio of two wastewaters was determined by set of batch experiments to identify the optimum nitrogen: phosphorous (N/P) ratio for *Chlorella vulgaris*. After this ratio was determined as 6, semi-continuous set of experiments were done with mixed wastewater prepared accordingly. Among 3 HRTs studied (5, 8, and 12 days), 12 days of HRT provided the best removal rates as 97.5% TAN, 97% PO_4-P, and 17.7 % CO_2 removal. Outcomes of this thesis study can be further used for large scale experimental sets to treat that specific wastewaters with *Chlorella vulgaris*.

Keywords: Carbon dioxide mitigation, *Chlorella vulgaris*, Coke factory wastewater, municipal wastewater, wastewater treatment.

ÖZ

MİKROALG SİSTEMLERİ İLE ENTEGRE KARBONDİOKSİT AZALTIMI VE EVSEL VE ENDÜSTRİYEL ATIKSULARDAN NÜTRİYENT GİDERİMİ

Kılıç, Gece Cansu
Yüksek Lisans, Çevre Mühendisliği Bölümü
Tez Yöneticisi: Doç. Dr. Tuba H. Bayramoğlu
Yardımcı Tez Yöneticisi: Prof. Dr. Sibel Uludağ Demirer

Ekim 2017, 160 sayfa

Bu yüksek lisans tezinin amacı tek hücreli *Chlorella vulgaris* kültürüyle, fotobiyoreaktörlerde çeşitli atıksulardan besiyer madde gideriminin ve karbondioksit azaltımının araştırılmasıdır.

Bu çalışmanın ilk kısmında, *Chlorella vulgaris* kültürü, kesikli reaktörlerde biyokütle miktarını artırmak ve algin büyüme evrelerini gözlemlemek için bold bazal besiyerinde yetiştirilmiştir. Daha sonra, iki parallel fotobiyoreaktör (FBR), *Chlorella vulgaris* kültürünün yarı-sürekli olarak kararlı halde yetiştirilebileceği en az sistem kontrolünü belirlemek için çalıştırılmıştır. Her besleme yapıldığında pH kontrolü ve sıcaklık düzenlemesi yapılması gerekliliği ortaya çıkmıştır.

İkinci olarak, evsel atıksudan *Chlorella vulgaris* ile nütriyent giderimi incelenmiştir. 3 adet FBR 2, 4 ve 8 gün hidrolik bekletme sürelerinden (HBS) nütriyent giderimine en uygununu bulmak amacıyla çalıştırılmıştır. 4 gün HBS ile çalışan FBR'de diğer HBS'lerde sağlanandan daha yüksek olacak şekilde %98-100 toplam amonyum azotu (TAN) ve %85-98 orto-fosfat (PO_4-P) giderimi sağlanmıştır. Yarı-sürekli deneylerin öncesinde ve sonrasında aklime olmamış ve olmuş kültürlerle kesikli deney setleri çalıştırılmıştır. En yüksek biyokütle büyüme hızı aklime olmamış kültür için 0,39 $gün^{-1}$ ve aklime olmuş kültür için 0,82 $gün^{-1}$ olarak ölçülmüş; bu sonuç aklimasyonun sistem verimliliği açısından önemli olduğunu göstermiştir.

Son kısımda, sisteme fosfor ve seyreltme sağlamak için birincil çamur yoğunlaştırma tankı süzüntü suyu ile karıştırılan kok fabrikası atıksuyunun, %4 karbondioksit ile zenginleştirilmiş hava verilerek *Chlorella vulgaris* ile arıtımı hedeflenmiştir. İki atıksuyun karışım oranı *Chlorella vulgaris* için en ideal nitrojen: fosfor (N/P) oranını belirlemek için yapılan kesikli deneylerle saptanmıştır. Bu oran 6 olarak belirlendikten sonra buna uygun şekilde hazırlanan karışım atıksuyu ile yarı-sürekli deneyler yapılmıştır. 5, 8 ve 12 gün HBS arasından 12 gün HBS'inde %97,5 TAN, %97 PO_4-P ve %17,7 CO_2 arıtımı ile en iyi giderim değerleri elde edilmiştir. Bu çalışmanın sonuçları bu spesifik atıksuların *Chlorella vulgaris* ile arıtımında büyük ölçekli deney setlerinde kullanılabilir.

Anahtar Kelimeler: Karbondioksit azaltımı, *Chlorella vulgaris*, Kok fabrikası atıksuyu, evsel atıksu, atıksu arıtımı.

ACKNOWLEDGMENTS

First of all, I would like to express my sincere gratitude to my advisor Assoc. Prof. Dr. Tuba H. Ergüder Bayramoğlu and my co-advisor Prof. Dr. Sibel Uludağ-Demirer for their guidance, patience and immense knowledge. Besides my advisors, I would like to thank the members of my thesis defense jury for their insightful comments and invaluable contributions to my thesis.

This thesis has emerged from the project funded by Scientific and Technological Research Council of Turkey (TUBITAK 111Y205) which I am especially grateful for. I also gratefully acknowledge the help, support, encouragement and guidance provided by our project coordinator Prof. Dr. Göksel Demirer and Assist. Prof. Dr. Barış Kaymak.

I am beyond grateful to my colleagues from the project, Özgül Çalıcıoğlu- Şengül and Burak Çakırlar and from the anaerobic laboratory, Eray Gür, İlke Çelik, Engin Koç, Melih Can Akman, Güneş Ekin Tunçay for their friendship and support. I would like to extend my gratitude to Fadime Kara Murdoch for her endless support, motivation and her friendship.

Last, but not least, I would like to express my thanks to my family. I am forever grateful to my parents for supporting and encouraging me for no matter what. My deepest gratitude is for my husband for his endless love, support, patience and guidance. Without him, this thesis could not exist.

TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

ABBREVIATIONS

μ	Specific growth rate (d^{-1})
ATP	Adenosine Triphosphate
BBM	Bold's Basal Medium
BOD	Biochemical Oxygen Demand
C	Carbon
$C_6H_{12}O_6$	Glucose molecule
CH_3CH_2OH	Ethanol molecule
CCS	Carbon Capture and Storage
CH_2O	Carbohydrates
CO_2	Carbon Dioxide
CO_3^{-2}	Carbonate Ion
COD	Chemical oxygen demand
C. vulgaris	*Chlorella vulgaris*
DO	Dissolved Oxygen
H_2CO_3	Carbonic Acid
H_2O	Water
HCO_3^-	Bicarbonate Ion
HRAP	High Rate Algal Ponds
HRT	Hydraulic retention time
N	Nitrogen
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
Na_2CO_3	Sodium Carbonate
$NaHCO_3$	Sodium Bicarbonate
NH_3	Free ammonia
NH_4^+	Ammonium Ion
NH_4^+-N	Ammonium nitrogen
NO_3^-	Nitrate

NO_3^--N	Nitrate nitrogen
NO_2^-	Nitrite
NO_2^--N	Nitrite nitrogen
NO_x	Nitrogen Oxide
O_2	Oxygen
OD	Optical Density
PO_4-P	Ortho-phosphate
PAR	Photosynthetically active radiation, (nm)
PBR	Photobioreactor
P	Phosphorus
PO_4^{3-}-P	Phosphate Ion
sCOD	Soluble Chemical Oxygen Demand
SO_x	Sulfur Oxide
tCOD	Total Chemical Oxygen Demand
TAN	Total ammonium nitrogen (NH_4^+-N + NH_3-N)
TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
TS	Total solids
TSS	Total suspended solids
TVS	Total volatile solids
WW	Wastewater
VFA	Volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids
vvm	Volume gas per volume of broth per minute

CHAPTER 1

INTRODUCTION

With the drastic increase in population and industrialization in the world, energy deprivation and anthropogenic pollution levels have been accelerating (Al-lwayzy et al., 2012). Nutrient (nitrogen and phosphorus) removal from wastewater is a neglected however an important issue. Only 33.8% of the municipal wastewaters and 44% of the industrial wastewaters undergo nutrient removal although it is obligated by regulations (TÜİK, 2015). In addition to nutrient removal, carbon dioxide (CO_2) emissions are important as well due to fast-growing concern about global warming. As Turkey also ratified Kyoto protocol, taking measures for CO_2 emissions from flue gasses become necessary. Technology reforms need to be emerged on sustainable and cost-effective wastewater treatment, CO_2 sequestration and energy production.

Microalgal systems are capable of providing solutions to all these problems mentioned above in one system since microalgae are photosynthetic microorganisms, which use nutrients and CO_2 from their environment while carrying out photosynthesis. Thus, microalgal systems are promising for being a CO_2 mitigation process and achieving nutrient removal at the same time.

Figure 1-1 shows a representation of such a system. After solids are removed from the wastewater with the help of screens, grit separators and/or primary clarifiers, it is directed to algal production reactors/ponds which operates like aeration tanks of activated sludge systems (Abdel-Raouf et al., 2012). In Photobioreactors (PBRs)/ponds, wastewater treatment and CO_2 capture are achieved simultaneously with the help of light owing to photosynthetic feature of algae (Znad et al., 2012).

Open algal ponds are most widely used ones as they are cheaper and easy to operate. However, PBRs provide higher production and treatment efficiencies, less contamination, and controlled environment (Christenson and Sims, 2011). Thereafter, treated water is separated from algal biomass by dewatering and harvesting. Treated wastewater can be treated further for polishing purposes before discharge. Harvested biomass, on the other hand, can be processed to produce biomethane, biohydrogen, biodiesel, bioethanol and fertilizer. The exhaust gas from the anaerobic digestion unit can be directed back to algal production system for CO_2 capture (Harun et al., 2010).

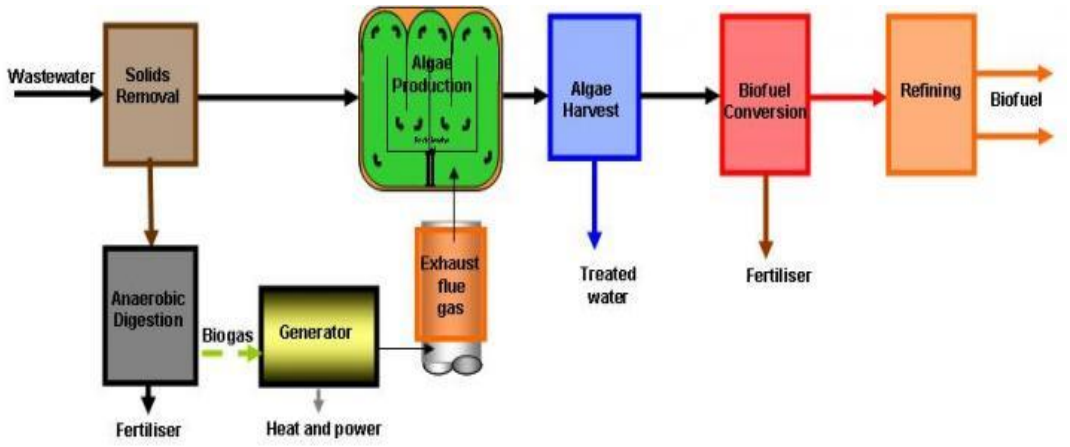

Figure 1-1 Scheme of algal system (Craggs, 2009)

Treating wastewater with algae is not a new idea. Even though Oswald designed the first open pond at 1957, technological improvements of the algal treatment system have emerged recently due to increase in greenhouse gas effect and cost of energy sources especially diesel or gasoline. Algal systems have two major bottlenecks; (i) harvesting to be energy intensive and (ii) diesel or gasoline to be cheaper than biodiesel obtained from algal biomass (Uduman et al., 2010). As harvesting technology improves and gasoline or diesel costs would be higher due to increasing progress of scarcity of the fossil fuel, algal technology will be needed more. Nonetheless, algal systems have undoubtable advantages over other technologies;

- High nutrient recovery can be achieved even in wastewaters with low COD content (Ruiz et al., 2013).
- Some of the algae strains can adsorb and remove heavy metals, organic solvents, aromatic hydrocarbons and phenols from wastewater. Furthermore, some algal species are capable of surviving at highly toxic environments (Muñoz and Guieysse, 2006).
- Algae cause no food versus energy conflict as it can be grown at non-arable lands (Lam and Lee, 2012a).
- Algae can double their biomass in less than one day and can sequester CO_2 100 times more efficiently than terrestrial plants (Lam and Lee, 2012b).
- Biofuels, fertilizers, pharmaceuticals, bioplastics, biochemical, non-animal-fish oil replacements, etc. can be produced from algae (Sterner, 2013).

Chlorella vulgaris has been chosen as the microalgal specie to be used in this thesis study. *Chlorella vulgaris* is a fast-growing, easily and quickly adaptive unicellular green algae (Li et al., 2013; Muñoz and Guieysse, 2006). *Chlorella* shows great promise on nutrient removal and lipid production (Widjaja et al., 2009). Studies have been conducted with municipal or urban wastewater (Boonchai et al., 2012; Li et al., 2013; Woertz et al., 2009). In these and similar studies, CO_2-enriched air was generally used to aerate culture for CO_2 supply since air is considered as insufficient carbon source for nutrient removal from municipal wastewater (Larsdotter et al., 2010). However, while treating municipal wastewater, providing flue gas for CO_2 source to the wastewater treatment plant can be highly costly as there might not be any factory chimney nearby to direct flue gas to the plant. On the other hand, aeration is also used at aeration tanks of conventional activated sludge systems, it would not be an additional economical burden for municipal wastewater treatment. Therefore, achieving high nutrient removal rates from municipal wastewater using just air with microalgae is an important issue.

In addition to municipal, variety of industrial wastewaters were treated with microalgae; dairy (Woertz et al., 2009), agroindustrial (Molinuevo-Salces et al.,

2016), piggery wastewater (Abou-Shana et al., 2013). Although steel-making plant wastewaters are problematic wastewaters, only one study has been conducted to treat influent of final treatment plant of steel-making facility with microalgae (Yun et al., 1997). However, no study was done with wastewater from coke factory of a steel-making facility as it has been aware of. Moreover, nutrient removal from two problematic wastewaters (coke factory wastewater and thickener supernatant) by mixing with microalgae has never been studied as it has been aware of.

The scope of this thesis study was to investigate the nutrient (nitrogen and phosphorus) removal from municipal and industrial wastewaters in batch and semi-continuous reactors. The objectives of this thesis were defined as follows;

1. To investigate the cultivation of axenic *Chlorella vulgaris* in batch and semi-continuous PBRs,

- to observe and investigate the growth phases of *Chlorella vulgaris* cultivating in nutrient media in batch PBR,
- to investigate the cultivation of *Chlorella vulgaris* with nutrient media in two parallel semi-continuous PBRs and to determine the operation requirements of the system.

2. To investigate the nutrient removal capability of *Chlorella vulgaris* culture from municipal wastewater

- to investigate the effect of different HRTs on nutrient removal efficiency and growth of *Chlorella vulgaris* culture with municipal wastewater in semi-continuous reactors,
- to investigate the growth kinetics of acclimated *Chlorella vulgaris* culture with municipal wastewater in batch reactors.

3. To investigate the nutrient removal capability of *Chlorella vulgaris* culture from coke plant wastewater mixed with thickener supernatant

- to determine the optimum nitrogen: phosphorus (N/P) ratio of mixed wastewater for nutrient removal with *Chlorella vulgaris* culture,
- to investigate the effect of different HRTs on nutrient removal efficiency and growth of *Chlorella vulgaris* culture with coke plant wastewater and thickener supernatant in semi-continuous reactors,
- to investigate the CO_2 fixation potential of *Chlorella vulgaris* culture in semi-continuous reactors.

CHAPTER 2

LITERATURE REVIEW

2.1. Microalgae

Microalgae are subgroup of a photosynthetic organism, algae, and they are often called "Phytoplanktons" (Larkum et al., 2012). The difference of the microalgae from the other subgroup of algae which are called macroalgae or seaweed is that microalgae are unicellular organisms even if they can be colonial sometimes while macroalgae are multicellular organisms (Johnson, 2009). Algae generally occur in aquatic environments such as freshwater, marine and brackish water, as well as terrestrial places. They can be found in anywhere on biosphere even with extreme conditions like hot springs, desert soils, etc (Kumar et al., 2011).

2.1.1. Physiology of Microalgae

Microalgae are photosynthetic unicellular (Schuler and Kargi, 2002) microorganisms like plants with no roots, steams or leaves (Brennan and Owende, 2010). Typical size of unicellular alga is 10-30 μm (Schuler and Kargi, 2002). They can be both prokaryotic and eukaryotic microorganisms. Prokaryotic microalgae are called cyanobacteria or blue-green algae which are lacking organalles with membrane (Brennan and Owende, 2010). Green algae (*chlorophyta*), red algae (*Rhodophyta*) and diatoms (*Bacillariophyta*) are the most important classes of eukaryotic microalgae (Lee, 2008).

Microalgae can be grown autotrophically, heterotrophically or mixotrophically. In the absence of light, microalgae replace its carbon source from fixing CO_2 from atmosphere to organic carbon from environment and grow heterotrophically. On the other hand, fixation of CO_2 and assimilating external organic carbon source simultaneously occur in mixotrophic growth regime of microalgae as photosynthesis and respiration, respectively (Octavio et al., 2011).

2.1.2. Microalgal Photosynthesis

Microalgae are photosynthetic organisms as mentioned in the previous section (Section 2.1.1. Physiology of Microalgae) and this attribute of microalgae is directly related with CO_2 capture mechanism, and therefore important. These organisms convert CO_2 in the presence of water by capturing sunlight with their chlorophyll and other pigments to chemical energy which is stored in carbonhydrate molecule (Octavio et al., 2011).

Photosynthesis is carried out by two types of reactions which are light (light-dependent) reactions and dark (light – independent) reactions. In the light reactions, two photosynthetic systems are used; PSII and PSI (Figure 2-1). By-product of the photosynthesis which is oxygen, is produced in this step. Photons with a wavelenght shorter than 680 nm are absorbed by PSII pigments to split water into H^+ (protons), e^- (electrons), and oxygen (O_2). Electrons are carried to the PSI system by electron carriers and cytochrome complex. Photons with a wavelenght shorter than 700 nm are absorbed by PSI to produce reduced NADPH and ATP (adenosine triphosphate). In the dark reactions; CO_2 is reduced with ATP and NADPH via calvin cycle to produce carbohydrates (CH_2O) (Figure 2-1) (Bryant and Frigaard, 2006; Znad et al., 2012). The overall reaction of photosynthesis is given in Equation 2-1.

$$CO_2 + H_2O + \text{sunlight} \rightarrow CH_2O + O_2 \qquad \text{Equation (2-1)}$$

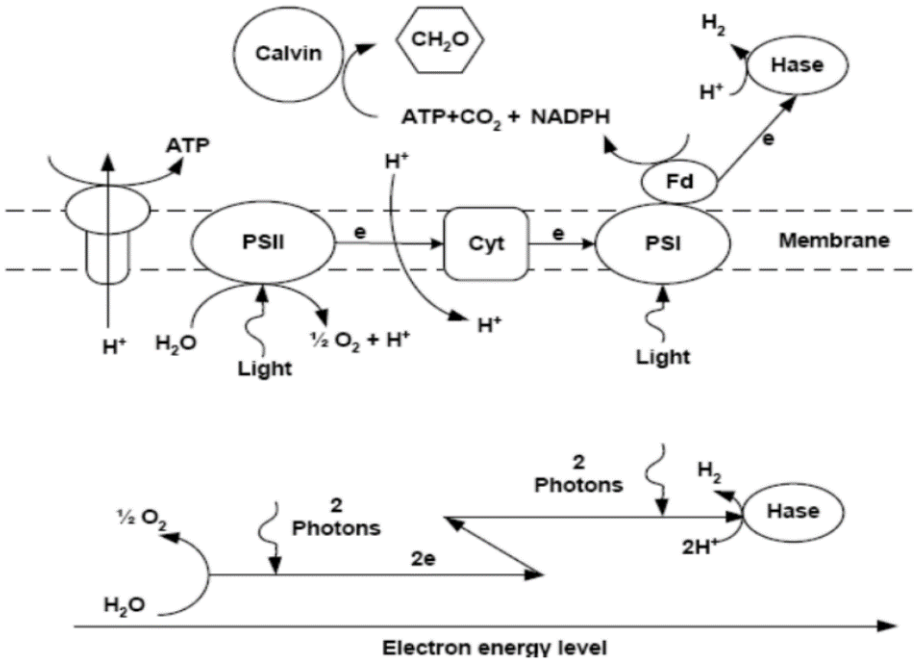

Figure 2-1 Schematic representation of photosynthesis mechanism (Znad et al., 2012)

2.1.3. Growth Kinetics of Microalgae

The algal growth has five different phases (Figure 2-2).

- (i) Lag phase: This is the adaptation period of newly inoculated culture to the environment.
- (ii) Exponential (logarithmic) growth phase: After the adaptation period, increase in algal biomass concentration per time is always proportional with the biomass population at any given time (Equation 2-2). At this phase, nutrient is not a limiting factor, so nutrient concentration does not affect growth rate. Relation between specific growth rate (μ) and change of biomass concentration (X) in time (t) is shown at Equation 2-2.

$$\frac{dX}{dt} = \mu . X \qquad \text{Equation (2-2)}$$

- (iii) Deceleration growth phase: Because of nutrient limitation, light limitation due to increased biomass concentration and/or toxic by-product

accumulation due to metabolic reactions, growth rate decelerates. This phase takes place in a short time period.

- (iv) Stationary phase: Stationary phase occurres when growth rate becomes zero or equal to the death rate. Biomass concentration reaches the maximum point and stays almost constant.
- (v) Death phase: Toxic metabolic compunds released into the medium due to cell deaths during stationary phase cause death phase to begin. Death rate becomes higher and growth rate becomes negligible (Schuler and Kargi, 2002).

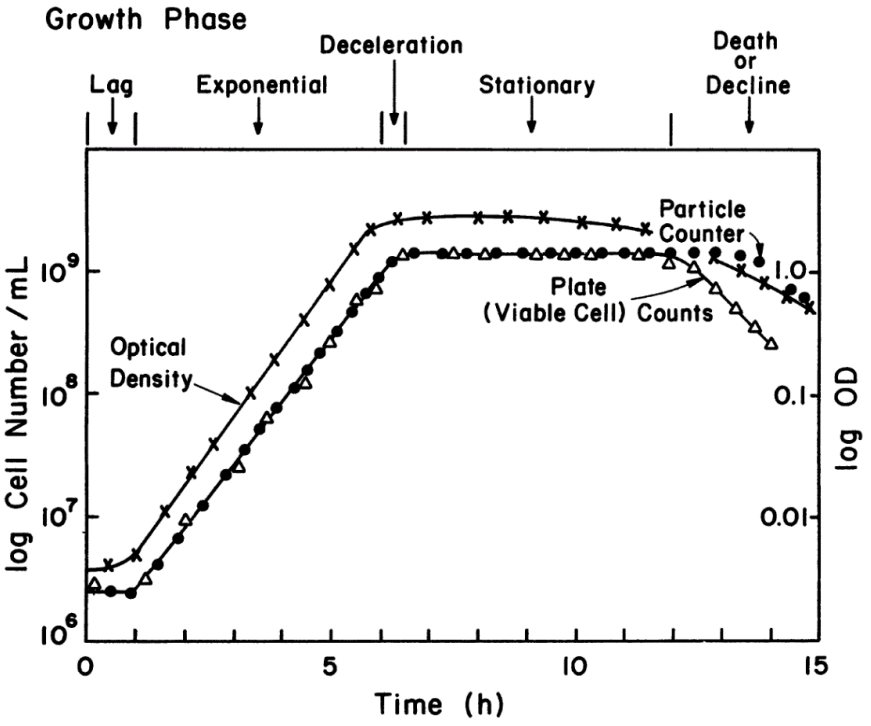

Figure 2-2 Typical growth phase of microorganisms (Schuler and Kargi, 2002).

2.1.4. Factors Affecting Microalgal Growth

In general, microalgal growth depends highly on key parameters. Keeping these parameters such as light, nutrient and carbon source, temperature and pH at optimum level is important for efficient microalgal cultivation (Travieso et al., 2006). Detailed

information on optimum growth conditions of *Chlorella vulgaris* will be further discussed in Section 2.1.5. Optimum Parameters for the Growth of *Chlorella vulgaris.*

2.1.4.1. Light

Light has a major impact on algal growth and should be provided at optimum in all conditions. While insufficient illumination causes low growth rates, excess illumination results in inhibitory effects to algal cells (Carvalho et al., 2011). Optimum light intensities vary depending on the algal strains. Optimum light intensities for *Chlorella kessleri* and *Chlorella protothecoide* were found as 120 µmol. $m^{-2}.s^{-1}$ and 30 µmol. $m^{-2}.s^{-1}$, respectively (Li et al., 2012). In another study, 100 µmol. $m^{-2}.s^{-1}$ was determined as the optimum light intensity for *Euglena gracilis* (Kitaya et al., 2005). Sforza et al. (2004) reported that maximum growth of *Scedenesmus Obliquus* was achieved at light intensity of 150 µmol. $m^{-2}.s^{-1}$ and efficiency started to decrease at light intensities beyond that value.

2.1.4.2. Nutrient Source

Nitrogen and phosphorous are the macronutrients that are very necessary for algal growth and metabolism. Nitrogen which corresponds to 7-20% of cell dry weight (Hu, 2004) constitutes vital organic molecules for microalgal cell which are nucleic acids and proteins (Juneja, et al., 2013). Inorganic nitrogen can be found in various aqueous forms such as ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) (A. Kumar et al., 2010). Urea ($CO(NH_2)_2$) is also used as a nitrogen source. However, NH_4^+ is the most preferred source for microalgae. NH_4^+ concentrations higher than 20 mg/L can cause ammonia toxicity. Although the second most preferrable nitrogen source is determined as nitrate, it is only used by microalgae at the absence of ammonium (Larsdotter, 2006).

Phosphorus is another important element that corresponds to the structures of ATP, nucleic acids, phospholipids, etc. and constitutes only 1% of the algal dry weight. Phosphorus is the primary limiting nutrient in nature (Juneja et al., 2013). Phosphorus is uptaken by microalgae in the PO_4-P form and stored in the form of polyphosphates in microalgal cells (Larsdotter, 2006).

Both nitrogen and phosphorus limiting conditions slow the growth down and reduce the chlorophyll-a and protein content; however, increase the lipid content in microalgal cells (Juneja et al., 2013; A. Kumar et al., 2010).

2.1.4.3. Carbon Source

Carbon is vital for growth as 50% of almost all microalgal cells consists of carbon (Carvalho, et al., 2006). General carbon sources for microalgae are; (i) atmospheric CO_2, (ii) CO_2 from flue gases, (iii) soluble chemically fixed CO_2 ($NaHCO_3$ and Na_2CO_3) (Znad et al., 2012). Inorganic carbon can exist in forms of $CO_2(aq)$, H_2CO_3, HCO_3^-, and CO_3^{-2} depending on pH and temperature of the culture medium (Carvalho et al., 2006). HCO_3^- and CO_3^{-2} are the inorganic carbon species that are most preferable by microalgae. Aeration is one way to supply CO_2 to microalgal culture. However, atmosferic concentration of CO_2 which is 0.039% (Putman, et al., 2016) may not be sufficient for maximum algal growth (Larsdotter, 2006). 1-10% CO_2 enriched air can be provided for carbon supply (Larsdotter, 2006; Siddiqui, et al., 2015). Even though CO_2 tolerance of the microalgae can change from one specie to another, at high CO_2 concentrations, it becomes hard to control pH and chemical precipitation of salts containing CO_3^{2-}, OH^-, PO_4^{3-} occurs, which causes cell injuries (Carvalho et al., 2006).

2.1.4.4. pH

The optimum pH of most of the microalgal species are between 7-9. However, optimum pH can be more acidic or basic depending on the microalgal specie (Wang

et al., 2012). pH of the culture is directly related with CO_2 concentration (Siddiqui et al., 2015). Nitrate ions and photosynthetic CO_2 assimilation generally increase pH (Larsdotter, 2006). Even though high pH values are advantageous for being inhibitory for pathogens, it can also be harmful for microalgae (Znad et al., 2012). On the other hand, pH can drop as low as 3 when ammonia is used as a nitrogen source which can be also inhibitory for microalgae (Larsdotter, 2006).

2.1.4.5. Temperature

Obtaining the optimum temperature for microalgal culture is vital for achieving maximum growth. Optimum temperature varies from one algal strain to another. Even though 20-24 °C is optimal for microalgae in general, several species can tolerate up to 60°C (Table 2-1). Growth slows down at temperatures lower than 16°C, and, temperatures higher than 35°C is deathly for most of the microalgal species (Siddiqui et al., 2015).

Table 2-1 Temperature tolerance of various microalgal species (Siddiqui et al., 2015).

Microalgal species	Maximum temperature (°C)
Cyanidium caldarium	60
Scenedesmus sp.	30
Synechococcus elongates	60
Chlorella sp.	45
Eudorina sp.	30
Chamydomonas sp.	35
Nannochloris sp.	25
Monoraphidiumminutum	25
Chaetoceros sp.	25
Rhodomonas sp.	30
Cryptomonas sp.	30
Isochrysis sp.	30
Phaeodactylum tricomutum	30
Chlorella ellipsoidea	30
Pavlovalutheri	30
Spirulinaplatensis	25

2.1.5. Optimum Parameters for the Growth of *Chlorella vulgaris*

Chlorella vulgaris are eukaryotic, unicellular, photosynthetic (contains chloroyphyll) and spherical green microalgae with cell diameter of 5-10 µm. Taxonomic group of *Chlorella vulgaris* is; kingdom *plantea*, division *Chlorophyta*, genus *Chlorella*, family *Oocystaceae*, order *Chlorococcales*, class *Trebouxiophyceae*. Division of one mature *Chlorella vulgaris* cell happens in every 16-20 hours to form four new cells

(Myers, 1953). For that cell formation to happen, namely, for *Chlorella vulgaris* to grow, nutrient supply according to composition of *Chlorella vulgaris*, pH, light supply, temperature, concentration of the CO_2 in enriched air and supply rate are important parameters.

2.1.5.1. Compostion of *Chlorella vulgaris*

Elemental composition of *Chlorella vulgaris* is reported inTable 2-2 (Mandalam and Palsson, 1998). Anjos et al. (2013) reported that *Chlorella vulgaris* cell contains 45.6 % carbon, 6.9 % hydrogen and 2.7% nitrogen. However, cell composition of a microalgae strain may change with respect to different cultivation conditions and harvesting at different growth stages as also shown in Table 2-2 (Brown, 1997).

Table 2-2 Elemental composition of *Chlorella vulgaris* (Mandalam and Palsson, 1998)

Element	% Range
Carbon	51.4-72.6
Oxygen	11.6-28.5
Hydrogen	7.0-10.0
Nitrogen	6.2-7.7
Phosphorus	1.0-2.0
Potassium	0.85-1.62
Magnesium	0.36-0.80
Sulfur	0.28-0.39
Iron	0.04-0.55
Calcium	0.005-0.08
Zinc	0.0006-0.005
Copper	0.001-0.004
Manganese	0.002-0.01

2.1.5.2. pH

pH range of *Chlorella vulgaris* that can grow in is pretty wide. *Chlorella vulgaris* could not grow pH under 2 at all, while maximum growth was achieved between pHs of 6 to 8. Growth rate is lower between pH 10-12 than the growth at pH 8 (Lustigman, et al., 1995). Continuous studies with *Chlorella vulgaris* reported pH values during operation as 8 to 10 (Feng et al., 2011), 8.5 to 10.3 (Cheng et al., 2006), 7.99 (Fan et al., 2007), 7.6 to 8 (Wang et al., 2010), 7.5 to 8.5 (Boonchai et al., 2012), 7 to 8 (Woertz et al., 2009) and 7 (Li et al., 2013a).

2.1.5.3. Light and Illumination

80 µmol. $m^{-2}.s^{-1}$ was determined as the optimum light intensity for *Chlorella vulgaris* (Khalili et al., 2015). 30 µmol. $m^{-2}.s^{-1}$ (de-Bashan et al., 2002), 40 to 60 µmol. $m^{-2}.s^{-1}$ (Li et al., 2013a), 50 µmol. $m^{-2}.s^{-1}$ (Boonchai et al., 2012), 120 µmol. $m^{-2}.s^{-1}$ (Wang et al., 2010), 3000 lux (Feng et al., 2011), 10800 lux (Fan et al., 2007), 4300 lux (Woertz et al., 2009) are the light intensity values reported by other studies conducted with *Chlorella vulgaris.*

Photoperiod is the time period that microalgae are illuminated in a day. Light/dark cycles can be arranged with artificial illumination. Even though continuous illumination (24h:0h) is generally preferred, 12 h:12 h (12 hours light and 12 hours dark period) (Cheng et al., 2006), 14 h: 10 h (Boonchai et al., 2012), 16 h: 8h (Woertz et al., 2009) photoperiods have been used at some of the studies.

2.1.5.4. Temperature

Optimum temperature for *Chlorella vulgaris* was determined as 30˚C in various studies (Azeez, 2009; Cassidy, 2011; Chinnasamy et al., 2009; Kitaya et al., 2005). Moreover, high nutrient removal and growth rates of *Chlorella vulgaris* were reported by studies operated at temperatures of 30 ˚C (Feng et al., 2011), 25 ˚C (Fan

et al., 2007; Li et al., 2013; Wang et al., 2010), 28 ˚C (de-Bashan et al., 2002), 26 ˚C (Boonchai et al., 2012) and 23 ˚C to 25˚C (Woertz et al., 2009).

2.1.5.5. Aeration Rate and Carbon Dioxide Concentration

According to the study of Anjos et al. (2013), 6% CO_2 concentration and 0.4 vvm are optimal for *Chlorella vulgaris* to grow (Table 2-3). Aeration rate of 0.07 vvm (Cheng et al., 2006), 0.5 vvm (Feng et al., 2011), 0.22 vvm (Fan et al., 2007), 0.67 vvm (Woertz et al., 2009) and 0.1 vvm (Li et al., 2013b) were also reported at continuous studies with *Chlorella vulgaris*. While some of the studies only supplied air to the system (Boonchai et al., 2012; Feng et al., 2011), other studies with *Chlorella vulgaris* preferred various percentages of CO_2-enriched air such as 0.04%-10% (Cheng et al., 2006), 1%-21% (Fan et al., 2007) and 2% (Li et al., 2013b; Wang, et al., 2010).

Table 2-3 Growth parameters of *Chlorella vulgaris* under different CO_2 concentration and aeration rates at 30 ˚C (Anjos et al., 2013).

Time (d)	Cultivation conditions		Growth parameters	
	CO_2 concentration (%)	Aeration rate (vvm)	X_{max}[a] (g L^{-1})	P_{max}[b] (g $L^{-1}d^{-1}$)
7.9	2	0.1	5.5 ± 1.7	0.7 ± 0.2
7.7		0.4	6.9 ± 1.2	0.9 ± 0.2
7.6		0.7	8.3 ± 2.8	1.1 ± 0.4
7.6	6	0.1	6.8 ± 0.5	0.9 ± 0.0
7.7		0.4	10.0 ± 0.5	1.3 ± 0.0
7.4		0.7	8.9 ± 0.8	1.2 ± 0.1
7.5	10	0.1	6.0 ± 1.9	0.8 ± 0.3
7.2		0.4	8.6 ± 2.4	1.2 ± 0.3
7.1		0.7	8.5 ± 0.1	1.2 ± 0.0

[a] Final biomass concentration.

[b] Maximum biomass productivity.

2.2. Microalgal Cultivation Systems

Several technologies have been developed for mass cultivation of microalgal biomass for commercial uses. These technologies can be categorized under open and closed systems.

2.2.1. Open Systems

The history of open ponds for algal cultivation and simultaneous wastewater treatment goes back to 1957 when Oswald designed the first high rate algal pond (HRAP) (Figure 2-3). When Oswald designed this system at that time, he aimed to accelerate the system by taking advantage from photosynthetic attribute of algal organisms to provide additional oxygen to the bacteria that consume organic matter in the wastewater. Moreover, nitrogen and phosphorus uptake from wastewater by algal organisms would have helped the nutrient removal (Craggs, 2009). Open ponds are cheaper to construct and operate and easier to scale up with respect to closed algal systems. As they can utilize sunlight directly, no artificial lightning is needed unlike in some of closed systems (Borowitzka, 1999).

Although, open ponds are the most common systems for algal cultivation in the world, they have disadvantages, too. One of the most important drawbacks of open ponds is the lack of control on the system. Lack of control hinders to improve system efficiency by changing its growth parameters such as temperature, light intensity, pH, and dissolved oxygen since these parameters are controlled by the environment. Therefore, algal biomass content of the ponds is relatively lower than the content of closed systems (Harun et al., 2010). Moreover, open ponds are more vulnerable to contamination risk. Algal culture in the pond can be contaminated by predators such as bacteria, fungi and zooplanktons. This contamination problem was tried to overcome by creating an even more selective environment for algal strains; however, this leads to survival of only limited range of algal species in the pond (Borowitzka, 2012).

Open ponds

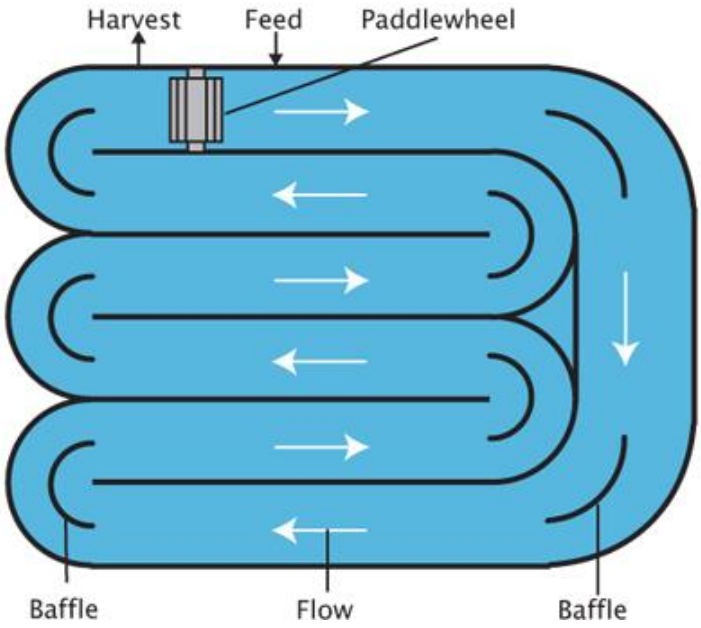

Figure 2-3 A representative raceway open pond (Johnson et al., 2009)

2.2.2. Closed Systems (Photobioreactors)

PBRs are designed for controlled photosynthetic biomass production so as to achieve higher biomass productivity. Despite being constantly compared to open ponds, PBRs have major advantages (Wang et al., 2012). First of all, PBR design overcomes the contamination problem by preventing direct contact with the environment which allows growing monocultures especially for producing complex biopharmaceuticals. Secondly, an easier control over substantial conditions that can easily be affected by

environmental factors such as pH, temperature, light, the supplied CO_2 concentration, etc., can be provided in closed systems unlike open ponds. Moreover, loss of water to evaporation is prevented. CO_2 produced from the reactor can be collected and directed back to the reactor and CO_2 mitigation can be achieved by this way (Singh and Sharma, 2012). Finally, since biomass growth is only limited to the surface of open ponds, lower photosynthetic efficiencies and biomass concentrations are obtained with open ponds when compared to closed systems (Wang et al., 2012). There are different types of PBRs that are designed by focusing on different design parameters.

2.2.2.1. Design Parameters of Photobioreactors

Design parameters are important for PBRs to be operated properly. While PBR is designed, literature values for parameters should be taken into consideration to provide maximum efficiency from the PBR.

Light Energy

Using light source with proper intensity, duration and wavelength is essential to improve microalgal growth (Carvalho et al., 2011). While low illumination causes insufficient growth, high intensity can cause photo-oxidation or photo-inhibition. In Figure 2-4, the part, where photosynthetic rate increasing with the irradiance, corresponds to light-limited region. Growth of microalgae can be limited there. It is the light-saturation area when photosynthetic rate reaches to P_{max} and does not change with the increasing light intensity. This is the optimum level for microalgal biomass to grow. However, beyond I_h, irradiance hinders the growth and photo-inhibition occurs (Carvalho et al., 2011). The photo-inhibition can be both reversible and irreversible depending on the exposure time and light stress (Wang et al., 2012).

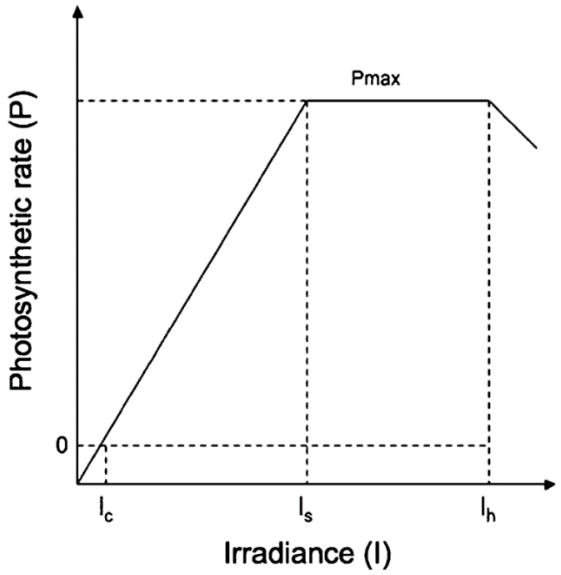

Figure 2-4 Photosynthesis rate (P) versus irradiance (I) curve for microalgae (Carvalho et al., 2011)

The wavelength of the light sources for microalgal growth should be between the range of 400 to 700 nm as this is the suitable spectrum for the chlorophylls and other photosynthetically active pigments (Suh and Lee, 2003). This spectral range is called photosynthetically active radiation (PAR) (Wang et al., 2012). The wavelength of the most light emitted from the fluorescent lamps are between 400-700 nm which is very suitable for microalgal growth as it is closer to the spectrum of daylight (Carvalho et al., 2011). According to the study of Blair et al., (2014), white (clear) light corresponds to the highest growth rate of *Chlorella vulgaris* culture. Red (650 nm) and green (510 nm) light are not absorbed by algae. Moreover, growth rates closer to the white light are obtained with blue light (475 nm).

The light regime is an important factor for algal growth (Merchuk et al., 1998). In nature, microalgae are limited with sunlight during day time. However, different photoperiods can be applied with artificial illumination. According to the studies in which various light/dark cycles were tried, continuous illumination was stated as the best one for microalgal growth (He et al., 2015; Jacob-Lopes et al., 2009). Although flashing light method was considered as a replacement for continuous illumination

(Park and Lee, 2001), Sforza et al., (2014) reported drastic decrease in the growth rate when illuminated with flashing light compared to continuous illumination.

Oxygen Removal and Carbon Dioxide Supply

Oxygen is produced as a by-product of photosynthesis in the reactors by consuming CO_2 using light energy. As PBRs are designed to produce high amounts of algal biomass, photosynthesis and oxygen production rates are also high. This situation causes accumulation of oxygen. Dissolved oxygen can supersaturate up to 400-500% even under good mixing conditions. High levels of dissolved oxygen are inhibitory for microalgal cells. In closed reactors such as tubular PBRs, accumulated oxygen should be removed by degasser (Suh and Lee, 2003; Wang et al., 2012).

CO_2, used as carbon source for photosynthesis, can be the limiting factor because of insufficient mixing (low mass transfer rate) and low CO_2 supplies to the culture. Diffusion of CO_2 from air into the water can only supply 0.039% CO_2. On the other hand, high dissolved CO_2 concentrations may lead to low pH which is inhibitory for algal growth. Hence, CO_2 level in the reactor should be kept at optimum values. Adaptation to the higher CO_2 values should be done slowly. CO_2 enrichment and oxygen stripping methods can be used for sustaining a balance between dissolved oxygen and CO_2 (Suh and Lee, 2003; Wang et al., 2012).

Mixing

Mixing is crucial and an important factor to keep microalgae in suspension so as to enhance utilization of light, gas exchange, nutrient distribution and to avoid thermal stratification. Mixing rate should be kept at the optimum. Insufficient mixing leads to settling of microalgal biomass, on the other hand; when mixing is too rigorous, hydrodynamic stress caused from bubble break-up or mechanical agitation may occur (Suh and Lee, 2003). Insufficient mixing can also cause dead zones, cell aggregation

and formation of multiphase systems (Siddiqui et al., 2015). Aeration, pumping or mechanical agitation or combination of these can be used for mixing depending on the chosen cultivation system and scale. Mixing system should be chosen with respect to algal species present in the culture as tolerance to different mixing systems can vary from one algal specie to another. Moreover, horizontal or vertical baffles can be used in the PBR for increasing mixing (Siddiqui et al., 2015).

Temperature Control

Temperature of the culture is directly related with growth rate since photosynthesis and respiration are fundamentally enzyme-based reactions (Suh and Lee, 2003). Outdoor PBRs are facing diurnal and seasonal variations (Wang et al., 2012) while indoor PBRs can be overheated due to the light source that has been used. Unsuitable temperature values may result in lethality of microalgal culture (Siddiqui et al., 2015). Whereas, at the optimum temperatures, cultures are more resistant to photoinhibition (Larsdotter, 2006). Therefore, temperature control is a vital matter. Evaporative cooling with spraying water on the PBR surface, controlling the temperature of the feed, placing the illumination unit in water pool or selecting heat-tolerant construction material can be the choices for temperature control (Siddiqui et al., 2015).

pH Control

pH control can be done at PBRs by CO_2 or chemical dosing. Chemical dosing is not practical especially in large scale systems due to economic reasons while CO_2 can also be used as a carbon source by the system. CO_2 can be provided from flue gasses which makes it more advantageous. If the flue gas has no inhibitory content for microalgal culture, it will be an economical control and mitigation method (Pawlowski et al., 2014).

2.2.2.2. Types of Photobioreactors

A variety of different PBRs has been designed with different reactor geometry to reduce the maintenance costs and energy utilization, illuminate the content of the reactor efficiently and uniformly, minimize fouling effect, provide optimum rates of mass transfer for gas exchange while not disrupting cell structures (Benemann, 2009; Singh and Sharma, 2012; Wang et al., 2012).

Vertical Column Photobioreactors

Vertical column PBRs consist of transparent upright cylindrical tube with air/gas supplied from the bottom (Singh and Sharma, 2012). The radii of the column can be up to 0.2 meters so as to increase surface-volume ratio and the height should be up to 4 meters not to limit gas transfer ratio and effect endurance of the construction material of the column. Higher column length may cause oxygen inhibition and CO_2 gradient throughout column (Wang et al., 2012). Supersaturation of the oxygen in the PBR due to high photosynthetic activity inhibits microalgae to grow. On the other hand, CO_2 gradient occurs when insufficient CO_2 is supplied to microalgae. Good overall mixing with less damage to the microalgal cells, high mass transfer of CO_2 and low residence time of oxygen are provided by air/gas supply. Vertical column PBRs consume less energy while they are sterilized easily. Moreover, photoinhibition and photooxidation problems are minimized. On the other hand, illumination surface area may decrease upon scaling-up (Ugwu et al., 2008). Depending on the liquid flow mode, bubble column and airlift PBRs are the two main types of vertical column PBRs (Singh and Sharma, 2012) (Figure 2-5). Bubble column reactors are simple, low cost tubular vessels that their height should be at least twice as their diameter. Airlift reactors are modified from bubble column reactors (Singh and Sharma, 2012). They provide higher mass transfer ratio and better mixing with same amount of aeration than bubble column reactors with the help of internal and external loop or vertical column in the reactor (Monkonsit et al., 2011). However, small illumination area is a problem for airlift reactors (Znad et al., 2012).

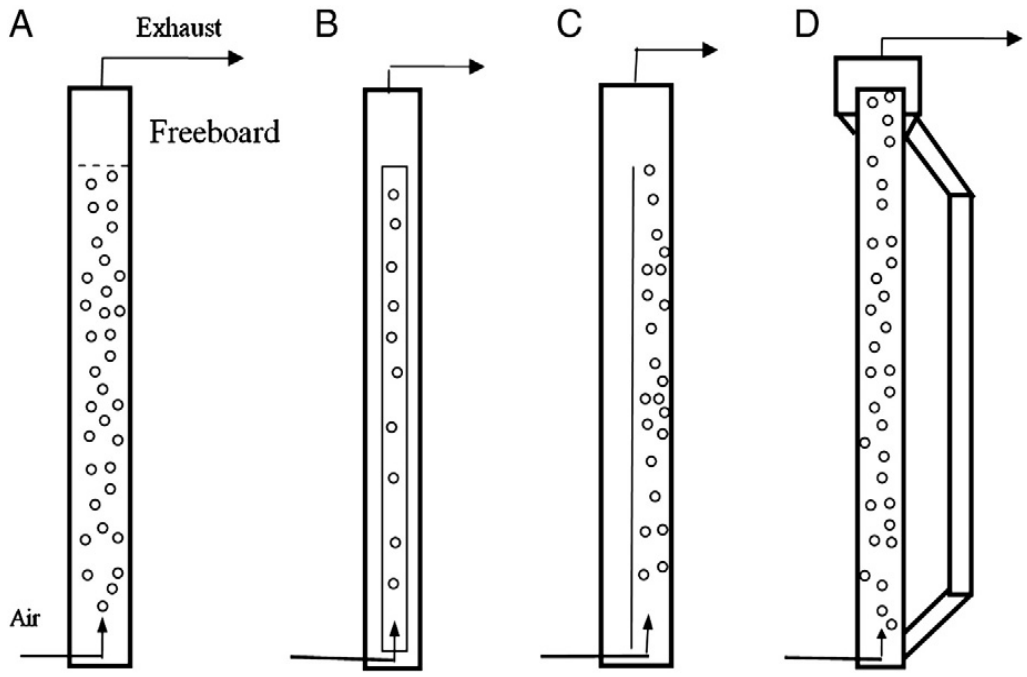

Figure 2-5 Schematic representation of different kinds of vertical column reactors: A. Bubble column PBR, B. Internal-loop airlift PBR, C. Split column airlift PBR, D. External-loop airlift PBR (Wang et al., 2012)

Tubular Photobioreactors

Tubular PBRs are one of the most commonly used type of PBRs (Wang et al., 2012). As is evident from its name, tubular PBRs are consist of thin transparent conduits of which diameter is 0.1 m or less (Figure 2-6). The tubular PBRs can be oriented horizontally, vertically or inclined (Wang et al., 2012). They are well suited for outdoor culturing of algae, thanks to its large illumination area. Airlift or air pump systems are used for aeration and mixing (Ugwu et al., 2008). Major problems related to tubular PBRs are low mass transfer, poor mixing, high pressure, concentration gradients, oxygen accumulation and temperature control (Moser, 1992). Moreover, large land areas are required for implementation. However, good biomass productivities are achievable with tubular PBRs (Brennan and Owende, 2010).

Figure 2-6 An example picture for a horizontal tubular PBR (Iersel et al., 2009)

Flat Plate Photobioreactors

Flat plate PBRs are transparent cuboidal tanks with the possible minimum light path (Figure 2-7). Air or gas mixture is supplied from the bottom with perforated tubes for mixing and aeration (Singh and Sharma, 2012). The most important characteristic of flat plate PBRs is their large surface area for illumination (Wang et al., 2012). The maximum utilization of illumination is the main purpose of flat plate PBR design. As high photosynthetic efficiencies can be accomplished, flat plate PBRs are convenient for mass cultivation of algae (Ugwu et al., 2008). The most important advantages of flat plate PBRs are ease of sterilization, low oxygen accumulation, high biomass productivities, low cost and being readily tempered. On the other hand, possibility of wall growth and hydrodynamic stress, lack of temperature control and difficulty in scaling-up are the limitations of using flat plane reactors (Brennan and Owende, 2010; Ugwu et al., 2008).

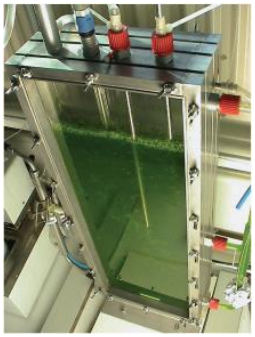

Figure 2-7 An example picture of flate plate PBR (Iersel et al., 2009)

2.3. Applications of Microalgal Biomass

2.3.1. Microalgal Wastewater Treatment

Microalgal wastewater treatment is an eco-friendly and cost effective way of treating nutrients in wastewater compared to other methods (Kligerman and Bouwer, 2015). Nitrogen in the wastewater can be removed by physico-chemical techniques (ammonia stripping, ion exchange and breakpoint chlorination) and biological techniques (nitrification and denitrification). Phosphorus in the wastewater;on the other hand, can be removed by physical methods (sedimentation, floatation and filtration), chemical methods (precipitation), and biological methods (uptake by phosphate accumulating organisms (PAOs)) (Tchobanoglous et al., 2003). Most of these processes are energy intensive. However, microalgal systems can work on low energy while reducing sludge production and greenhouse gas emissions (Sirin and Sillanpaa, 2015).

Microalgae based treatment methods are efficient to remove BOD, pathogens, toxic metals and nutrients compared to other biological treatment methods. These systems can be used in terms of secondary treatment or tertiary treatment (Kligerman and Bouwer, 2015; Sirin and Sillanpaa, 2015). It has been reported that algal systems can treat municipal wastewater (Boonchai et al., 2012; Kim et al., 2010; Renuka et al., 2013; Sirin and Sillanpaa, 2015; Sriram and Seenivasan, 2012; Wang et al., 2010), textile azo dye (Acuner and Dilek, 2004; Lim et al., 2010), rubber wastewater (Bich

et al., 1999), dairy farm wastewater (Wang et al., 2010; Woertz et al., 2009), paper and pulp industry wastewater (Tarlan et al., 2002), livestock wastewater (Park et al., 2010), and settled piggery wastewater (Travieso et al., 2006). Some of the batch reactor studies peformed with microalgae are summarized in Table 2-4.

As it can be seen in Table 2-4, variety of growth rates and removal efficiencies were reported. In the study of Aslan and Kapdan (2006), the effect of initial concentration of nutrients on removal performance was investigated. After 10-day of batch operation, 100% TAN (NH_4^+-N + NH_3-N) removal was sustained at initial concentrations of 13.2 - 21.2 mg/L. Moreover, effective PO_4-P removal was observed at PO_4-P concentrations lower than 7.7 mg/L. Results indicated that removal performance decreases with increasing nutrient concentrations. The reason of low nutrient removal performances at higher concentrations was explained with light limitation due to excess biomass concentration. It was also concluded that *Chlorella vulgaris* culture can remove nitrogen better than phosphorus.

Table 2-4 Summary of microalgal batch reactor studies

Microalgae Species	**Wastewater (ww) type**	**Growth rate (d)**	**N removal (%)**	**P removal (%)**	**N influent (mg/L)**	**P influent (mg/L)**	**Ref[a]**
C. vulgaris	Secondary effluent	0.103	TN:61.7	TP:78.52	TN:28.7	TP:0.149	1
C. vulgaris	Agroindustrial ww	-	TAN:30-95	PO_4:20-55	TAN:3-36	PO_4:112	2
C.vulgaris	Municipal ww	0.2-0.374	TAN:98 TN:91-94	TP:90-92	TAN:50 TN:55	TP:3	3
C. vulgaris	Dairy ww	-	TAN:96	PO_4:>99	TAN:16-31	PO_4:2-3	4
C. vulgaris	Artificial ww	-	TAN:90	PO_4:94	TAN:18	PO_4:4	5
C. vulgaris	Artificial ww	0.377	TAN:74.3	PO_4:70.2	TAN:32.5	PO_4:2.5	6
	Urban ww	0.186	TAN:60.1	PO_4:80.3	TAN:34-48	-	
Scenedesmus obliquus	Artificial ww	0.401	TAN:100	PO_4:60	TAN:32.5	PO_4:2.5	
	Urban ww	0.285	TAN:100	PO_4:83.3	TAN:34-48	-	
C. vulgaris	Synthetic ww	-	TAN:99	PO_4:0	TAN:3	PO_4:12	7
Microalgal Polyculture [b]	Urban ww with high TAN	0.143	TAN:100	PO_4:94-100	TAN: 250	PO_4:8	8
	with low TAN	0.086		PO_4:100	TAN:80	PO_4:23-36	
C.reinhardtii	Synthetic ww	-	TAN:42-83	PO_4:13-14	TAN:129	PO_4:120	9
Chlorella sp.	Permeate of aerobic membrane bioreactor (MBR)	0.059	NO_3:49	PO_4:92	TAN: 0.78 NO_3:70 NO_2:18	PO_4:16	10
C.vulgaris		0.072	NO_3:55	PO_4:82			
S. quadricauda		0.067	NO_3:43	PO_4:71			
S.dimorphus		0.083	NO_3:51	PO_4:75			
C. vulgaris	Artificial medium	-	TAN:23-100	PO_4:46-94	TAN:13-410	PO_4:5-8	11

[a] Ref: 1. Boonchai et al. (2012); 2. Gonzfilez (1997); 3.Li et al. (2013); 4.Woertz et al. (2010); 5. Feng et al. (2011); 6. Ruiz-Marin et al. (2010); 7. de-Bashan et al. (2002); 8.Molinuevo-Salces et al. (2016); 9. Kong et al. (2010); 10. Singh and Thomas (2012); 11.Aslan and Kapdan (2006).

[b] *C. reinhardtii, Scenedesmus obliquus, C. vulgaris (Chlorella vulgaris)*

Singh and Thomas (2012) evaluated the nutrient treatment performance of different microalgal species (*Chlorella sp., Chlorella vulgaris, Scenedesmus quadricauda and Scenedesmus dimorphus*). Some of the concluded remarks can be summarized as below:

- Nutrients can be removed from wastewater by microalgae.
- Nutrient recovery (type of nutrient and removal rate) changes from one specie to another.
- Highest removal of nutrients achieved by the species can vary with time. Water composition and environmental conditions are indicated as possible reasons for that.
- Due to the reasons above, no specie could be determined as the best performancing one for nutrient removal.
- Nutrient removal increases with HRT; however, it is not favorable in water treatment. Reducing the HRT is essential.
- *Chlorella vulgaris* was selected for further experiments due to its high growth rate within 2-day period, which could reduce HRT of the system.

Molinuevo-Salces et al. (2016) stressed out how removal mechanism of nitrogen was determined by initial TAN load. Study was conducted with urban wastewater with high TAN load (250 mg/L) and low TAN load (80 mg/L). It was stated that ammonia volatilization was observed at higher TAN loads. 17-29% and 6-12% of ammonia volatilization were observed in cases of high and low TAN loads, respectively. Although ammonia is toxic to microalgae on some levels, no ammonia inhibition was stated. Moreover, for high TAN loads less nitrogen uptake efficiencies were reported with respect to low TAN loads.

Chlorella vulgaris is among the fastest growing microalgae (Kim et al., 2010) and its treatment ability has been studied in several research papers. In a study, inorganic nitrogen and phophorus removal efficiencies of acclimated *Chlorella vulgaris* from primary settled wastewater was found as 86% and 70%, respectively (Lau et al., 1996). In another study, when *Chlorella vulgaris* was cultivated in municipal

wastewater treatment plant effluent in batch PBR, half of the nitrogen concentration of the wastewater (7.7±0.19 mg/L TAN) was removed in 48 hours after a 24-hour lag phase (Kim et al., 2010). In batch mode, after 48 hours of treatment, free cells of *Chlorella vulgaris* was able to remove 60.1% TAN and 80.3% PO_4-P of urban wastewater, and 74.3% TAN and 70.2% PO_4-P of artifical wastewater (Ruiz-Marin et al., 2010). In a study of Wang et al., (2010), *Chlorella vulgaris* was cultivated semi-continuously to treat digested and undigested dairy manures. Removal rates of TAN, TN, TP and sCOD for undigested dairy manure were determined as 99.7%, 89.5%, 92.0%, and 75.5%, respectively under 5-day HRT. Nevertheless, 100% TAN, 93.6% of TN, 89.2% of TP, and 55.4% of sCOD removal efficiencies for digested dairy manure were achieved at 20-day HRT. As a result of operation in batch, semi-continuous and continuous modes, it was reported that 79.8-90% sCOD, 83.4-88.4% BOD, 90.9-93.6% N and 89.9-91.8% P removal efficiencies were achieved by *Chlorella vulgaris* from municipal wastewater (Li et al., 2013). Yun et al. (1997) studied ammonia removal from steel-making plant wastewater with *Chlorella vulgaris* culture. As wastewater had no phosphorus content, phosphate salts were added to the system. 100% of ammonia and almost half of the nitrate removal were achieved by *Chlorella vulgaris*. Some important continuous reactor studies with microalgal cultures were summarized in Table 2-6.

Table 2-5 Summary of microalgal continuous reactor studies

Microalgae Species	**Wastewater (ww) type**	**HRT (d)**	**N removal (%)**	**P removal (%)**	**N influent (mg/L)**	**P influent (mg/L)**	**Ref[a]**
Chlorella vulgaris	Primary effluent	2	TN:30	TP:53	TN:38.76	TP:3.17	1
	Secondary effluent		TN:44	TP:84.2	TN:24	TP:0.68	
Chlorella vulgaris	Undigested dairy manure	5	TAN:99.7 TN:89.5	TP:92	TN:52-85 TAN:48-68	TP:8-13	2
	Digested dairy manure	20	TAN:100 TN:93.6	TP:89.2	TAN:80-90 TN:80-100	TP:5.5-6.5	
Chlorella vulgaris	Municipal ww	2-4	TAN:98.4 TN:93.6	TP:91.8	TAN:50 TN:55	TP:3	3
Chlorella vulgaris	Municipal ww	2-4	TAN:84-100	PO_4:93-99	TAN:39	PO_4:2.1	4
Chlorococcales	SAMBR[d] effluent	2	TAN: 67.2	PO_4:97.8	TAN:45-80	PO_4:5-10	5
Scenedesmus obliquus (immobilized)	Artificial ww	1.46 (35 h)	TAN:30-97	PO_4:30-85	TAN:32.5	PO_4:2.5	6
	Urban ww		TAN:10-90	PO_4:18-64	TAN:34-48		
Chlorella vulgaris (immobilized)	Synthetic ww	2	TAN:67-100	PO_4:14-83	TAN:3.4	PO_4:15	7
Microalgal polyculture[b]	Urban ww	8	TAN:99	PO_4:82	TAN: 300	PO_4:30	8
Chlorella vulgaris	Synthetic ww	1.7-5.5	TAN:34-93	-	TAN:10-20	-	9
Microalgal polyculture[c]	Piggery ww	2	TAN:7.54-60.37	TP:2.81-28.16	TN:53	TP:7.1	10

[a]Ref: 1. Boonchai et al. (2012); 2. Wang et al. (2010); 3.Li et al. (2013); 4.Woertz et al. (2010); 5.Ruiz-Martinez et al. (2012); 6. Ruiz-Marin et al. (2010); 7. de-Bashan et al. (2002); 8.Molinuevo-Salces et al. (2016); 9. Kapdan and Aslan (2008); 10. Abou-Shanab et al. (2013)

[b] *Chlamydomonas reinhardtii, S. obliquus, Chlorella vulgaris (C. Vulgaris)*

[c] *Ourococcus multisporus, Nitzschia cf. pusilla, Chlamydomonas mexicana, Scenedesmus obliquus (S. Obliquus), Chlorella vulgaris (C. Vulgaris), Micractinium reisseri*

[d] *SAMBR : Submerged anaerobic membrane bioreactor*

The study of Ruiz-Martinez et al. (2012) cultivated *Chlorococcales* (microalgal polyculture) in submerged anaerobic membrane bioreactor effluent for 42 days in semi-continuous mode. High nutrient removals were achieved. At the beginning of the operation, 96.65% Cyonobacteria, 3.35% Chlorophycease and no diatoms were detected at the polyculture. However, at the 40th day operation, cell count of culture showed that 72.13%, 27.72%, and 0.15% of the microalgae were Chlorophycase, Cyanobacteria and diatoms, respectively. To conclude, this study showed that strain selection happened naturally between the species in polyculture and evolved with varying conditions.

In the study of Wang et al. (2010), C*hlorella vulgaris* culture was used to treat diluted undigested and digested dairy manure at semi-continuous reactors. Reactors were oprated at 3.3, 5, 10 and 20 days of HRT to obtain the best removal efficiencies. Wang et al. (2010) mentioned that nutrient removal efficiency depends highly on cellular retention time which is HRT as solar radiation and temperature were kept almost constant at indoor studies. In the meantime, high HRT resulted in more operation costs and it is undesirable. It was stated in the study that loading rate of nitrogen and phosphorus corresponds to lower HRT and higher productivity. However, loading rates beyond certain point can cause microalgal system to collapse via nutrient build-up.

To conclude, Table 2-4 and Table 2-6 show that microalgae, in general, can remove nutrients effectively from various types of wastewaters with different influent concentrations. To improve the efficiency of microalgal systems, further studies reseaching the development of operational conditions, such as decreasing HRT, are needed. In addition, potential use of microalgal systems for different wastewater types remains to be researched.

2.3.2. Carbon Dioxide Sequestration and Mitigation

One of the main causes of the global warming is considered as carbon dioxide emissions (Schneider, 1989). 68% of the total green gas emissions constitutes carbon dioxide (Stewart and Hessami, 2005). From 1850 to 1989, carbon dioxide level in the atmosphere increased 25% due to fuel combustion and deforestation (Schneider, 1989). International energy agency (IEA) projected a growth of primary energy requirement of world by 55% between 2005 to 2030 with an annual rate of 1.8% (IEA, 2007). Therefore, an international movement for carbon dioxide reduction has been set in motion by imposing carbon tax (Yun et al., 1997). There are three major options established for reduction of carbon dioxide emissions related with fossil fuels; improvement of energy production efficiency, reduction of fuel carbon content, and carbon dioxide sequestration (Stewart and Hessami, 2005).

Carbon dioxide sequestration is an important tool for reducing atmospheric carbon dioxide emissions (Olaizola, 2003). There are number of options for carbon dioxide sequestration. (i) Monoethanolamine (MEA) scrubbing process is one of them. This process aims to scrub carbon dioxide from flue gas with a solvent called MEA. This process is generally seen uneconomic for requiring high energy and large equipment. (ii) Membrane technology is another option for Carbon capture and storage (CCS). Membrane works as an assistant to increase the mass transfer area rather than serving as a separator. Amine is used as the separator, however, it can cause blockage on the membrane surface. (iii) A carbon fiber molecular sieve is used to separate carbon dioxide from mixture of gasses based on their molecular weight or size. (iv) By using zeolite as desiccant, carbon dioxide can be removed by desiccant adsorption at normal pressure. Then, carbon dioxide can be regenerated under depressurization by heating the adsorbent. However, reacting of desiccant with the SO_x in the flue gas can be a problem. (v) Direct injection of carbon dioxide into a sink is another option. The sink should be able to store mega-tones of gas for a long period of time. Oceans and geologic reservoirs are being used as a sink. Nevertheless, leakage possibility from the sinks after some time is a problem. (vi) Carbon dioxide pumping into the

oceans to provide carbon source for marine phytoplankton which are the food source of fish species is called ocean fertilization. Ocean fertilization is among biological sequestration of CCS. It aims to reduce greenhouse gas concentration as well as increasing the fish stocks. Long term effects of this method on ocean eco-system is debatable. (vii) CCS by photosynthetic microorganisms is also a solution for CO_2 problem (Stewart and Hessami, 2005).

Microalgal Carbon Dioxide Mitigation

The early atmosphere of the earth consisted similar composition to volcano emissions (CO_2, CO, H_2O, N_2, and H_2) with no oxygen. Cyanobacteria, prokaryotic microalgae, caused carbon dioxide to be reduced from the atmosphere and oxygen to be emitted. Once oxygen became saturated in the ocean (after 300.000 years of the first appearance of cyanobacteria), rest of the oxygen started to accumulate in the atmosphere. Today, oxygen concentration in the atmosphere is 20%, thanks to algal photosynthesis (Holland, 1984). Microalgae can grow 10-50 times faster than terrestrial plants hence they can fix carbon dioxide faster (Wang et al., 2008). Production of 100 tons of algal biomass can fix 183 tons of carbon dioxide (Chisti, 2008). Some of the studies of CO_2 mitigation with microalgae are summarized in Table 2-6.

Keffer and Kleinheinz (2002) reported that *Chlorella vulgaris* culture was very effective for carbon dioxide sequestration. *Chlorella vulgaris* culture, which was exposed to an airstream with over 1850 ppm CO_2, was able to remove 74% of CO_2 of the airstream. 63.9 $g/m^3/h$ CO_2 was removed from the bulk air stream. Keffer and Kleinheinz (2002) stated that properties of CO_2 and the airstream may have limited the bioavailability of CO_2 in PBR. As no dissolved CO_2 was detected, pH in the PBR was maintained at 9 (no conversion to carbonic acid), all free CO_2 in the medium was assimilated by biological activity. More efficient air-to-water distribution system was recommended to make CO_2 more bioavailable for removal. In addition to CO_2 removal, 11% volatile organic carbon (VOC) removal was observed, when 2330 ppm

of VOC was added to the airstream. It has been stated that removal of the VOC from the system could not be surely attributed to *Chlorella vulgaris*, some abiotic factors or photooxidation could be the reason for this removal. However, no evidence was observed that VOC in the system hindered the growth of *Chlorella vulgaris* and the removal of CO_2.

In the study of Yun et al. (1997), 15% CO_2-enriched air was supplied to the *Chlorella vulgaris* culture, which was acclimated to 5% CO_2-enriched air, to treat steel-making industry wastewater. Carbon dioxide fixation rate was determined as 26 $g/m^3/h$ CO_2. pH was not controlled; however, 2 g/L HEPES buffer was used. Nevertheless, the growth in the unbuffered raw wastewater was better than buffered one. Yun et al. (1997) indicated the possibility of algal cultivation without buffering or pH control.

1% CO_2-enriched air was supplied to the *Chlorella vulgaris* culture at presence and absence of membrane in the PBR (Cheng et al., 2006). CO_2 fixation was measured as 80 mg/l/h in ordinary PBR while 260 mg/l/h CO_2 was fixed with membrane-PBR. Cheng et al. (2006) stressed the importance of the inlet CO_2 concentration. Low inlet CO_2 concentration would compensate the carbon need of microalgae while high one would cause great loss of CO_2 to air. Moreover, dissolved oxygen (DO) accumulation was a problem for the system which was overcome when the membrane module was integrated. DO decrease and increase in O_2 outlet were observed due to the increase in gas exchange efficiency. However, it was also stated that fouling and pressure resistance problems of membrane modules should be resolved for extended periods of operation.

Table 2-6 Summary of some microalgal CO_2 mitigation studies

Microalgae species	**Wastewater type**	**Growth rate (1/d)**	**Airflow rate (vvm)**	**CO_2 of feeding gas**	**CO2 fixation rate ($g/m^3/d$)**	**CO_2 removal efficiency (%)**	**REF[a]**
Chlorella vulgaris	Double-strength mineral medium	-	0.5	1850 ppm	64	74	1
Chlorella vulgaris	Steel-making plant ww	0.46	2	15%	26	-	2
Chlorella vulgaris	Medium	0.95	0.1-0.7	2-10%	48-95	-	3
Chlorella vulgaris	Double-strength mineral medium	-	0.3	1%	80 (bubble column)-260 (membrane)	70	4
Chlorella sp.	Modified f/2 medium [b]	0.58-0.66	0.25	2-15%	-	16-58	5
Spirulina sp.	Modified Zarrouk medium [c]	0.33-0.44,	0.3	0, 6, 12%	-	27.14-37.9, 6.7-17.06	6
Scenedesmus obliquus		0.14-0.22				7.4-13.45, 4.39-8.63	
Spirulina sp.	Modified Zarrouk medium[c]	0.11	0.3	12% CO_2	270	-	7
		0.09		12%CO_2+100 ppm NO+60 ppm SO_2	130		
Scenedesmus obliquus		0.15		12% CO_2	220	-	
		0.04		12%CO_2+100 ppm NO+60 ppm SO_2	110		
Spirulina sp.	Modified Zarrouk medium [c]	0.026	0.3	Flue gas (102 g/L CO_2)	-	24	8
Scenedesmus obliquus		0.013				13	

[a] 1. Keffer and Kleinheinz (2002), 2. Yun et al. (1997), 3. Anjos et al. (2013), 4. Cheng et al. (2006), 5.Chiu et al. (2008), 6.de Morais and Costa (2007), 7. Morais et al. (2011), 8.Costa et al. (2015),

[b] Synthetic medium derived from f/2 medium by Guillard and Ryther (1962),

[c] Zarrouk medium (Zarrouk, 1966).

Tolerance of *Chlorella sp*. KR-1 to the high CO_2 concentrations was investigated by Sung et al. (1999). 10%, 30%, 50% and 70% CO_2-enriched air was supplied to the culture. Maximum growth rate was found in the presence of 10% CO_2-enriched air. Although high growth rates were also observed in the presence of 30% and 50% CO_2-enriched air, little growth was observed in the presence of 70% CO_2-enriched air. 70% CO_2-enriched air is a very high and toxic CO_2 concentration for most of the microalgae. Therefore, it was concluded that *Chlorella sp.* is a highly tolerant microalgae specie to CO_2.

In the study of Chiu et al. (2008), effect of CO_2 concentration and initial cell density on microalgal growth were investigated. Air, 2%, 5%, 10%, and 15% CO_2- enriched air were introduced to high density and low density *Chlorella sp.* in batch PBR. 10% and 15% CO_2-enriched air inhibited both of the cultures. To overcome inhibition, pre-adapting microalgal culture to lower CO_2 concentrations was suggested. 2% CO_2-enriched air was the best aeration option among all cases. Air was the second best option for low density culture; on the other hand, 5% CO_2-enriched air was better than air for high density culture. Although, growth rates of low density and high density cultures were almost the same when aerated with air, growth rate of high density cultures was 2.5 times of low density culture when aerated with 5% CO_2-enriched air. The explanation of this situation was stated as the requirement of higher carbon source for high density culture. It was also claimed that CO_2 tolerance of microalgal culture depended on cell density.

In the study of Morais and Costa (2007), 0%, 6% and 12% CO_2-enriched air were supplied to the *Scenedesmus obliquus* and *Spirulina sp.* in serial tubular PBRs. *Spirulina sp.* showed better growth results than *Scenedesmus obliquus*. The highest CO_2 fixation rate, biomass productivity rate and growth rate were observed in the presence of 6% CO_2-enriched air. In another study of the same research group (Morais et al., 2011), synthetic flue gas was used to aerate *Scenedesmus obliquus* and *Spirulina sp.* cultures in serial tubular PBRs. 12% CO_2, 100 ppm NO and 60 ppm SO_2 was the content of synthetic combustion gas. 12% CO_2-enriched air or synthetic

flue gas were introduced to both microalgal culture to evaluate the difference. It was reported that no decrease in cell density was observed, which showed microalgal cultures were SO_2 and NO resistant. However, maximum biomass productivity, growth rate and CO_2 biofixation results were better for both culture when aerated with only 12% CO_2-enriched air. These results support the idea of Olaizola et al. (2004) stating that microalgal-based CCS had some advantages over other CCS methods. Neither CO_2 needs to be separated from flue gas, nor high purity CO_2 is required for algae. Other major flue gas components, nitrogen oxides (NO_x) and sulphur oxides (SO_x), can also be used by microalgae as nutrient like a flue gas scrubber (Olaizola et al., 2004).

Microalgal CO_2 mitigation is a promising subject to overcome high CO_2 emissions. However, related studies are limited. To analyze the mechanisms and understand the practical, further studies are needed.

2.3.3. Downstream Processing of Microalgal Biomass for Biofuel Production

Sustainability is the key factor at today's technology. Even though wastewater treatment can be well achieved by microalgal systems as well as conventional systems, microalgal systems provide a more environment-friendly and sustainable way to do it by biofuel production from microalgae (Wang et al., 2010). Before biofuel production, harvesting might be needed. Therefore, microalgal wastewater treatment should not be considered without downstream processes.

2.3.3.1. Harvesting of Microalgal Biomass

Besides treating the wastewater feature, one of the most important advantage of algal system is to use algal biomass as an energy source by turning it into bioethanol, biodiesel, biomethane and biohydrogen by downstream processing of algal biomass. However, to obtain the biofuels; first, algal biomass has to be harvested. Unfortunately, harvesting of algal biomass is considered as a bottleneck in the algal system. It constitutes 20-30% of the total production cost of algal biomass, as, unlike

bacterial systems, there is no suitable and economical harvesting method for every algal specie, in general (Mata et al., 2010). One or combinations of chemical, biological and physical methods should be chosen according to the features of algal specie present in the system such as density and size of the algae and the end-product desired to be obtained from it (Chen et al., 2011). Even though filtration and centrifugation along with the sedimentation are the most common methods, combining these methods with flocculation or coagulation improves the efficiency (Mata et al., 2010). Generally, microalgae harvesting is a two-step process including bulk harvesting and thickening. Bulk harvesting method intends to seclude algal biomass from bulk suspension using processes such as flocculation, floatation and gravity sedimentation. 2-7% total solid matter can be reached by this method depending on the applied processes and the former biomass concentration. The second step, thickening, consumes more energy than bulk harvesting step due to the nature of the thickening processes such as centrifugation and filtration (Brennan and Owende, 2010).

2.3.3.2. Biofuel Production

Biodiesel

Biodiesel is basically methyl esters produced by transesterification of triglyceride molecules which is also known as parent oil in the presence of methanol (Figure 2-8) (Chisti, 2007). The by-product, glycerol, is removed from biodiesel by phase separation (Harun et al., 2010).

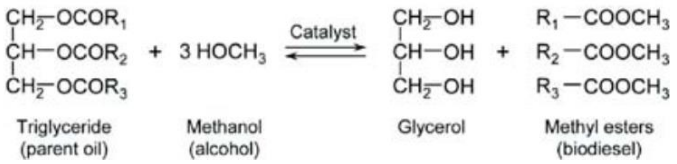

Figure 2-8 Production of biodiesel by transesterification. R_{1-3} groups are referred to hydrocarbons (Chisti, 2007)

Biodiesel is a successful replacement for petro-diesel as it is a renewable, biodegradable and non-toxic energy source which is free of pollutant such as sulfur and aromatics (Demirbas, 2011). Current sources of commercial biodiesel production are soybeans, canola oil, animal fat, palm oil, corn oil, waste cooking oil and jathropha oil (Chisti, 2007). However, food versus fuel conflict affects the usage of vegetable based raw materials for biodiesel production. Occupying fertile lands for biofuel production instead of obtaining food can disrupt food supply chain in global levels (Demirbas, 2011). On the other hand, microalgae need far less land to cultivate, have doubling times as short as 3.5 hours during exponential growth phase and have high oil content with respect to vegetable oils (Chisti, 2007). Being able to grow at arid lands, having higher oil yield, less land requirement for meeting 50% of all transport fuel needs of U.S. make microalgae an advantageous resource for biodiesel

Table 2-7 Comparison of biodiesel sources (Chisti, 2007)

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

[a] For meeting 50% of all transport fuel needs of the United States.

[b] 70% oil (by wt) in biomass.

[c] 30% oil (by wt) in biomass.

Bioethanol

Generally, bioethanol is produced from fermentation or gasification processes. Basically fermentation process occurs fermenting carbohydrates into ethanol by bacteria, yeast or fungi under anaerobic conditions (Equation 2-3). Even though most common feedstock for bioethanol production are sugar and corn, their high food value and requirement for large land area for their cultivation are important disadvantages for them. Nevertheless, microalgae provide a carbon source for the fermentation and creates no food versus fuel conflict while requiring less land. However, only little research has been conducted on bioethanol production from microalgae (Harun et al., 2010).

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \qquad \text{Equation (2-3)}$$

Biomethane

Biomethane from microalgae can be produced with anaerobic digestion. Anaerobic digestion is transformation of organic matter into methane, CO_2 and other trace gasses such as hydrogen and hydrogen sulfide (Brennan and Owende, 2010). Organic matters with high moisture content (80-90%) are well suitable for anaerobic digestion as so microalgal biomass (Mckendry, 2002). No lignin and low cellulose of microalgae provides a stable process and high digestion efficiencies for anaerobic digestion. Moreover, residual biomass from anaerobic digestion can be further used as fertilizer after a conversion process which is cost-efficient and sustainable (Harun et al., 2010).

The first ones to use microalgal biomass as a feedstock for anaerobic digestion was Golueke et al. (1957). They found out that digestibility of algal biomass is relatively lower than wastewater sludge due to resistance of cell walls of microalgae to bacterial degradation. In addition, ammonia toxicity is a big problem due to high protein content, namely, low C/N ratio of microalgal biomass. Co-digestion of microalgal biomass with high carbon content organic wastes such as wastewater sludge or waste

paper solve the problem by adjusting C/N ratio around 20-25:1 (Abdel-Raouf et al., 2012).

Biohydrogen

Hydrogen is a non-polluting, harmless energy source. Hydrogen, which canbe produced biologically; is called biohydrogen. It can be produced biologically from microalgae, too. Biohydrogen from microalgae can be produced by 2 ways; (1) direct photobiolysis, (2) indirect bio-photolysis. In direct photobiolysis, water molecules are split into hydrogen and oxygen molecules in the presence of hydrogenase enzyme with sunlight and photosynthetic reactions by microalgae. However, this is a very fragile and short-lived process due to accumulation of oxygen in the system causing oxygen inhibition of hydrogenase enzyme (Benemann, 2000). On the other hand, indirect biophotolysis was proposed to overcome the oxygen inhibition problem of direct biophotolysis. Indirect photolysis is a two-stage process in which oxygen and hydrogen exist at different stages (Rashid et al., 2013). Coupling with wastewater treatment and CO_2 fixation by recycling the excess CO_2 emitted from the system, hydrogen can be gained as a valuable by-product. Feasible bio-hydrogen production from microalgae shows no promise due to practical and commercial strains (Benemann, 2000; 2009).

CHAPTER 3

MATERIALS AND METHODS

This thesis study consists of three stages which are investigation of; (i) cultivation of *Chlorella vulgaris* culture, (ii) nutrient removal from municipal wastewater, (iii) nutrient removal from industrial wastewater and thickener supernatant, and carbon dioxide mitigation. The inoculum, synthetic media, wastewaters, PBRs, analytical methods and experimental procedures that are used in the thesis study are explained in this chapter.

3.1. Inoculum

Axenic culture of green microalgae *Chlorella vulgaris* (Sams Research Services Ltd, CCAP No: 211/11B) was purchased from Culture Collection of Algae and Protozoa (CCAP), England (Figure 3-1). The culture was cultivated in Bold's Basal Medium with 3-Fold Nitrogen and Vitamins (3N BBM + V) as it is recommended by CCAP. The details about the cultivation medium are explained in Section 3.2.

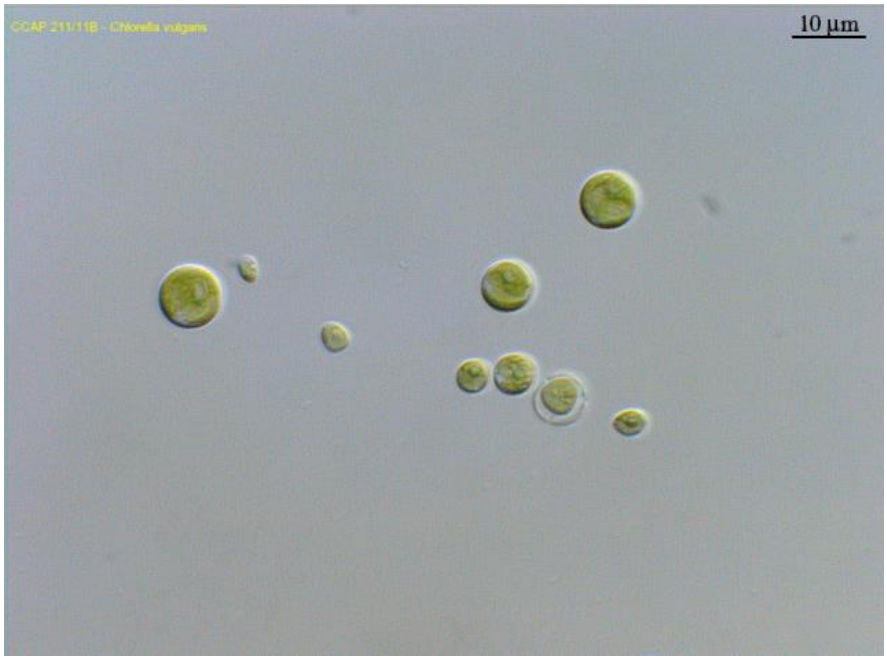

Figure 3-1 *Chlorella vulgaris* (CCAP, 2013)

3.2. Synthetic Media

In order to enrich the purchased algal culture, Bold's Basal Medium with 3-Fold Nitrogen and Vitamins (3N BBM + V) was used for cultivation (Andersen, 2005). The content of the medium is provided in Table 3-1.

Table 3-1 Constituents of 3N BBM + V (Andersen 2005)

Constituents	Concentration (mg/L)	Constituents	Concentration (mg/L)
$NaNO_3$	0.75	$FeCl_3.6H_2O$	5.84×10^{-4}
$CaCl_2.2H_2O$	0.025	$MnCl_2.4H_2O$	2.46×10^{-4}
$MgSO_4.7H_2O$	0.075	$ZnCl_2$	3×10^{-5}
$K_2HPO_4.3H_2O$	0.075	$CoCl_2.6H_2O$	1.2×10^{-5}
KH_2PO_4	0.175	$Na_2MoO_4.2H_2O$	2.4×10^{-5}
NaCl	0.025	Vitamin B1	1.2×10^{-3}
Na_2EDTA	4.5×10^{-3}	Vitamin B12	1×10^{-5}

3.3. Wastewaters

Three different types of wastewater were used to operate PBRs in this thesis study. None of the wastewaters were autoclaved before usage. The general information about these wastewaters are given below.

3.3.1. Municipal Wastewater

The municipal wastewater used in this study was obtained from primary sedimentation tank effluent of Greater Municipality of Ankara Tatlar Municipal Wastewater Treatment Plant, located in Ankara, Turkey. 0.3 mm pore size sieve was used to screen the obtained wastewater to remove larger particles. Wastewater was stored in 0˚C at dark. Characteristics of the municipal wastewater are provided in Table 3-2.

Table 3-2 Characteristics of municipal wastewater

Parameters[a]	Value	Parameters[a]	Value
TS (mg/L)	413 ± 17	TN (mg/L)	42.1 ± 2.1
VS (mg/L)	269 ± 17	TKN (mg/L)	42 ± 5.9
%VS in TS	65	Organic-N (mg/L)	11.5
Chlorophyll-a (mg/L)	0	TAN (mg/L)	30.5 ± 1.2
pH	7.95	NO_3-N (mg/L)	< 0.1
tCOD (mg/L)	254 ± 2.5	NO_2-N (mg/L)	< 0.01
sCOD (mg/L)	78.5 ± 0.3	PO_4-P (mg/L)	4.9 ± 0.3

[a] TS: Total solids, VS: Volatile solids, tCOD: Total chemical oxygen demand, sCOD: soluble chemical oxygen demand, TN: Total nitrogen, TKN: Total kjeldahl nitrogen, TAN: Total ammonium nitrogen, NO_3-N: Nitrate nitrogen, NO_2-N: Nitrite nitrogen, PO_4-P: Ortho-phosphate.

3.3.2. Industrial Wastewater

Industrial wastewater was obtained from KARDEMİR A.Ş. Karabük Steel and Iron Facilities, Coke Plant Main Channel. 0.3 mm pore size sieve was used to screen the obtained wastewater to remove larger particles. Wastewater was stored in 0˚C at dark. Characteristics of the coke wastewater are provided in Table 3-3.

Table 3-3 Characteristics of coke wastewater

Parameters[a]	Values	Parameters[a]	Values
TS (mg/L)	8471 ± 311	Organic-N (mg/L)	244
VS (mg/L)	136 ± 4	PO_4-P (mg/L)	1 ± 0.1
%VS in TS	2	Sulfate (mg/L)[b]	1509
Chlorophyll-a (mg/L)	0	Cyanide (µg/L)[b]	12.5
tCOD (mg/L)	11827 ± 150	Arsenic (µg/L)[b]	767.2
sCOD (mg/L)	10225 ± 61	Mercury (µg/L)[b]	3.27
TN (mg/L)	3600 ± 90	Iron (mg/L)[b]	9.26
TAN (mg/L)	3352 ± 78	Cadmium (µg/L)[b]	17
NO_3-N (mg/L)	4 ± 0.2	Total Chromium (µg/L)[b]	7.8
NO_2-N (mg/L)	< 0.01	Phenol (mg/L)[b]	950

[a] TS: Total solids, VS: Volatile solids, tCOD: Total chemical oxygen demand, sCOD: soluble chemical oxygen demand, TN: Total nitrogen, TAN: Total ammonium nitrogen, NO_3-N: Nitrate nitrogen, NO_2-N: Nitrite nitrogen, PO_4-P: Ortho-phosphate.

[b] Indicated parameters were measured by an accredited laboratory (Encon Çevre Danışmanlık Ltd. Şti.).

3.3.3. Primary Sludge Thickener Supernatant (Thickener Supernatant)

Thickener supernatant is a problematic wastewater originated from sludge thickeners in conventional wastewater treatment plants. Generally, thickener supernatants are directed to secondary treatment (aeration tank) for treatment. However, due to its high pollution load, it creates a huge burden on the system (Wang, et al., 2010). Thickener supernatant was used for phosphorus source and dilution of high strength coke wastewater in this study. This way, it was also aimed to treat two problematic wastewaters together.

Thickener supernatant was obtained from wastewater line of primary sludge thickener from Greater Municipality of Ankara Tatlar Municipal Wastewater Treatment Plant. 0.3 mm pore size sieve was used to screen the obtained wastewater to remove larger particles. Wastewater was stored in 0˚C at dark. Characteristics of the thickener supernatant are provided in Table 3-4.

Table 3-4 Characteristics of the thickener supernatant

Parameters[a]	Values	Parameters[a]	Values
TS (mg/L)	880 ± 42	TN (mg/L)	47.2 ± 1.5
VS (mg/L)	488 ± 20	TAN (mg/L)	41.7 ± 2.1
%VS in TS	55	NO_3-N (mg/L)	< 0.1
Chlorophyll-a (mg/L)	0	NO_2-N (mg/L)	< 0.01
tCOD (mg/L)	587 ± 3.6	Organic-N (mg/L)	5.48
sCOD (mg/L)	328 ± 6.2	PO_4-P (mg/L)	19.9 ± 0.2

[a]TS: Total solids, VS: Volatile solids, tCOD: Total chemical oxygen demand, sCOD: soluble chemical oxygen demand, TN: Total nitrogen, TAN: Total ammonium nitrogen, NO_3-N: Nitrate nitrogen, NO_2-N: Nitrite nitrogen, PO_4-P: Ortho-phosphate.

3.4. Photobioreactors (PBRs)

Two different bubble column PBRs were used for the experiments. The PBRs used for cultivation of microalgal biomass had 3 L volume, 9 cm diameter and 40 cm height (Figure 3-2). PBRs used in treatment studies, on the other hand, had 1 L volume, 8 cm diameter and 24 cm height (Figure 3-3).

Figure 3-2 PBRs for cultivation of microalgal biomass

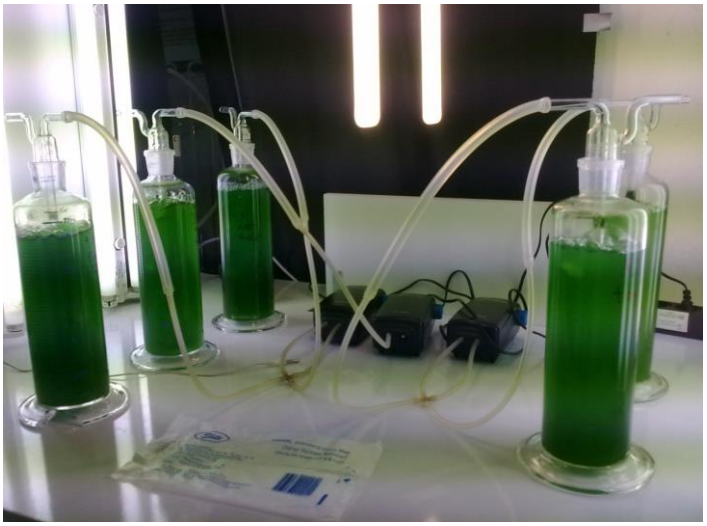

Figure 3-3 PBRs used in wastewater treatment studies

3.5. Analytical Methods

During the experimental studies, density, pH, temperature, photosynthetically active radiation (PAR), total solids (TS) and volatile solids (VS), total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD), total nitrogen (TN), total ammonia nitrogen (TAN), ortho-phosphate (PO_4-P), nitrate-nitrogen (NO_3-N),

nitrite-nitrogen (NO_2-N), chlorophyll-a were measured. For the analyses of sCOD, total soluble nitrogen, TAN, PO_4-P, NO_3-N and NO_2-N, soluble portion of the samples were required. Therefore, before analyses, samples were filtered through 0.45 μm cellulose-acetate filter (Sartorius Stedim, 1110647-N) by using filtration unit (Millipore, WP8 11 2250).

pH: pH meter (Eutech, CyberScan, pH510) and pH probe (Sensorex, p350) were used to measure pH value.

Temperature: Temperature values of the reactors were measured with 9263 A Plus digital thermometer.

Optical Density: HACH spectrophotometer DR 2800 with 1-cm light path was used to measure optical density values at optimum wavelength determined for cultured *Chlorella vulgaris* culture. To determine this optimum wavelength, optical density values were read at different wavelengths and the highest absorbance value was obtained at 685 nm. Detection limit is between 0.1 and 1, so for samples with optical densities higher than 1, dilution is necessary.

Photosynthetically Active Radiation (PAR): Li-Cor LI-250A light meter was used for the PAR measurements.

Total Solids (TS) and Volatile Solids (VS): Total solids and volatile solids measurements were done according to the Standard Methods 2540 B and 2540 E, respectively (APHA, 1998).

Total and Soluble Chemical Oxygen Demand (tCOD and sCOD): tCOD and sCOD analyses were done with E.P.A. approved micro-COD method. Medium-range (0-1500 mg/L) and low-range (0-150 mg/L) test kit vials (Catalog No: 2 42 07 20/2 42 07 21, Lovibond GmbH, Aqualytic, Germany) were used for measurements.

Thermoreactor RD 125 was used to heat vial up to 150˚C. Cooled vials were measured with Multidirect photometer (Lovibond, Aqualytic, Germany).

Total Nitrogen (TN): Low-range test kit vials (Catalog No: 535560, Lovibond GmbH, Aqualytic, Germany) were used for the measurement of TN.

Total Ammonia Nitrogen (TAN): pH of the samples was adjusted around 7 before analysis to be able to measure $NH_4^+ + NH_3$ together as TAN. Test kit vials (Catalog No: 53600, Lovibond GmbH, Aqualytic, Germany) were used for the measurement.

Nitrate-Nitrogen (NO_3-N): Nitrate test kit vials (Catalog No: 535580, Lovibond GmbH, Aqualytic, Germany) were used to measure NO_3-N.

Nitrite- Nitrogen (NO_2-N): Nitrite test vials (Catalog No: 512310, Lovibond GmbH, Aqualytic, Germany) were used to measure NO_2-N.

Ortho-Phosphate ($O.PO_4^{-3}$-P): Low range phosphorus tablet packs (Catalog No: 515810, Lovibond GmbH, Aqualytic, Germany) were used for measuring $O.PO_4^{-3}$-P.

Chlorophyll-a and Pheophitine-a: Chlorophyll-a and Pheophitine-a measurements were done according to the Standard Methods 10200H (APHA, 1998). Optical density ratio of $664_b/665_a$ (OD ($664_b/665_a$)) gives insight about health of microalgal culture. Ratio of 1.7 represents the healthiest situation while 1.0 represents death of culture. When chlorophyll-a content of the culture is higher, the ratio would be closer to 1.7; however, when pheophitine-a concentration is high, the ratio would be closer to 1. Pheophitine-a is the chlorophyll-a molecule that lost its Mg^{+2} ion and cannot function in photosynthesis reactions anymore.

Microscopic Analysis and Cell Counting: Microbial analyses were conducted using Automated Inverted Microscope for Life Science Research (Leica, DMI4000 B).

Utermöhl method was used for cell counting (Paxinos and Mitchell, 2000). Samples from experiment sets in cultivation studies (Section 4.1.1 and Section 4.1.2), semi-continuous municipal wastewater study (Section 4.2.2), and semi-continuous industrial wastewater study (Section 4.3.2) were examined (Appendix-A). Samples were taken at 12^{nd} day of batch cultivation, at 110^{th} day of semi-continuous cultivation, at 35^{th} day of semi-continuous municipal wastewater and at 52^{nd} day of semi-continuous industrial study. Only *Chlorella vulgaris* could be identified in the samples of cultivation studies. This was an anticipated result as inoculum used for both studies were axenic cultures and reactors were run under hygienic conditions and autoclaved equipment were used. Organisms (diatoms, *Scenedesmus obliquus*, oocyst) detected at wastewater studies were less than 0.01% of Chlorella *vulgaris*. Hence, *Chlorella vulgaris* dominates the culture as nutrient-rich environments are selective for *Chlorella* (Brennan and Owende, 2010).

Gas Chromatograph (GC): Gas composition measurements were done by gas chromatograph (GC) Agilent 6890N equipped with a thermal conductivity detector and capillary column CP-Sil 8 (CP8752, Varian) to detect CO_2 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. Calibration curve for CO_2 measurements was presented in Appendix-B.

3.6. Experimental Procedure

3.6.1. Cultivation of *Chlorella Vulgaris*

This part of the study focuses on cultivation of the axenic *Chlorella vulgaris* culture to augment the biomass content first in batch and then in semi-continuous PBRs for further experiments. Batch PBRs were run to determine the characteristics and growth phases, while semi-continuous PBRs were run to observe and analyze the steady-state growth conditions of the *Chlorella vulgaris* culture.

3.6.1.1. Cultivation of Stock *Chlorella Vulgaris* Culture in Batch PBR

Axenic culture of green microalgae *Chlorella vulgaris*, which was purchased from CCAP (Section 3.1.), was transferred to a sealed 250 mL Erlenmeyer flask and cultivated in 3N BBM +V which was mentioned in Section 3.2 to prepare stock culture for *Chlorella vulgaris*. Aeration may cause disruption of some microalgal cells and this may hinder the growth of culture especially when concentration of the culture is low (Eriksen, et al., 1998). Therefore, no air was supplied to the Erlenmeyer flask until green color of *Chlorella vulgaris* culture started to be observable (Figure 3-4).

After that, seed taken from the flask was transferred to 3N BBM+V in 1-L PBR to amplify and observe the growth phases of *Chlorella vulgaris* culture. Inoculation of *Chlorella vulgaris* seed was handled at sterile environment with the help of open flame. Inoculation process was handled via transferring 100 mL seed from stock *Chlorella vulgaris* culture in Erlenmeyer flask, concentration of which was $4.8x10^9$ cells/L, to 700 mL basal medium in 1-L PBR. The 250 mL Erlenmeyer flask, 1-L PBR, tubes and basal medium, which were sealed with sterile cotton and aluminum foil, were autoclaved at 121^oC and 15 psi for 20 minute before usage in order not to contaminate the culture.

1-L batch PBR was operated for 12 days until the growth reached the stationary phase. The reactor was illuminated with 2 cool-white 18 W fluorescent lamps (OSRAM, L 18W/685) at 200 $\mu mol.m^{-2}.s^{-1}$ (Degen et al., 2001) for 16 hours per day (Li et al., 2013a; Woertz et al., 2009). Adaptation to artificial illumination with day-to-night cycle was stated to be beneficial before switching to continuous illumination (Lee and Lee, 2001). 0.5 L/min (0.625 vvm) air was supplied from air pump (RESUN Air Pump AC-9602) to the reactor only at the light cycle periods (Anjos et al., 2013; Ruiz et al., 2013). At the end of air inlet and outlet pipes, 0.2 μm filters (Hidrofobic Minisart Syringe Filter) were used to avoid contamination (Figure 3-4). Optical density and VS concentrations were measured each day.

Figure 3-4 The experimental set of cultivating stock *Chlorella Vulgaris* (The reactor on the left is the reactor of axenic *Chlorella vulgaris* culture and the Erlenmeyer flask on the left of the picture is the stock *Chlorella vulgaris* culture).

3.6.1.2. Cultivation of *Chlorella vulgaris* Culture in Semi-Continuous PBRs

Chlorella vulgaris culture was grown in two parallel 3-L semi-continuous PBRs (R1 and R2), which were mentioned in Section 3.4, at steady-state to use the reactor outputs as seed for further experimental studies. Steady-state condition was defined to be achieved when the change in optical density values was less than 10% in three consecutive days.

Both PBRs were operated at 10-day HRT. Autoclaved 3N BBM+V (Section 3.2) was used as feed. Reactors were operated in 16:8 hours' day-to-night cycle until the 100th day of operation. Photoperiods are beneficial for microalgae culture to adapt artificial illumination (Lee and Lee, 2001). After the 100th day, reactors were illuminated and aerated continuously to observe whether culture would adapt the continuous illumination and could preserve its steady-state conditions. Light was provided to the reactors with eight cool-white 18 W fluorescent lamps (OSRAM, L 18W/685).

Provided PAR was 200 μmol.m^{-2}.s^{-1}. Air flowrate of 1.07 L/min was supplied to the reactors with air pump (RESUN Air Pump AC-9602). The ends of air inlet and outlet pipes were sealed with 0.2 μm filters (Hidrofobic Minisart Syringe Filter) to prevent contamination.

Both reactors were operated for 110 days (corresponding to 11 HRTs). During this period, optical density, pH, dissolved oxygen and temperature parameters were monitored daily. During the first 40 days, it was aimed to operate the PBRs with minimum intervention as much as possible; thus, no pH and temperature control was made. After 40 days (4 HRTs), pH of the reactors was started to be adjusted to pH of 7.5 with 1 N H_2SO_4.

NO_3 and PO_4-P measurements were done before and after the feeding procedure at 4^{th}, 8^{th}, 10^{th}, 60^{th}, 61^{st}, 62^{nd}, and 63^{rd} days of operation to observe the nutrient removal capability of the system. No ammonium measurements were done as 3N BBM+V contains only nitrate for nitrogen source.

3.6.2. Treatment of Municipal Wastewater via Microalgal Culture

The main objective of this part of the study is the nutrient removal from municipal wastewater via *Chlorella vulgaris* culture.

3.6.2.1. Selection of the Cultivation Reactor for Inoculum

The purpose of this study is to determine the inoculum amount and the cultivation reactor (that is, either R1 or R2 (Section 3.6.1.2)) from which the inoculum would be taken for semi-continuous PBRs that would be run for treatment of municipal wastewater.

It should be noted that acclimation behavior of each cultivation reactor (R1, R2) to the municipal wastewater may differ as they may have reached different steady-state

conditions at the end of 3-month operation time. Regarding that, 4 batch PBRs with different dilution ratios and seed type from different cultivation reactors (R1 and R2, Section 3.6.1.2) were operated (Table 3-5). Each PBR was inoculated with either 50 mL or 100 mL microalgal seed taken from one of the two cultivation reactors (R1 and R2). All PBRs were later filled up to 1 L with municipal wastewater. Municipal wastewater used was the effluent of primary sedimentation tanks as mentioned in Section 3.3.1. As seen in Table 3-5, two inoculation ratios (1/10 and 1/20) were studied. Inoculation ratio of 1/10 may cause self-shading effect and slow down the nutrient removal and biomass growth rate; therefore, 1/20 inoculation ratio was also studied (Luo and Al-Dahhan, 2004). Self-shading effect is that high concentration of algae prevents light to reach other algae and causes a decrease in photosynthesis rate.

Initial pH values of the four reactors, namely, B1-50, B1-100, B2-50, B2-100, were set to 6±0.05 (Powell et al., 2009). Batch reactors were operated for 4 days with continuous light and air supply. As mentioned in Section 3.6.1.2., *Chlorella vulgaris* culture well-adapted to the continuous illumination and steady-state of the culture was not disrupted. Therefore, it was decided to use continuous illumination for further treatment studies as it was the most efficient among photoperiods (Jacob-Lopes et al., 2009). Light was provided to the reactors with eight cool-white 18 W fluorescent lamps (OSRAM, L 18W/685). Provided PAR in the reactors was 200 $\mu mol.m^{-2}.s^{-1}$. Air supply was provided with RESUN Air Pump AC-9602 at 1 vvm.

Optical density was measured daily while TAN and PO_4-P were measured bi-daily. Obtained results were analyzed in terms of biomass production rate and nutrient removal rate to determine the cultivation reactor from which inoculum would be taken and used for further treatment experiments.

Table 3-5 Experimental design of cultivation reactor performance comparison

Reactor Name	The amount of inoculum taken from cultivation reactors (mL)		The amount of municipal wastewater added (mL)
	R1	R2	
B1-50	50	-	950
B1-100	100	-	900
B2-50	-	50	950
B2-100	-	100	900

3.6.2.2. Nutrient Removal from Municipal Wastewater in Semi-Continuous PBRs

The aim of this study was to investigate the nutrient (N and P) removal from municipal wastewater with microalgal culture. Moreover, it was also aimed to determine the optimum HRT(s) that nutrient removal and biomass growth can be achieved at steady-state conditions. At this part of the study, three 1-L PBRs were operated at three different HRTs (2, 4, 8 days) (Table 3-6). These HRTs were selected considering similar studies using *Chlorella vulgaris* and close nutrient content to our study (Boonchai et al., 2012; C. Li et al., 2013; Wang, et al., 2010; Woertz et al., 2009). Steady-state condition was defined to be achieved when the change in optical density values was less than 10% in three consecutive days.

Table 3-6 Nomenclature of semi-continuous reactors fed with municipal wastewater

PBR Names	HRT (days)		
	2	4	8
X1	+		
X2		+	
X3			+

As mentioned previously in Section 3.6.2.1., batch PBRs (B1-50, B1-100, B2-50, B2-100) were operated to select the inoculum amount and cultivation reactor to be used in this study. The results of these batch experiments (discussed further in detail in Section 4.2.1.) indicated that the reactors B1-50 and B1-100 inoculated with R1 cultivation seed resulted in higher nutrient removal rate and biomass production compared to those of B2-50 and B2-100 inoculated with R2 cultivation seed. Thus, R1 cultivation reactor was determined as inoculation reactor in this study and further experiments.

As X1, X2 and X3 reactors should be identical at 0^{th} day of operation, content of reactors (4-L of total) was prepared together. Optical density close to 1 was aimed for the start-up conditions of reactors in order to sustain a sufficient algal culture in wastewater. However, to obtain an optical density of mixture closed to 1, around 450 mL of algal culture should be inoculated from R1 reactor. Due to the high nutrient content of cultivation medium (Section 3.2.), this amount of inoculation would have caused high initial background concentrations of NO_3 and PO_4-P at the start-up. To avoid that situation and still use acclimated culture, the leftover content of the B1-50 and B1-100 reactors was also used as seed source. Seed from B1-50 and B1-100 was withdrawn after the 4^{th} day of reactors operation in order to minimize the background nutrient concentration.

As seen in Table 3-7, required volumes of municipal wastewater and seed from R1 reactor were mixed with leftovers of B1-50 and B1-100 reactors according to the theoretical calculations made with respect to the measured optical densities. Only 85 mL of culture from R1 cultivation reactor was used. The effect of initial nutrient concentration in 85 mL was negligible considering 4 L of total volume of 3 reactors' mixture. Final optical density of 4-L of mixture was measured as 0.946, which is close to optical density 1 as predicted. Homogenous mixture was shared to three 1-L PBRs and 1 L remaining mixture was used for preliminary analysis.

Table 3-7 Initial constituents of X reactors

	Optical Density (685 nm)	Volume (mL)
R1 Reactor	8.15	85
(B1-50) + (B1-100)	2.017	1640
Wastewater	0.09	2275
X-final	0.946	4000

X1 and X2 reactors were operated for 35 days while operation of the X3 reactor was ended at the 17th day as its performance was relatively lower than the other reactors. After every feeding protocol, pH was set to 6 with 5N H_2SO_4 in each reactor. Every feeding protocol was done with the same municipal wastewater (Table 3-2, Section 3.3.1). Light was provided to the reactors with eight cool-white 18 W fluorescent lamps (OSRAM, L 18W/685). Provided PAR in the reactors was 200 μmol.m^{-2}.s^{-1}. Air supply was provided with RESUN Air Pump AC-9602 as 1 vvm.

Optical density and pH were measured daily before the feeding protocol while TS, VS, TAN and PO_4-P were measured 3 days a week. After the 17th day of operation, sCOD, TN, chlorophyll-a and pheophitine-a values were also started to be measured. For the X3 reactor, sCOD and TN measurements were not done, chlorophyll-a and pheophitine-a measurements were done at the 17th day.

3.6.2.3. Kinetic Study with Microalgal Culture Acclimated to Municipal Wastewater

In order to determine the growth and nutrient removal rate of algal culture acclimated to municipal wastewater, kinetic studies were conducted with acclimated microalgal culture taken from semi-continuous reactors mentioned in Section 3.6.2.2 (X1 and X2). 900 mL municipal wastewater was inoculated with 100 mL microalgal culture obtained from 21st day outputs of semi-continuous PBRs, X1 and X2 (Table 3-8).

This inoculation ratio (1/10) was determined according to the results of the study mentioned in Section 3.6.2.1. Outputs were taken when PBRs were at steady-state and they were operated long enough to ensure microalgal culture had been acclimated to the municipal wastewater.

pH of the reactors was set to 6.00 ± 0.05 at the start-up of the operation (Powell et al., 2009). Reactors were aerated with RESUN Air Pump AC-9602 as 1 vvm. Light was provided to the reactors continuously with eight cool-white 18 W fluorescent lamps (OSRAM, L 18W/685). Provided PAR in the reactors was 200 $\mu mol.m^{-2}.s^{-1}$. Batch reactors were operated for 72 hours. sCOD and TN were measured every 24 hours while pH, optical density, TS, VS, TAN and PO_4-P were measured at varied time spans.

Table 3-8 Experimental design of the kinetic study with microalgal culture acclimated to the municipal wastewater

Reactor Name	Added Amounts (mL)			
	Inoculation Reactors		Wastewater	Total
	X1	X2		
XB-1	100	-	900	1000
XB-2	-	100	900	1000

3.6.3. Treatment of Industrial Wastewater via Microalgal Culture

The main objective of this part of the study was the nutrient removal from industrial wastewater via microalgal culture.

3.6.3.1. Determination of the Optimum Nitrogen: Phosphorus Ratio of Wastewater

As industrial wastewater, coke wastewater, which was described in Section 3.3.2, was used. Analyses' results showed that coke wastewater is highly polluted (Table 3-3). Even though ammonium is a perfect source of nitrogen for microalgae, it also has inhibitory effects on growth (Azov and Goldman, 1982). Tam and Wong (1996) reported no significant difference in specific growth rates at ammonium concentrations between 20-250 mg/L. Beyond these limits, less growth was observed. Moreover, coke wastewater has low phosphorus content that will limit the growth of algae. Because of these reasons, direct use of the coke wastewater can cause inhibition and it can also be unsuitable due to high N/P ratio. In addition, coke wastewater is high in phenol and heavy metal content which are toxic to microalgal growth even though some microalgae are known as resistant. These problems about coke wastewater were handled by mixing it with another wastewater with high phosphorus content which is thickener supernatant (Section 3.3.3). Problems of inhibition due to high heavy metal, phenol and nitrogen content in addition to insufficient phosphorus content for algal growth were eliminated by this way.

Mixing ratio of the two wastewaters is an important aspect for proper nutrient (both N and P) removal to be achieved. Therefore, the purpose of this part of the study was set to determine the optimum N/P ratio for mixed wastewater so that maximum N and P removal would be achieved in semi-continuous microalgal system.

To determine the optimum N/P (g/g) ratio of mixed wastewater, two wastewaters were mixed in three different volume ratios as their N/P ratios would be 6, 8, and 10 (Table 3-9). These N/P ratios were selected considering the study of Kapdan and Aslan (2008). In that study, N/P ratio of 8 was determined as optimum for *Chlorella vulgaris* culture. Moreover, N/P ratio of the typical microalgal cell is 7 (Kapdan and Aslan, 2008). Hence the possible N/P ratio range in this study were widen with N/P ratios of 6 and 10.

Two wastewaters were then mixed with respect to these three N/P ratios. Then, 900 mL was taken from every mixed wastewater and inoculated with 100 mL microalgal culture obtained from the outputs of X1 and X2 (Section 3.6.2.2) to be operated in 1-L semi-continuous PBRs. Operation of the 1-L PBRs, CB6, namely, CB8 and CB10, was not ended until at least one nutrient was consumed almost completely. Operation of CB6 and CB10 reactors were ended at the 10^{th} day of operation while operation of CB8 reactor was ended at 12^{th} day.

Table 3-9 Mixing ratios of thickener supernatant and coke (industrial) wastewater (ww) with respect to N/P ratios

N/P (g/g)	Total WW (mL) / Coke WW (mL)	Reactor Name
6	50	CB6
8	34	CB8
10	25	CB10

In order to treat coke wastewater, CO_2-enriched air was supplied to the PBRs to be able to remove high nutrient content from wastewater. Therefore, 4% CO_2 – enriched air was supplied to PBRs at 0.5 vvm, which balanced the pH of the reactors without any additional control. Also, it provided appropriate mixing. In some studies, 2% CO_2-enriched air was supplied to *Chlorella vulgaris* culture while treating different type of wastewaters (Li et al., 2013b; Wang et al., 2010). However, nutrient content in these wastewaters was approximately half of the nutrient content measured in this study. In addition, another study which provided 10% CO_2 – enriched air to *Chlorella vulgaris* culture indicates that 4% CO_2 – enriched air has no inhibitory effects on microalgal growth (Anjos et al., 2013). Therefore, 4% CO_2 – enriched air was decided to be used.

4% CO_2 – enriched air was obtained with the help of rotameters as it can be seen in Figure 3-5. For each reactor 2 rotameters were used. One was for the control of ambient air, which was pumped with RESUN Air Pump AC-9602, and the other was for the control of pure CO_2, which was supplied with pressurized pure CO_2 cylinders. After the flows were regulated by rotameters, CO_2 and air pipes were combined with T- tube and CO_2-enriched air was supplied to the PBRs. To confirm that 4% CO_2-enriched air was supplied to the reactors, air samples were collected from the combined pipe once every week into an impermeable medical bag and analyzed by GC.

As mixed wastewaters contained heavy toxic metals and volatile organics (phenols) that can volatilize with neutral pH and above, the experiments with coke wastewater were conducted in a fume hood (Figure 3-5). Four by four placed eight cool-white 18 W fluorescent lamps (OSRAM, L 18W/685, Korea) were used to provide light for PBRs. Each of the lamps was oppositely aligned. Each lamp was parallel and 6 cm away from each other. PBRs were 10 cm away from each other. From one side, the distance between PBRs and the lamps were 5 cm and from the other side it was 35 cm due to extra 3 identical PBRs (Figure 3-5). 120 $\mu mol.m^{-2}.s^{-1}$ PAR was provided to the PBR from this lighting system, continuously. Temperature rise due to heat generated from fluorescent lamps was controlled by ventilation system of the hood. The temperature of the reactors was maintained at 28 ± 2°C.

pH, optical density, TS, VS, TAN and PO_4-P analyses were done daily while chlorophyll-a and pheophitine-a analyses were done in every two days.

Figure 3-5 A photograph of the set up for the determination of optimum N/P ratio of mixed wastewater

3.6.3.2. Determination of the Optimum HRT Leading to Maximum Nutrient Removal from Coke Wastewater

This study was conducted in order to determine the optimum HRT(s) that the highest nutrient removal and biomass growth rate would be achieved and to investigate CO_2 mitigation at steady-state. 1-L PBRs which were mentioned in Section 3.4 were used for this semi-continuous study. Three 1-L semi-continuous PBRs were run with 5, 8 and 12 days of HRT (Table 3-10). Different HRTs ranging from 5 to 13 days were used in various studies treating high strength wastewaters or industrial wastewaters (Li et al., 2013a; McGriff and McKinney, 1972; Tam and Wong, 1989; Wang et al., 2010; Woertz et al., 2010). Therefore, PBRs were decided to be run at 5, 8 and 12 days of HRTs. Steady-state condition was defined to be achieved when the change in optical density values was less than 10% in three consecutive days.

As mentioned in previous section, optimum N/P ratio of the mixed wastewater (thickener supernatant and coke wastewater) was determined so that microalgal culture could remove both of the nutrient (N and P), more efficiently and none of the nutrient would be limiting for the treatment of other. N/P ratio of 6, which means

dilution of coke wastewater with thickener supernatant 50 times, was determined to be the most suitable for microalgal culture. Therefore, mixed wastewater for this study was prepared considering N/P ratio of 6.

Contents of PBRs, namely, C5, C8 and C12, were prepared together for each reactor to start operation with the same content as in Section 3.6.2.2. Initial pH and optical density value of all reactors were 7.63 and 1.006, respectively. The inoculation seed was obtained from reactor CB-6 (mentioned in previous section, Section 3.6.2.1), which was fed with the mixed wastewater with an N/P ratio of 6 and run as batch PBRs. 400 mL of inoculum was added to the 3.6 L mixed wastewater. 1 L of this mixture was used for initial measurements, remainder part was split into three 1 L-PBRs i.e., C5, C8 and C12. At the beginning of each day, 200, 125 and 83.3 mL of the C5, C8 and C12 reactor contents were wasted and replaced with mixed wastewater.

Table 3-10 Nomenclature of semi-continuous PBRs

Reactor Names	HRT (day)		
	5	8	12
C5	+		
C8		+	
C12			+

4% CO_2-enriched air at 0.5 vvm was supplied to the PBRs with the help of rotameters. Air flow from an air pump (RESUN 9602, China) was controlled by one rotameter while CO_2 flow from pressurized pure CO_2 cylinder was controlled by another rotameter, and their outlets were connected with a pipe to each other before supplied to the system. Rotameters of the air pump and pure CO_2 were adjusted to 0.48 L/min and 0.02 L/min, respectively. CO_2-enriched air concentration was measured with GC

in order to be sure if CO_2 was 4%. The system was operated in a fume hood due to safety reasons as industrial wastewater contains hazardous pollutants (Section 3.3.3.).

Four by four placed eight cool-white 18 W fluorescent lamps (OSRAM, L 18W/685, Korea) were used to provide light for PBRs. Each of the lamps was oppositely aligned. Each lamp was parallel and 6 cm away from each other. PBRs were 10 cm away from each other. From one side, the distance between PBRs and the lamps were 5 cm and from the other side it was 35 cm due to extra 3 identical PBRs (Figure 3-6). 120 $\mu mol.m^{-2}.s^{-1}$ PAR was provided to the PBRs from this lighting system, continuously.

Figure 3-6 A photograph of the set-up run for determination of optimum HRT leading to maximum nutrient removal

pH control was achieved with 4% CO_2-enriched air supplied to the reactors. Temperature rise due to heat generated from fluorescent lamps was controlled by ventilation system of the hood. The temperature of the reactors was maintained at 28 ± 2°C.

pH and optical density were measured daily for each reactor. TS and VS, TAN and PO_4-P were measured every other day. sCOD was measured every other day after reactors reached steady-state. For C8 and C12 reactors, TN, chlorophyll-a and pheophitine-a were measured once in every week after steady-state was reached. Outlet CO_2 concentration was measured at 24^{th}, 26^{th} and 28^{th} days of operation for each reactor. After the 31^{st} day of operation, CO_2 concentration was measured once in every three days for C8 and C12 reactors.

When steady-state conditions were achieved in C8 and C12 reactors, possible improvements in CO_2 mitigation was tried. For C8 reactor, pH adjustments were made to increase CO_2 mitigation. pH of C8 reactor was 6 -7, which was decreased to 4-5 to increase CO_2 solubility. pH was adjusted to the values indicated in Table 3-11 with the help of 5 N H_2SO_4 after 43^{rd} day of operation. Moreover, in C12 reactor, 4% CO_2-enriched air supply rate was decreased from 0.5 vvm to 0.2 vvm after the 52^{nd} day of operation to observe whether lower air flow rate would increase the residence time of gas bubbles in the reactor and cause more CO_2 capture by microalgae.

Table 3-11 pH adjustments for C8 reactor at different operation days

Operation days	43	44	45	46	47	48	49	50	51
Adjusted pH	4	4	4	4.5	5	5	5	5	5

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Cultivation of *Chlorella Vulgaris*

Before treatment studies, *Chlorella vulgaris* was, first, cultivated in batch reactors and then steadily produced in semi-continuous reactors.

4.1.1 Cultivation of Stock *Chlorella Vulgaris* Culture in Batch PBR

The purpose of this study was to observe the growth curve of axenic culture *Chlorella vulgaris* while amplifying culture for further studies. Growth curve of *Chlorella vulgaris* culture was shown in Figure 4-1. The changes in growth parameters, i.e., optical density and VS concentration, were parallel to each other as expected. During the first 4 days of the operation, culture stayed at lag phase. After the 4^{th} day and until 10^{th} day of operation, culture grew exponentially. The change in optical density and VS concentration in the first 4 days were only 0.04 absorbance and 7.72 mg/L, respectively. On the other hand, at the exponential growth phase, the increase in optical density and VS concentration were 1.87 absorbance and 687 mg/L, respectively. Biomass production rate (P) in this phase was calculated as 114.4 mg/L/d, while logarithmic growth rate was calculated as 0.71 d^{-1} (Appendix-C). After the 10^{th} day of operation, change in growth parameters almost stopped and stationary phase began. The operation was stopped at 12^{th} day of operation.

In the study of Feng et al. (2011), *Chlorella vulgaris* culture was also cultivated in a batch reactor and growth curve was obtained. Culture reached stationary phase at 7^{th} day of operation. As lag phase only took 1 day, remaining 6 days of operation

indicates exponential growth phase of the culture. Likewise, in this study, exponential growth phase took 6 days of operation. However, culture reached stationary phase at 10th day of operation as lag phase took 4 days of operation. NH_4Cl in the medium of Feng et al. (2011)'s study could be the reason for the shortened lag phase, because *Chlorella vulgaris* prefers NH_4 (TAN) over NO_3 as a primary N source (Cai et al. 2013; Feng et al., 2011). In the study of Feng et al. (2011), TAN was depleted at the end of day 2, and culture tried to adapt to NO_3 as the other nitrogen (N) source, microalgal growth slowed down. In present study, the only N source in the medium was NO_3; therefore, no adaptation needed after the lag phase. Higher growth rate in the present study (0.71 d^{-1}) with respect to that of Feng et al. (2011)'s study (0.46 d^{-1}) supports this explanation.

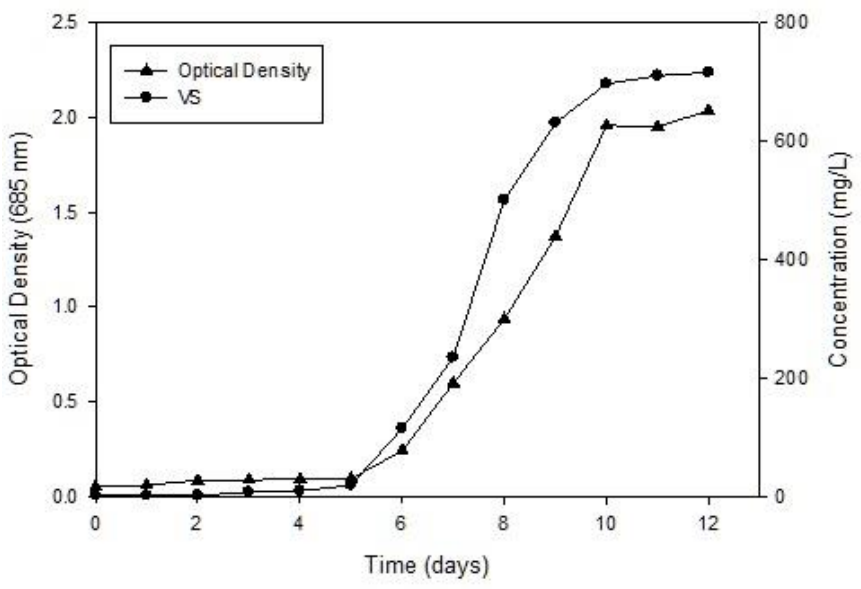

Figure 4-1 The change in optical density and VS concentration in batch cultivation reactor of *Chlorella vulgaris*

4.1.2. Cultivation of *Chlorella Vulgaris* Culture in Semi-Continuous PBRs

Two identical 3-L semi-continuous cultivation PBRs (R1 and R2) were operated at 10 days of HRT for 110 days. The results are given in Figure 4-2, Table 4-1, Figure

4-3, and Table 4-2. The aim of this study was to cultivate a steady-state axenic *Chlorella vulgaris* culture for the following experiments. This study also helped to define the conditions to be prevailed and controlled during the operation of semi-continuous reactors.

For the first 40-day period, no intervention was made to control any of the environmental parameters as it was aimed to operate the PBRs with minimum intervention as much as possible (Figure 4-2 and Figure 4-3). During that period, optical density value of R1 reactor dropped from 4.08 at 8^{th} day of operation to 2.52 at 10^{th} day of operation, and stayed at around this level (2.45±0.25) at steady-state for 18 days. The reason of this sudden drop can be associated with the self-shading effect (Sforza et al., 2014). Optical density of R1 reactor indicates that it was a highly dense culture before 10^{th} day of operation, which might have led to low light penetration in the PBR and created a light-limiting environment.

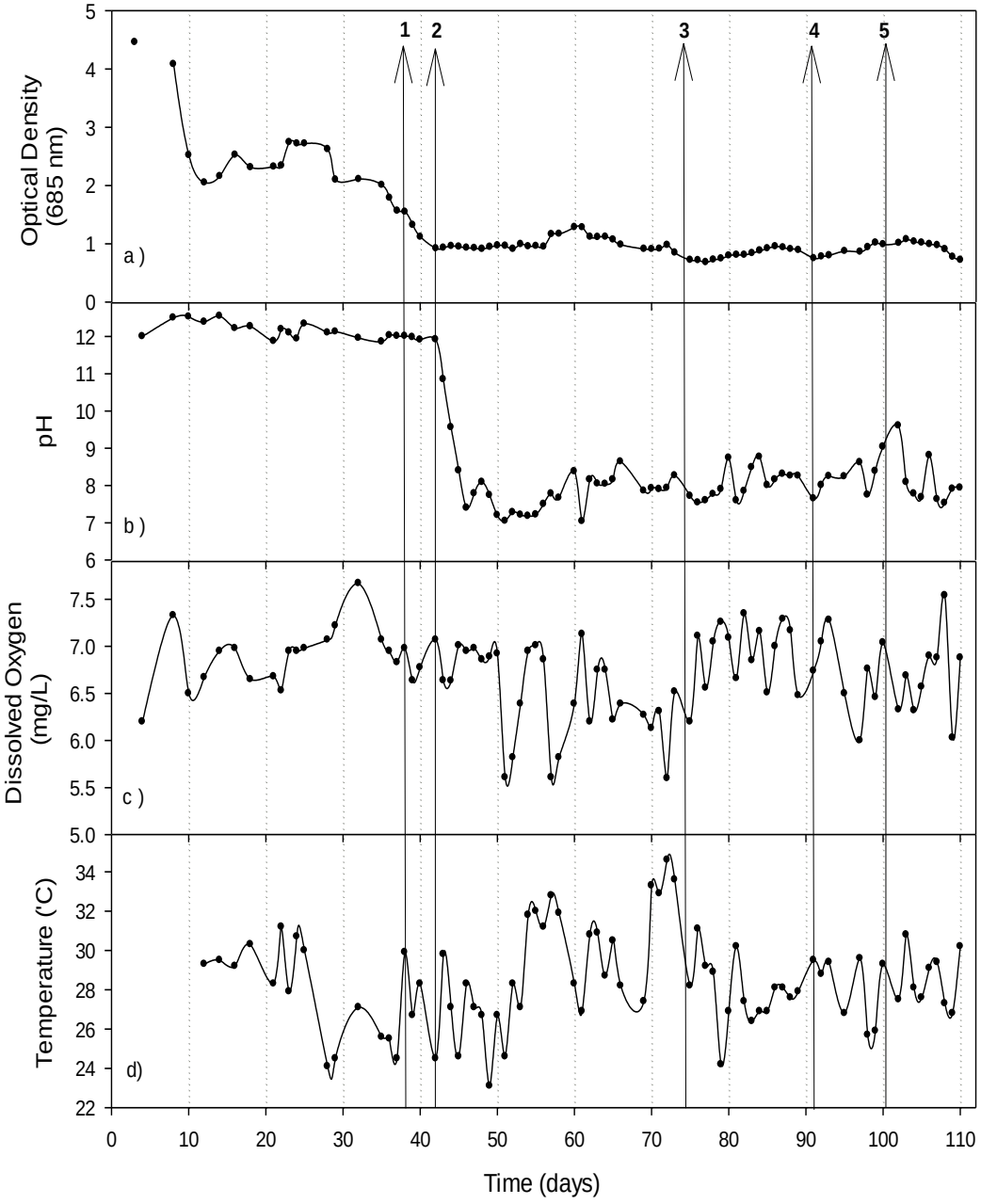

Figure 4-2 The change in physical parameters; a) Optical Density, b) pH, c) Dissolved Oxygen, d) Temperature, in R1 cultivation reactor with respect to time. ([1] wasting procedure stopped, [2] wasting procedure started, [3] temperature control was started, [4] OD decrease due to air pump breakdown, [5] continuous illumination started)

After 40 days of operation, optical density values of R1 started to decrease rapidly. Temperature values, which are present in Table 4-1 (Figure 4-2.d), were between the optimum ranges according to the study of Mayo (1997). Average DO concentrations during that period were below saturation values defined for the corresponding average temperatures (Figure 4-2.c) ("Ambient Water Quality Criteria for Dissolved Oxygen", 2014). In other words, no oxygen supersaturation was observed. Therefore, inhibition of the microalgal growth by oxygen accumulation in the system was not the reason for this decrease in optical density (Chisti, 2007). Moreover, no oxygen accumulation is an indication of good gas transfer rate of the system (Suh and Lee, 2003). After eliminating temperature and DO for the probable causes for optical density decrease, high pH of the culture (12.2± 0.22) was predicted to be the reason (Yeh and Chang, 2012). The optimum pH for *Chlorella vulgaris* is 6.5-7.0 (Wang et al., 2010). *Chlorella vulgaris* culture to survive at pH 12 for 30-day is an unusual situation for microalgal cultivation (Figure 4-2.b). One of the reason for this is that ammonia is present in its free form beyond pH 12 which is toxic for microalgae (Azov and Goldman, 1982). In this study, NH_4Cl was not present in the basal medium; therefore, no inhibitory effect of free ammonia due to high pH was possible. The photoperiod of 16 h day: 8 h night cycle might be the other reason for that. When the photosynthesis dominates at day period, pH increases as CO_2 is captured from the medium; on the other hand, when only respiration occurs at night period, pH decreases as CO_2 released to the medium (Muñoz and Guieysse, 2006). pH variation during each cycle due to day: night periods might have prevented potential irreversible inhibitory effect of high pH. However, this buffering effect was not a sustainable one for microalgal cultivation and high pH might have resulted in decrease in optical density.

Table 4-1 Average values of parameters of R1 reactor at each HRT cycle

HRT No	Time (d)	Optical Density	pH	Dissolved Oxygen	Temperature (˚C)
1	0-10	3.69±1.	12.5±0.0	6.9±0.6	-
2	10-20	2.26±0.	12.4±0.1	6.8±0.2	29.6±0.5
3	20-30	2.51±0.	12.1±0.2	6.9±0.2	28.1±2.9
4	30-40	1.64±0.	12.0±0.1	7.0±0.3	26.8±1.8
5	40-50	0.94±0.	8.80±1.7	6.9±0.2	26.4±2.1
6	50-60	1.04±0.	7.50±0.4	6.3±0.6	29.8±2.8
7	60-70	1.08±0.	8.00±0.4	6.5±0.4	29.6±2.2
8	70-80	0.77±0.	7.90±0.4	6.6±0.5	30.0±3.4
9	80-90	0.88±0.	8.20±0.3	6.9±0.3	27.7±1.1
10	90-100	0.88±0.	8.20±0.5	6.7±0.4	28.1±1.7
11	100-110	0.97±0.	8.10±0.5	6.7±0.5	28.3±1.3

Similar problems were observed in R2, as well (Figure 4-3). First of all, optical density dropped from 3.048 at the 8th day to 1.95 on 10th day of operation due to self-shading effect. For the next 18 days, until 29th day of operation, optical density values were at steady-state as indicated in Table 4-2 and Figure 4-3.a. After 29th day of operation, steady-state was disrupted as also observed in R1. Optical density started to decrease. Optical density values continued to be monitored for both of the reactors until the 38th operation day. Wasting procedure was stopped for 3 days for both of the reactors to avoid the much more decrease in optical density.

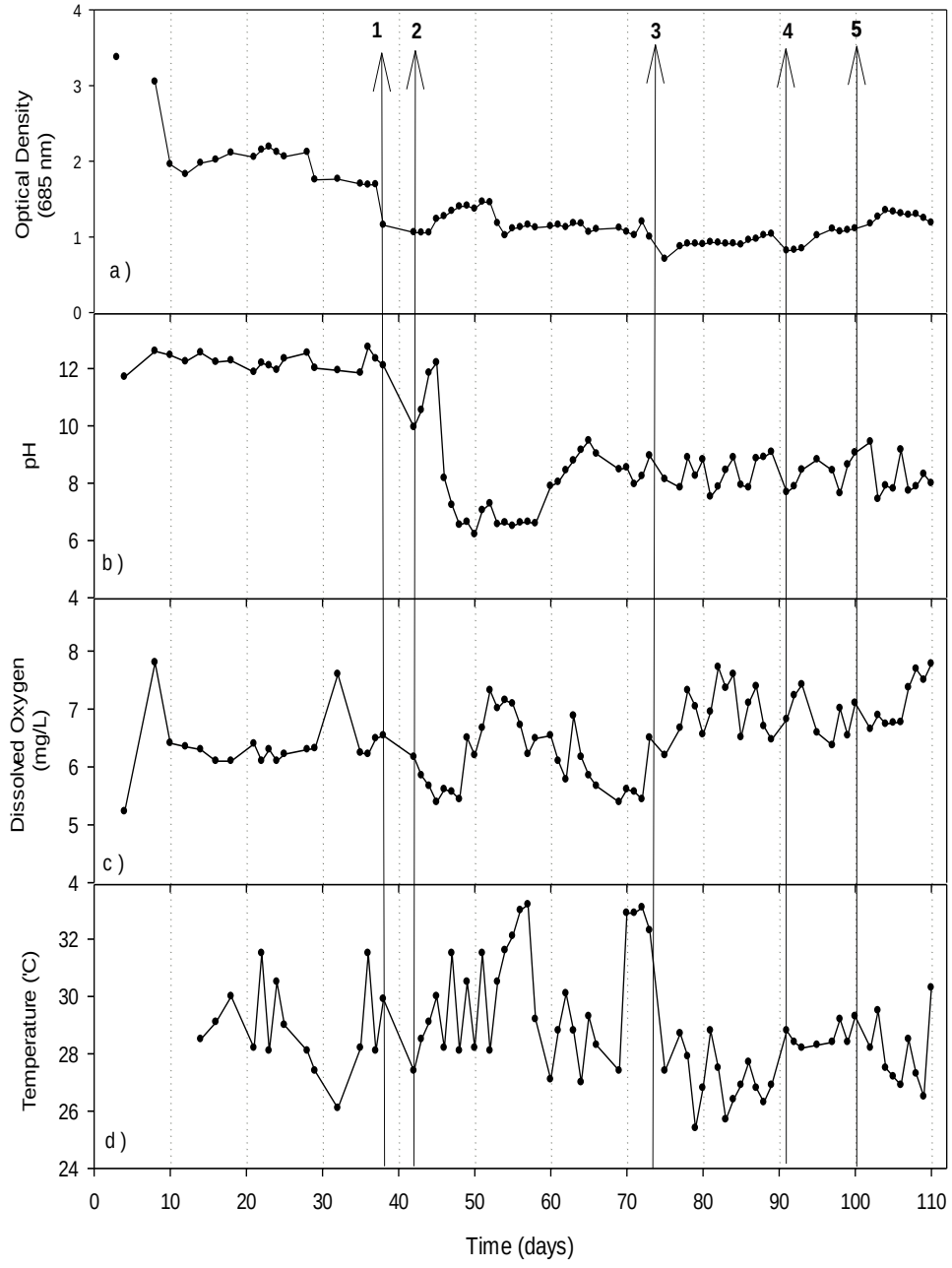

Figure 4-3 The change in physical parameters; a) Optical Density, b) pH, c) Dissolved Oxygen, d) Temperature, in R2 cultivation reactor with respect to time. ([1] wasting procedure stopped, [2] wasting procedure started, [3] temperature control was started, [4] OD decrease due to air pump breakdown, [5] continuous illumination started)

1 L algal culture was taken from R2 reactor and operated alternatively in an identical semi-continuous reactor with 10-day HRT in order to check if pH decrease would solve the decrease in optical density. Thus pH of the alternative 1 L reactor was adjusted to 7.5 after every feeding. Optical density of this reactor increased from 1.16 to 1.38 after the 1st day and 1.56 after the 3rd day of operation. This indicated that system needed pH control. Therefore, pH was adjusted to 7.5 after each feeding protocol for R1 and R2 reactors. After Day 42 (marked as 2 on Figure 4-2 and Figure 4-3), wasting procedure was restarted. R2 was operated with 2 L active volume after this point, no medium addition was made to R2 to avoid dilution. Steady-state was achieved on Day 44 for R1 and Day 43 for R2. As from Day 46, pH values were in the appropriate range (6-9) for microalgal growth according to Mayo (1997).

Due to seasonal temperature increase, temperature of R1 and R2 increased over 30°C (Figure 4-2.d and Figure 4-3.d). Steady-state optical density of R1 and R2 reactors were disrupted on Days 73 and 75, respectively. With the instillations of two ventilators to the reactor apparatus after Day 75 (8th HRT period; marked as 3 Figure 4-2.d and Figure 4-3.d), the temperature of the reactors was kept under 30°C which is the limit of optimum temperature range (Mayo, 1997). After the 79th day of operation steady-state optical density values (0.75 for R1 and 0.91 for R2) were achieved again.

At the 91st day of operation (marked as 4 on Figure 4-2 and Figure 4-3), due to breakdown of the air pump, aeration stopped for 16 hours (one-day cycle). This situation resulted in a decrease in the optical density, however, optical density increased afterwards and steady-state was achieved again in 2 days.

At the last HRT period (between Days 100-110) , instead of 16:8 day: night cycle, the reactors were started to illuminate continuously so as to get a maximum efficiency from the system(marked as 5 on Figure 4-2 and Figure 4-3) (Jacob-Lopes et al., 2009). Optical density values showed that cultures adapted well to the continuous illumination. While optical density values increased slightly, steady-state of the

system was not disrupted. Increase in the optical density, especially in R2, indicated that continuous illumination is favoring microalgal growth.

Table 4-2 Average values of parameters of R2 reactor at each HRT cycle

HRT No	Time (d)	Optical Density	pH	Dissolved Oxygen	Temperature (˚C)
1	0-10	2.79±0.7	12.5±0.1	7.1±1.0	-
2	10-20	1.98±0.1	12.3±0.2	6.2±0.1	29.2±0.8
3	20-30	2.06±0.1	12.1±0.2	6.2±0.1	29.0±1.5
4	30-40	1.60±0.3	12.2±0.4	6.6±0.6	28.8±2.0
5	40-50	1.24±0.2	8.80±2.4	5.8±0.4	29.1±1.3
6	50-60	1.20±0.2	6.90±0.5	6.8±0.4	30.7±2.1
7	60-70	1.12±0.0	8.70±5.0	5.9±0.5	29.1±1.8
8	70-80	0.94±0.1	8.40±0.4	6.4±0.7	29.3±3.0
9	80-90	0.95±0.1	8.40±0.6	7.1±0.5	27.0±0.9
10	90-100	0.99±0.1	8.30±0.5	6.9±0.4	28.6±0.4
11	100-	1.28±0.1	8.20±0.7	6.9±0.4	28.0±1.0

Outcomes of this study are summarized as below;

- pH control of the microalgal culture at the feeding procedure is a requirement.
- Temperature of the reactors should be regulated with ventilation according to seasonal temperature variations.
- Gas transfer rate of the system is appropriate to avoid oxygen accumulation.
- Switching to continuous illumination was better than photoperiods as optical density increased after the switch.
- Steady-state *Chlorella vulgaris* culture was obtained for the following experiments.

Nutrient Removal in Cultivation Reactors

As operation of cultivation reactors did not aim to remove nutrients, no optimization was made to improve the treatment performance of reactors. The reactors were fed with basal medium (Section 3.2) which was developed to provide nutrient-rich, non-limiting environment for microalgal biomass. However, it should be noted that, air is not a sufficient carbon dioxide source for the nutrient removal from a concentrated medium. Therefore, it is impossible for microalgal culture to treat entire nutrient content of the medium with that carbon content in a reactor operating at 10-day HRT.

Nitrogen and phosphorus measurements were done at the first HRT period and 7^{th} HRT period. Even though microalgal biomass concentration was higher at the first HRT period, nutrient uptake rates for both reactors were higher for both of the reactors at 7^{th} HRT period (Table 4-3). This indicates that decrease in optical density did not affect the system efficiency, on the contrary, nutrient uptake rate of the system increased. This might be attributed to self-shading effect at higher biomass concentrations defined with higher optical density values. These results also indicated that seeding with microalgal culture obtained from a steady-state system would work more efficient.

Table 4-3 Nutrient removal of cultivation reactors

Reactor	Operation days	HRT No	Optical Density	NO_3-N Concentration (mg/L)		*N uptake rate (mg N /mg VS.d)	PO_4-P Concentration (mg/L)		*P uptake rate (mg P /mg VS.d)
				Initial	Final		Initial	Final	
R1	4	1	4.46		15.3			26.1	
	8		4.08	58.4	16.1	0.014	40.9	23.0	0.006
	10		2.52	58.8	27.7	0.017	39.0	39.3	0
	60	7	1.29		111.0			68.6	
	61		1.28	170.2	109.5	0.066	77.0	65.9	0.012
	62		1.12	168.9	113.0	0.069	74.6	64.1	0.013
	63		1.12	172.0	108.0	0.079	73.0	64.3	0.011
R2	4	1	3.37		14.7			26.2	
	8		3.05	58.0	14.6	0.015	40.9	25.6	0.0052
	10		1.96	58.0	29.2	0.016	40.6	39.6	0.0006
	60	7	1.14		75.5			58.8	
	61		1.16	138.3	74.0	0.069	68.2	59.1	0.010
	62		1.13	136.9	81.5	0.068	68.5	58.2	0.013
	63		1.18	143.7	85.5	0.072	67.7	58.1	0.012

*Nitrogen and phosphorus uptake rates were calculated by subtracting final concentration from initial concentration and dividing this value to VS concentration of that day. VS concentrations were calculated according to the correlations graph in Appendix-D.

4.2. Treatment of Municipal Wastewater via Microalgal culture

After the cultivation experiments, microalgal culture obtained from semi-continuous cultivation reactors were used to investigate the treatment of municipal wastewater in batch and semi-continuous reactors. The results of these experimental set-ups are discussed in this section.

4.2.1. Selection of the Cultivation Reactor for Inoculum

To determine the cultivation reactor (R1 or R2), that is the seed, to be used in the removal of nutrients from the municipal wastewater was the main objective of this study. Moreover, the effect of different inoculation ratios was also studied. The comparisons among the reactors were made with respect to the biomass growth rate and nutrient removal results.

As mentioned previously, B1-50 and B1-100 reactors were inoculated with the output of R1 cultivation reactor. The analyses results of these reactors are given in Figure 4-4. According to the optical density curves, for the cultures in both reactors, lag phase ended at the second day and logarithmic growth phase started. At the logarithmic growth phase, biomass production rates (P) based on optical density of B1-50 and B1-100 reactors were calculated as 0.49 abs/d, and 0.52 abs/d, respectively. The highest optical density (680 nm) was reported as 2.07 in the study of Li et al. (2013) that also used *Chlorella vulgaris* as microalgae. The highest optical densities (685 nm) reached in B1-50 and B1-100 reactors were measured as 2.42 and 2.86, respectively. Optical density values measured at 680 nm and 685 nm can be comparable since only 0.006 standart deviation and lower than 5% standard error was determined between the measurements with these two different absorbances for *Chlorella vulgaris* culture (Appendix-E). In the study of Li et al. (2013), production rate was reported as 0.49 abs/d when biomass concentration reached the highest concentration. This is same with the production rate of B1-50 reactor. Nevertheless, higher productivity rate was achieved in B1-100 reactor.

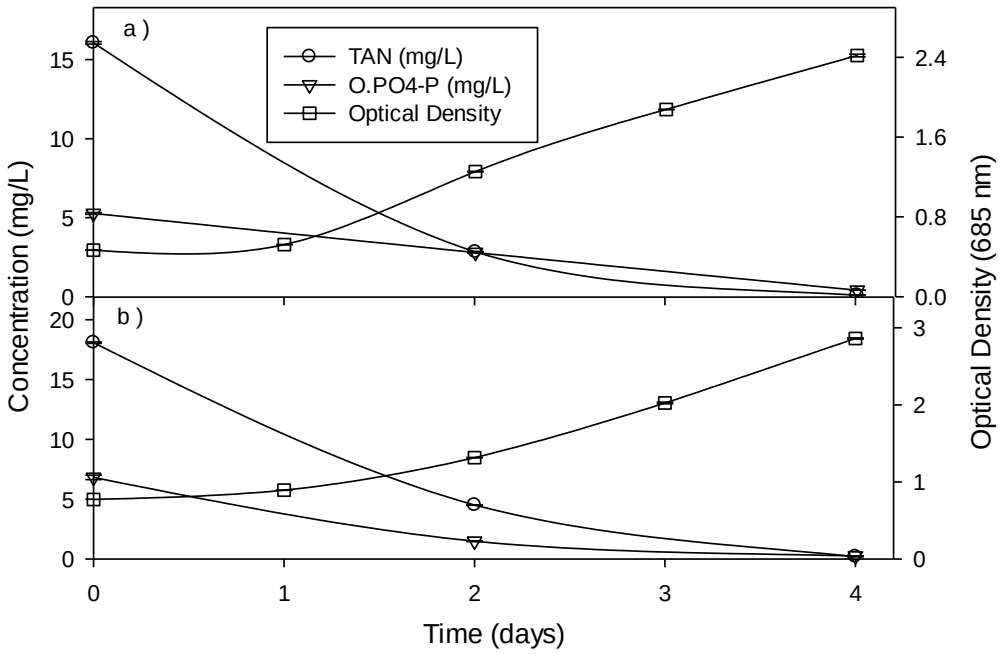

Figure 4-4 The change in TAN, PO_4-P and optical density values in reactors a) B1-50, b) B1-100 with respect to time

Parallel to production rates, nutrient removal rates of B1-100 reactor were higher than B1-50 reactor. TAN removal rates of B1-50 and B1-100 reactors were 4 and 4.5 mg/L.d, while PO_4-P removal rates were 1.22 and 1.64 mg/L.d, respectively. In the study of Li et al. (2013), where the system was also fed with municipal wastewater, 4-day's TAN removal rate was reported much higher as 9.81 mg/L.d. Removal efficiencies of B1-50 and B1-100 reactors at the end of 4^{th} day was calculated as 99.4% and 98.6%, respectively, while TAN removal efficiency was reported slightly lower as 98.1% in the study of Li et al. (2013). Initial TAN concentration in B1 reactors were around 18 mg/L, while, it was 50 mg/L in the study of Li et al. (2013). Aslan and Kapdan (2006) reported that removal rate decreases with increasing TAN concentration. As likewise, other study that reported average TAN removal efficiency of 72% from recalcitrant wastewater with 3-8 mg/L of initial TAN concentration (Valderrama et al., 2002). Low initial TAN concentration could be the

reason of low TAN removal efficiency (Valderrama et al., 2002). On the other hand, another study, which also worked with *Chlorella vulgaris*, reported that almost 100% TAN removal efficiency was achieved when the initial TAN concentration was 21.2 mg/L (Aslan and Kapdan, 2006). However, this removal efficiency was achieved at 10^{th} day of operation time, which indicates their system was much slower than the system in this study. One of the reasons for that could be different media composition (Aslan and Kapdan, 2006).

As mentioned above, the study of Aslan and Kapdan (2006) reported high TAN removal efficiency when the initial TAN concentration was 21.2 mg/L; however, low P (phosphorus) removal efficiency was reported at the corresponding initial PO_4-P concentration which is 15.4 mg/L. It was reported that the highest P removal efficiency would have been achieved when initial PO_4-P concentration was lower than 7.7 mg/L (Aslan and Kapdan, 2006). As being compatible with that, B1-50 and B1-100 reactors with initial PO_4-P concentrations of 5.3 and 6.8 mg/L, achieved 92.2% and 96.2% removal efficiencies, respectively. In the study of Valderrama et al. (2002), like TAN removal efficiency, low P removal efficiency (28%) due to low initial concentrations (1.5-3.5 mg/L) was reported.

When results of B1-50 and B1-100 reactors are compared, it can be easily said that the results of B1-100 reactor is slightly better than the results of B1-50 with respect to the biomass growth and nutrient removal efficiencies. This means that 1/10 inoculation (dilution ratio) caused no shadowing effect (Aslan and Kapdan, 2006). This inoculation ratio was decided to be used for further experimental sets.

B2-50 and B2-100 reactors were inoculated with the output of R2 cultivation reactor. The analyses results of these reactors are given in Figure 4-5. As it can be seen in Figure 4-5, neither of the B2 reactors could reach logarithmic growth phase. Optical density of B2-100 reactor constantly decreased while no significant TAN removal was observed (0.1 mg/L.d). Similarly, no significant change in the optical density or TAN concentration (0.15 mg/L.d) at B2-50 reactor was observed. As no algal growth

or TAN removal was observed, PO_4-P was not measured. Based on these results, it was calculated that culture from R2 reactor could not adapt to municipal wastewater. Therefore, seed will be taken from R1 cultivation reactors for the further experiments with municipal wastewater.

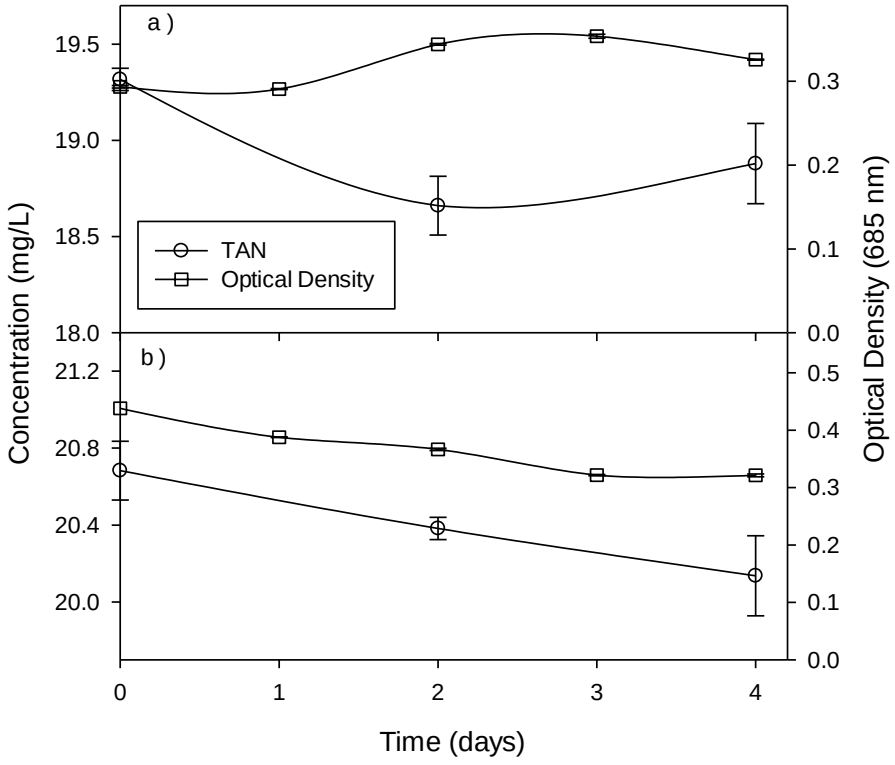

Figure 4-5 The change in TAN and optical density values in reactors a) B2-50 b)B2-100 with respect to time

4.2.2. Nutrient (N and P) Removal from Municipal Wastewater in Semi-Continuous PBRs

The aim of this study was to investigate the nutrient removal from municipal wastewater and to determine the optimum HRT(s) that the highest nutrient removal and biomass growth can be achieved at steady-state conditions. Three photobioreactors, X1, X2 and X3 were operated at HRTs of 2, 4 and 8 days, respectively (Figure 4-6, Figure 4-7, Figure 4-8).

Optical density (685 nm) was one of the parameters used to observe microalgal growth. According to the optical density values, after the 12^{th} and 10^{th} day of operation of X1 and X2 reactors, steady-state was achieved (Figure 4-6.b, Figure 4-7.b), respectively. Before steady-state was achieved in X1 reactor, high fluctuations were observed in optical density, solids, TAN and PO_4-P (Figure 4-6.b, c, f and g). After a sharp increase in the optical density in the first two days from 0.95 to 1.65, it decreased to 0.35 at 7^{th} day of operation. Similar decline showed itself in nutrient removal. TAN removal efficiency decreased from 94% to 55% and PO_4-P removal efficiency dropped from 61% to 47%. A decline was also observed in solids concentrations; however, volatile portion of solid concentration increased. After the 7^{th} day, optical density and nutrient removal efficiencies started to increase. On Day 12, steady-state was achieved. High fluctuations in parameters before the steady-state can be related with the short HRT of X1 reactor. 2-day HRT may have led to fast replenishment and thus, harder adaptation of the culture for semi-continuous operation.

X1 reactor was operated for 35 days, and it was at steady-state for 22 days of operation. During steady-state conditions, average optical density, TS and VS concentrations were 0.99±0.14, 1003±54 mg/L, 599±52 mg/L, respectively. Average TAN and PO_4-P removal efficiencies were 87±2 % and 72±2 %, respectively. At 17^{th} day of operation, chlorophyll-a, pheophitine-a, TN, sCOD started to be measured. Average chlorophyll-a concentration of X1 reactor at steady-state was measured as 26.6±1.5 mg/L. OD($664_b/665_a$) ratio was 1.64 on the average. As it is close to ratio of 1.7, X1 reactor was a functioning and healthy culture.

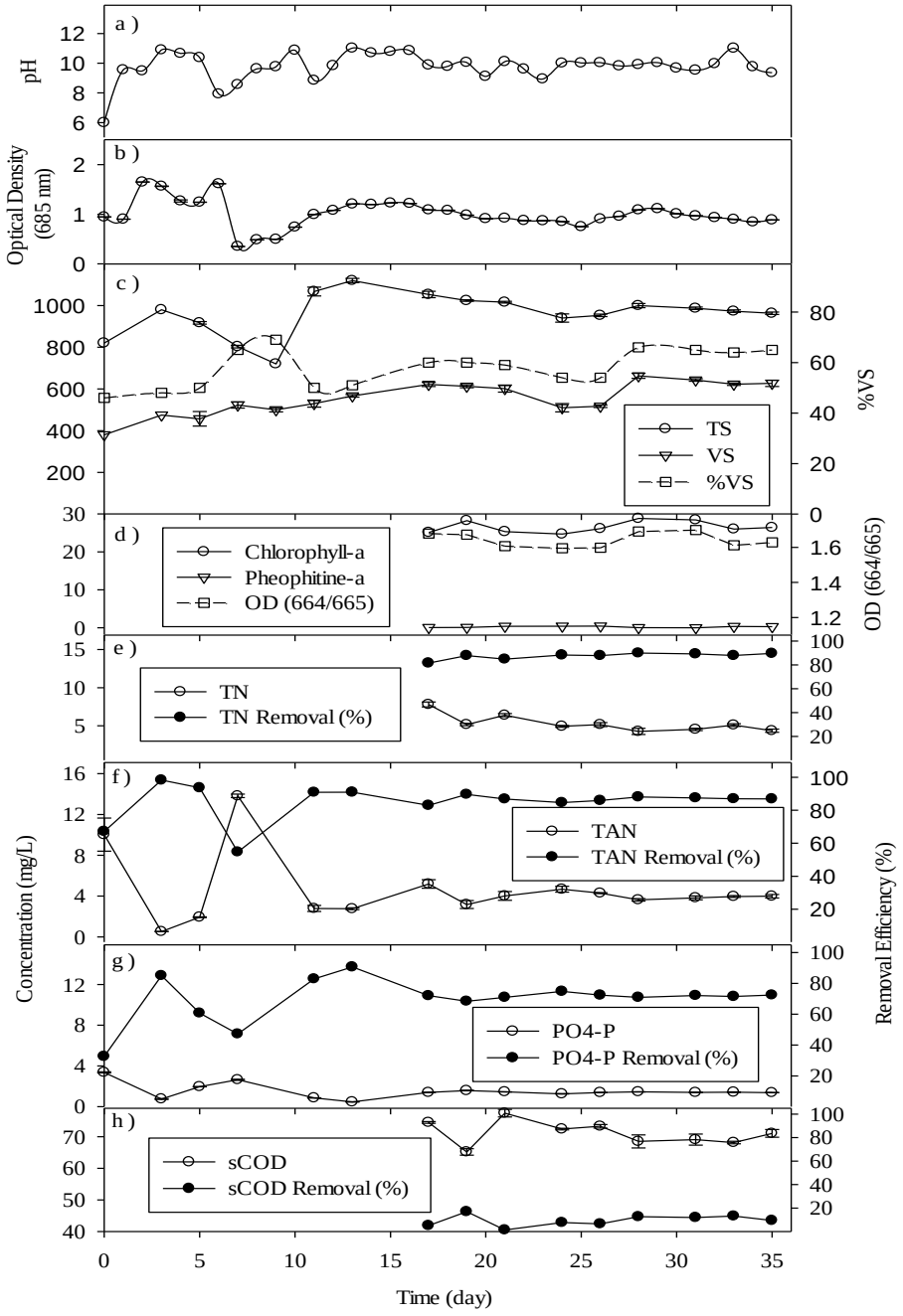

Figure 4-6 The change in parameters of X1 Reactor with respect to time; a) pH, b) Optical density, c) TS concentration, VS concentration, %VS, d) Chlorophyll-a, Pheophitine-a, OD ($664_b/665_a$), e) TN effluent concentration, TN removal efficiency, f) TAN effluent concentration, TAN removal efficiency, g) PO_4-P effluent concentration, PO_4-P removal efficiency, h) sCOD effluent concentration, sCOD removal efficiency.

TN removal efficiency was averagely 87±3%. Despite the high nutrient removal, average sCOD removal was 9±5%. Microalgae can be both autotrophic and heterotrophic. Moreover, some microalgae, such as *Chlorella*, can be mixotrophic, as well. *Chlorella vulgaris* can utilize CO_2 as a carbon source in the presence of other carbon sources unlike other mixotrophic species (Heredia-Arroyo et al., 2011). Under continuous illumination conditions, heterotrophic growth and carbon assimilation could be suppressed by autotrophic growth and carbon capture. This might limit the sCOD removal from wastewater with microalgae. Moreover, by-products (glycolic acid) formed as a result of photosynthetic activity might increase the sCOD effluent (Wang et al., 2010).

X2 reactor was operated at 4-day HRT in semi-continuous PBR. Optical density value increased rapidly at 3rd day of operation similar to X1 reactor. However, unlike X1 reactor, fluctuations of the parameterswere minor before steady-state conditions were achieved. After the 10th day of operation, steady-state was achieved. Short HRT of X1 reactor had been claimed to be the reason of high fluctuations in X1 reactor before steady-state. HRT of X2 reactor, on the other hand, was twice of the HRT of X1 reactor. The effect of that HRT difference could be realized easily as very slight fluctuations in the parameters (optical density, TS, VS, TAN and PO_4-P concentrations) of X2 reactor. Adaptation of the culture of X2 reactor to semi-continuous operation was easier than culture of X1 reactor. Requiring a shorter period (i.e., 10 days) than X1 reactor to achieve steady-state is another indicator of easier adaptation of the culture with longer HRT to the system.

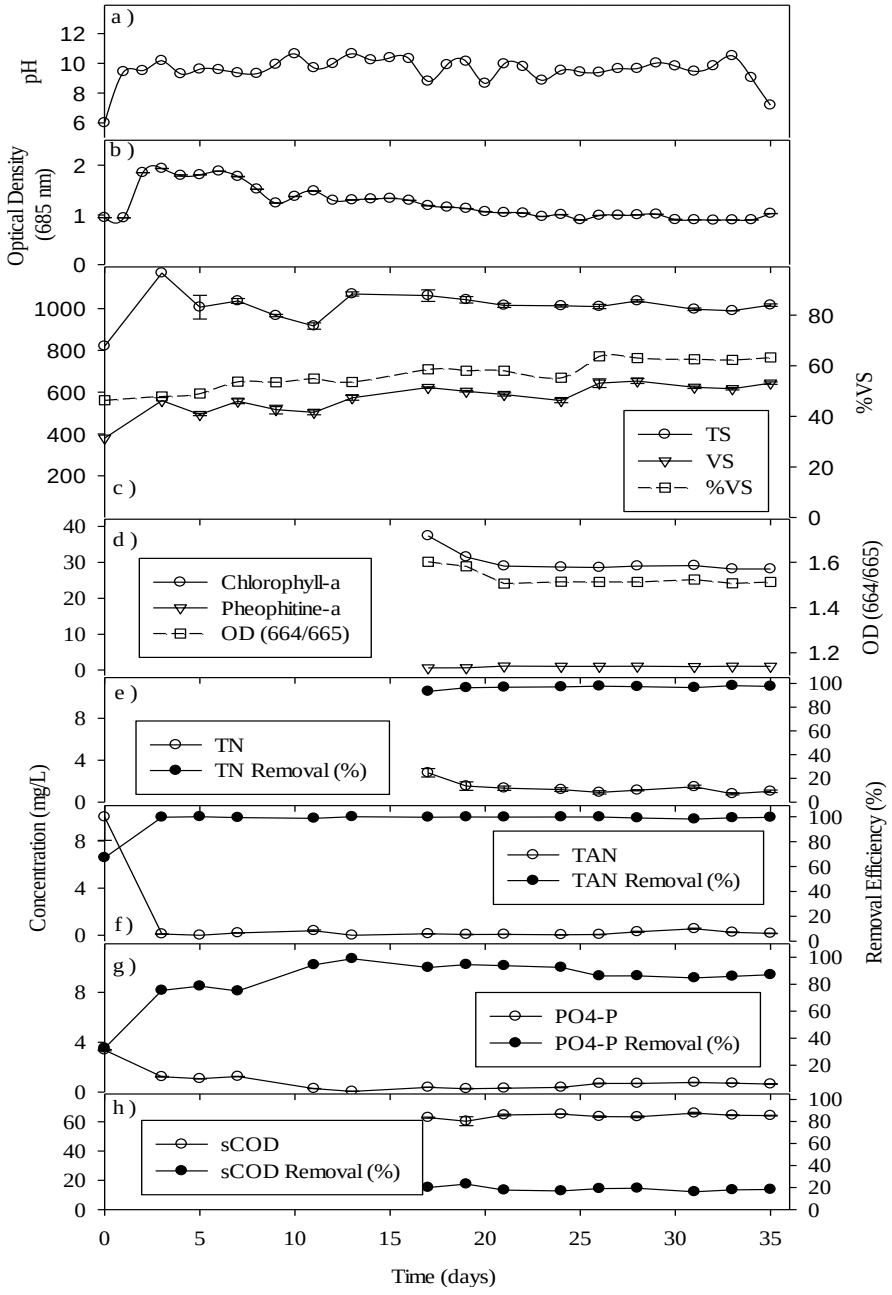

Figure 4-7 The change in parameters of X2 Reactor with respect to time; a) pH, b) Optical density, c) TS concentration, VS concentration, %VS, d) Chlorophyll-a, Pheophitine-a, OD ($664_b/665_a$), e) Total Nitrogen effluent concentration, Total Nitrogen removal efficiency, f)TAN effluent concentration, TAN removal efficiency, g) PO_4-P effluent concentration, PO_4-P removal efficiency, h) sCOD effluent concentration, sCOD removal efficiency

Higher HRT results in higher microalgal biomass hence higher removal efficiencies (Larsdotter, 2006). Average steady-state optical density, TS and VS concentrations were 1.08±0.17, 1015±41 mg/L, and 603±44 mg/L, respectively (Figure 4-7b and c). These values are slightly higher than values of X1 reactor, as expected. Average chlorophyll-a value was 29.95±2.9 mg/L, which is also higher than the average chlorophyll-a value of X1 reactor. Average OD($664_b/665_a$) ratio was 1.53 (Figure 4-7.d). This ratio is lower than the ratio of X1 reactor; however, ratio value of 1.53 still indicated a healthy microalgal culture as it is close to 1.7. Faster wash-out of dead microalgal cells from X1 reactor due to lower HRT could be the reason of higher OD($664_b/665_a$) ratio of X1 reactor (1.64) was higher than X2 reactor (1.53).

As mentioned before, higher HRT leads to higher removal rates (Larsdotter, 2006). As expected, TAN, TN, PO_4-P and sCOD removal rates of X2 reactor were higher than X1 reactor. Almost complete (98-100%) TAN removal was achieved in X2 reactor (Figure 4-7.f). Likewise, TN removal was also high (96-98%) (Figure 4-7.e). Average PO_4-P removal was 90%±5 (Figure 4-7.g).

TAN can be removed from the system also by ammonia stripping and by nitrification besides microalgae. Ammonia stripping depends on temperature, pH and initial TAN concentration (Molinuevo-Salces et al., 2016). The free ammonia concentrations were calculated according to Anthonisen, et al. (1976) (Equation 4-1 and 4-2). Increased temperature (28-30 ^oC) due to illumination and elevated pH (8-10) due to photosynthetic activity were taken into consideration to evaluate TAN consumption (Figure 4-6.a, Figure 4-7.a). The highest free-ammonia volatilized from X1 and X2 PBRs at steady-state were calculated as 31.2 mg/L and 30.8 mg/L, respectively. This corresponds to 97.2% of TAN for X1 reactor and 98.5% of TAN for X2 reactor. Nevertheless, Molinuevo-Salces et al. (2016) states that only 6-12% of the TAN can be removed by ammonia stripping in the case of steadily growing microalgal biomass and low TAN loads. Hence, only 0.46-0.92 mg ammonia could be stripped on average from 7.63 mg TAN load daily. This is a negligible portion of initial TAN load, thus, it might be accepted that most of the removal was done by microalgal culture.

Moreover, TAN removal trends and microalgal development curves (optical density, TS, VS, chlorophyll-a) overlap, microalgal culture had healthy green color and chlorophyll-a and pheophitine-a concentrations also indicated healthy culture; therefore, the major TAN removal mechanism is believed to be the microalgal activity (Boonchai et al., 2012). To make another control, mass balance for X_2 reactor based on nitrogen was performed (Appendix-F). Mass balance was performed only for X_2 reactor because nitrogen composition of microalgal culture was analysed for X_2 reactor as its nutrient removal performance was the best one as mentioned below. According to the mass balance, highest ammonia stripped to air was determined as 6% for X_2 reactor at steady-state. This corresponds to highest 0.44 mg/d stripped ammonia from X_2 reactor.

$K_b/K_w = e^{(6344/(273+T(°C))}$.....Equation (4-1)

NH_3-N (mg/L) = (TAN (mg/L) x 10^{pH}) / ($e^{Kb/Kw}+10^{pH}$) .....Equation (4-2)

Where K_b is Base dissociation constant and K_w is self-ionization constant of water.

In the nitrification process, NH_4 is converted to NO_2^- and then to NO_3^- biologically. In present study, NO_2^- and NO_3^- (NO_x) were measured regularly (operation days of 0, 7, 13, 35) for each reactor. No NO_x could be detected. Moreover, continuous illumination and optimum temperature favors microalgal growth. Therefore, TAN removal by nitrification was not possible.

PO_4-P removal ranges were between 68-75% for X1 reactor and 85-92% for X2 reactor at steady-state. Biological removal and chemical precipitation are the two methods of phosphorus removal. Biological phosphorus removal can be achieved by phosphate accumulating organisms (PAOs). PAOs remove PO_4-P with mechanism called “luxury uptake”. To achieve that PAOs should be introduced to anaerobic conditions so as to convert internally stored poly-phosphates to PO_4-P and release them from the cell. When the system is oxygenated later, PAOs uptake more PO_4-P than they released (Morse, et al., 1998). In the present study, PBRs were aerated homogeneously and no anaerobic zones present in the system to allow that

mechanism to occur. Chemical precipitation of phosphorus can occur at high alkaline pHs similar to the pHs X1 and X2 reactors (Cai et al., 2013). However, below 1-2 mg/L P, phosphorus precipitation become challenging (residual phosphorus) (Mohammed and Shanshool, 2009). For the removal of 1 mole PO_4-P, 1 mole of Al^{3+} and Fe^{3+} is required (Tchobanoglous et al., 2003). In the present study, daily PO_4-P feed to the X1 and X2 reactors were 2.5 mg/L and 1.25 mg/L, respectively. Therefore; 1.08-2.16 mg/L Al^{3+} and 2.36-4.48 mg/L Fe^{3+} should be present in the wastewater for phosphorus precipitation. However, aluminum and iron concentrations in municipal wastewater were reported as 0.05-0.2 mg/L and 0.07-0.4 mg/L, respectively (Popa et al., 2012). This amount of aluminum and iron was insufficient to form phosphorus precipitates. For phosphorus to precipitate with calcium, calcium (or lime) should be 1.4-1.5 times of total alkalinity (mg/L as $CaCO_3$). Typical total alkalinity of municipal wastewater is around 1eq/m^3 or 150 mg/L as $CaCO_3$ (Takawira, et al., 2014; Tchobanoglous et al., 2003). That corresponds to the calcium requirement of 210-225 mg/L as $CaCO_3$ for phosphorus to precipitate. However, calcium concentrations between 62-98 mg/L as $CaCO_3$ were reported for municipal wastewater (Bincy, et al., 2015).

sCOD removal in the X2 reactor was low due to autotrophic conditions mentioned above. However, removal efficiency of X2 reactor (19±2%) was twice as the sCOD removal of X1 reactor (9±5%). Longer HRT of X2 reactor had an improving effect on sCOD removal so as on nutrient removal.

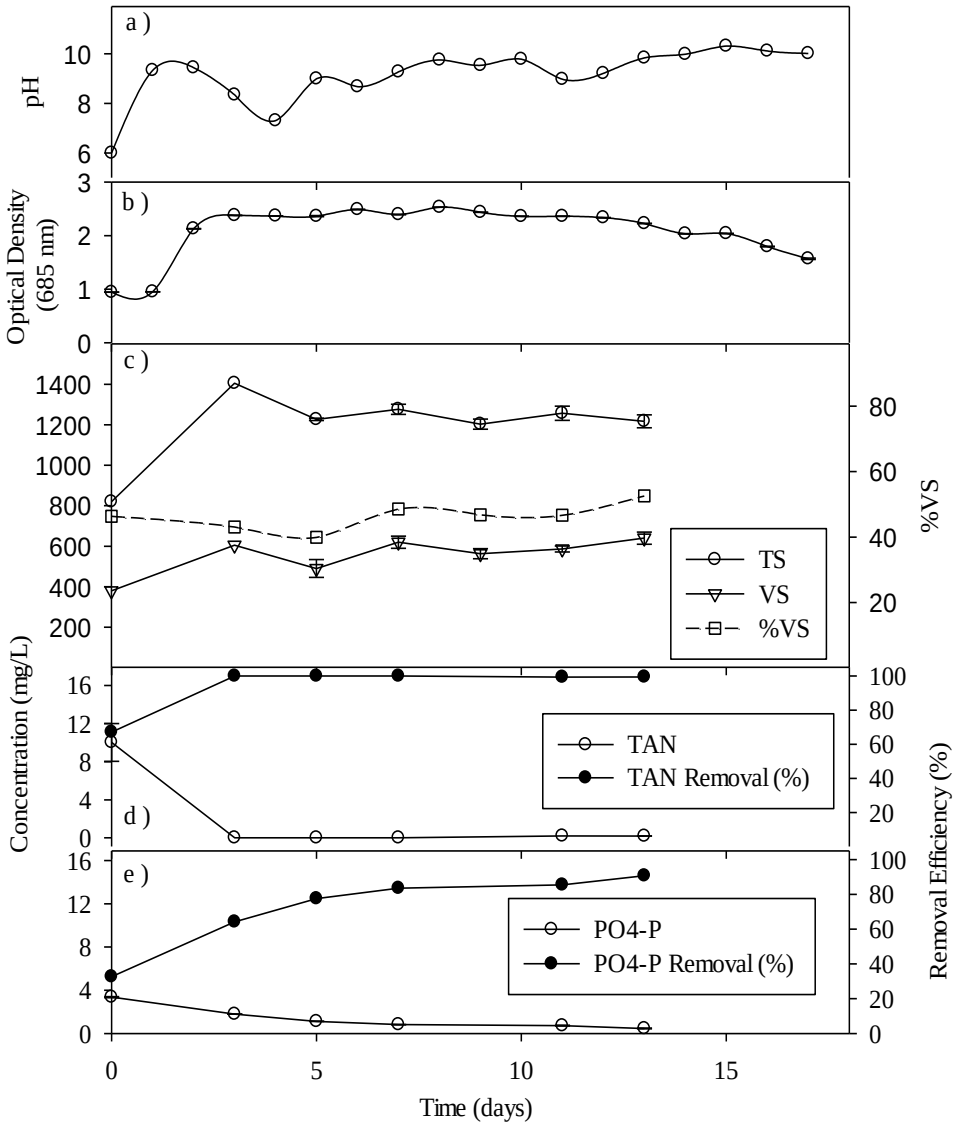

Figure 4-8 Change in parameters of X3 reactor with respect to time; a) pH, b) Optical density, c) TS concentration, VS concentration, %TVS, d) TAN effluent concentration, TAN removal efficiency, e) PO_4-P effluent concentration, PO_4-P removal efficiency.

Treating municipal wastewater in semi-continuous reactors has been studied with various algal species as well as *Chlorella vulgaris* at different retention times. In the study of Perez-Garcia et al., (2011), 2-68.2% TAN removal was achieved with *Chlorella vulgaris* culture at retention times ranging from 3 to 10 days. Li et al. (2013) reported 98% TAN, 90.9-93.6% TN, 90% sCOD and 89.9-91.8% P removal from municipal wastewater with *Chlorella vulgaris* culture. 99.7% TAN, 89.5%TN, 92% TP and 75.5% sCOD removal from undigested dairy manure was achieved by Wang et al. (2010) at 5-day HRT. 2-day HRT provided good results according to other studies mentioned above considering it is a short retention time. However, it is concluded that 4-day HRT provided better results with *Chlorella vulgaris* due to higher removal rates and biomass production.

Optical density of X3 reactor, which was operated with 8-day HRT, increased rapidly in 2 days from 0.95 to 2.13 and continued to increase to 2.53 until 8th day of operation (Figure 4-8.a). After the 8th day, optical density started to decrease slowly. Even though optical density values were still high (around 2.00), the color of the culture became an unhealthy yellow. Absorbance curve (Appendix-E) had been prepared with healthy green *Chlorella vulgaris* culture; therefore, 685 nm was not a suitable wavelength to measure the optical density value of unhealthy yellow culture of X3 reactor. Therefore, chlorophyll-a content of the X3 reactor was measured to determine the healthy microalgae concentration in the system. Chlorophyll-a concentration at the 17th day of operation was measured as 0.54 mg/L; however, initial chlorophyll-a concentration of the reactor was measured as 22.24 mg/L. Moreover, pheophitine-a concentration of the culture increased from 0.38 mg/L to 21.8 mg/L at the 17th day. OD ($664_b/665_a$), on the other hand, was 1.02. OD ($664_b/665_a$) indicates healthiness of the culture. The ratio is close to 1 meaning unhealthy culture. These analyses showed that microalgal culture was mostly lost; therefore, the operation of X3 reactor was terminated at 17th day of operation.

The reason of the culture loss was associated with the high HRT that the reactor was operated. In spite of 100% TAN and high PO_4-P removal efficiencies, nutrient

concentration that entered to the system at each feeding was not enough to sustain microalgal growth at 8-day HRT (Figure 4-8.c and d). In the present study, 30.5 mg/L TAN and 5 mg/L PO_4-P were the influent concentrations. As mentioned before, even though longer HRT is favorable for easier adaptation of the culture to the semi-continuous system, longer than a certain HRT might cause nutrient-limiting environment (Larsdotter, 2006). This statement was also proved in our study; 4-day HRT was favorable than 2-day HRT; however, 8-day HRT was not appropriate. To illustrate, Molinuevo-Salces et al. (2016) who studied continuous urban wastewater treatment with 8-day HRT, achieved high removal rates. Because, influent TAN and PO_4-P concentrations (300 mg/L TAN and 30 mg/L PO_4-P) were much higher than the present study, which verifies the potential explanation on initial nutrient concentration and HRT relationship.

According to the Table 2 of urban wastewater treatment regulation, total nitrogen and total phosphorus discharge concentrations should be below 10 and 1 mg/L or removal rates of the parameters should be 80% and 70-80% or more, respectively (MoFWA, 2006). TN and PO_4-P effluent concentrations of X2 reactor were between 0.8-2.8 mg/L N and 0.27-0.77 mg/L P, respectively which are below discharge criteria. TN effluent concentration of X1 reactor was complied with the criteria (4.3-7.8 mg/L N). Average phosphorus removal of X1 reactor (72%) is between 70-80%, yet PO_4-P effluent concentration was between 1.26-1.58 mg/L P. Nevertheless, X2 reactor performed best and its effluent concentrations complied with the regulation.

The results of the present study were summarized below;

- Good nutrient removal rates were achieved with 2-day HRT such as 83-91% TAN, 85-90% TN and 68-75% PO_4-P removal.
- High nutrient removal rates were achieved with 4-day HRT such as 98-100% TAN, 93-98% TN and 85-99% PO_4-P removal.
- Even though 99-100% TAN and 64-91% PO_4-P removal efficiencies were achieved at 8-day HRT, microalgal biomass was determined to be lost with

respect to chlorophyll-a content of culture and the operation of X3 reactor was terminated.

- X2 reactor (4-day HRT) performed better than X1 reactor (2-day HRT). 4-day HRT was determined to be the optimum HRT for the nutrient removal from municipal wastewater in semi-continuous PBRs, which were aerated with air.

4.2.3. Kinetic Study with Microalgae Culture Acclimated to Municipal Wastewater

At this part of the study, it was aimed to determine the growth rate and nutrient removal rate of microalgal culture that was acclimated to municipal wastewater. Acclimated culture was obtained from the semi-continuous experiment set mentioned in previous section.

XB-1 reactor was inoculated with the seed from the output of semi-continuous X1 reactor. Optical density values showed a linear increase (Figure 4-9.b). Optical density (OD) value reached to 0.91 from 0.29 at the end of the first 24-hour, then increased to 1.49 after another 24-hour and at the end of the operation (72-h), OD was 2.04. Almost a linear increase was also observed for TS and VS concentrations. TS and VS concentrations increased from 635 mg/L and 445 mg/L to 1735 mg/L and 940 mg/L, respectively (Figure 4-9.c). At the end of 56 hours, 100% TAN removal was achieved while PO_4-P and TN removal was 83% and 99.7% at the end of 72 hours, respectively (Figure 4-9.d, e and f.). No sCOD removal was observed (Figure 4-9.g).

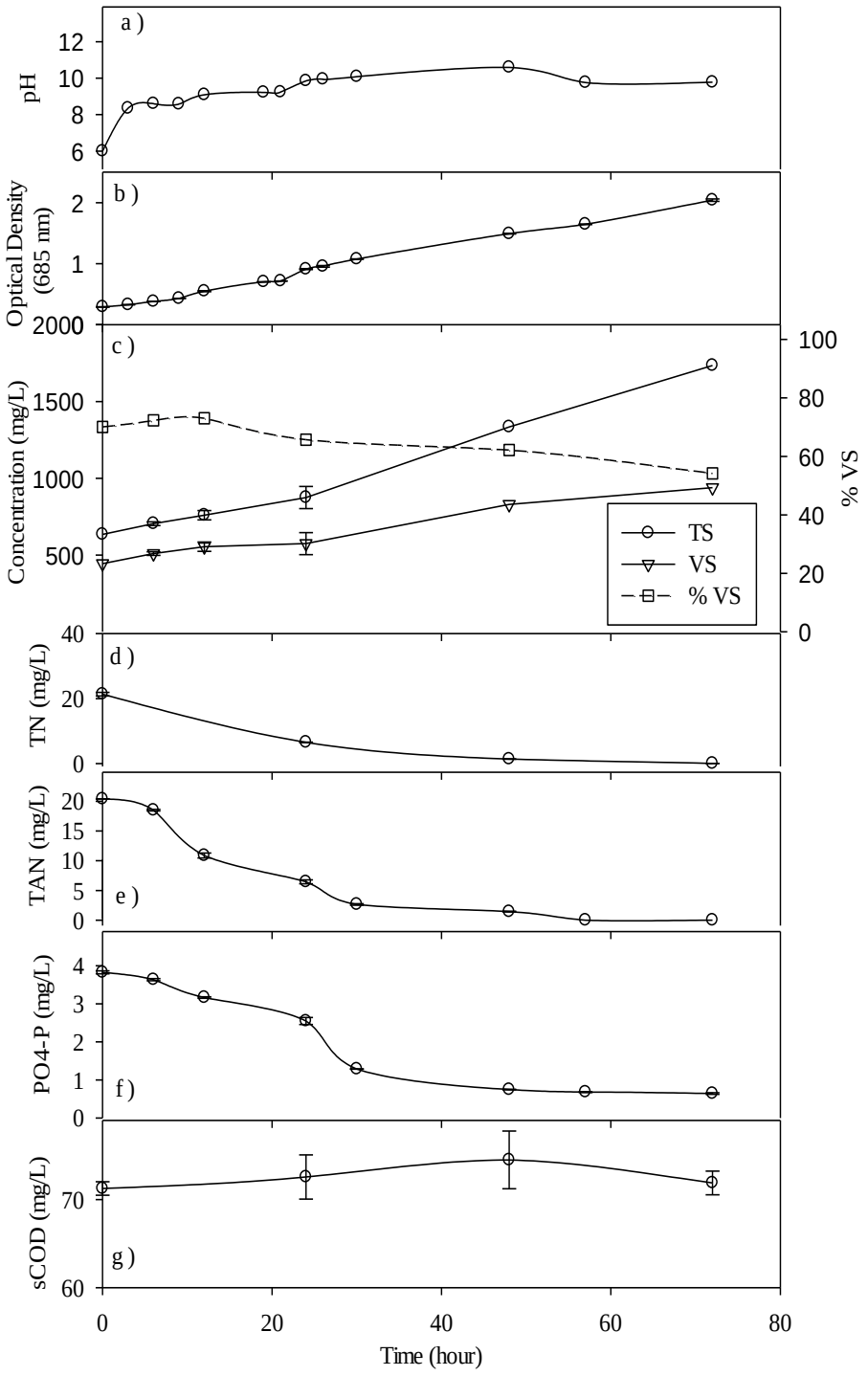

Figure 4-9 The change in parameters a) pH, b) Optical density, c) TS concentration, VS concentration, %VS, d) TN, e) TAN, f) PO_4-P and g) sCOD in XB-1 reactor with respect to time

XB-2 reactor was inoculated with the seed from the output of X2 reactor. A linear increase was observed at optical density, TS, and VS concentrations until 56th hour until almost all TAN was depleted (Figure 4-10.b, c and e.). Optical density started with 0.26 and reached to 1.6 at the end of 72 hours (Figure 4-10.b). Meanwhile, the initial concentrations of TS and VS were 715 and 535 mg/L, respectively. At the end of 72 hours, these values reached to 1810 mg TS/L and 1005 mg VS/L. Even though 100% TAN was removed, only 85% TN and 55% PO_4-P could be removed (Figure 4-10.d, e and f). No sCOD removal was observed (Figure 4-10.g).

According to the growth rate calculations, the net specific growth rate of the XB-1 reactor which is 0.31 1/d is higher than the growth rate of XB-2 reactor which is 0.12 1/d. However, TAN uptake rate of XB-2 is 0.018 mg N/mg VS.d which happens to be slightly higher than TAN uptake rate of XB-1 reactor, 0.015 mg N/mg VS.d^{-1}. Nonetheless, TN and PO_4-P uptake rates of XB-2 are lower than those of XB-1 reactor. As XB-1 reactor was inoculated from X1 reactor (2-day HRT) and the replenishment rate of the culture from X1 reactor would be faster due to low HRT, biomass concentration and removal performance of the culture of XB-1 reactor were expected to be slightly better than XB-2 (Larsdotter, 2006).

TAN removal rates of XB-1 and XB-2 reactors were determined as 8.6 and 10.4 mg/L.d, respectively. Moreover, XB-1 and XB-2 reactors had 9.9 and 8.8 mg/L.d TN removal rates, respectively. PO_4-P removal rates were 1.54 and 1.02 mg/L.d for XB-1 and XB-2 reactors, respectively. This values are compatible with the literature values.To illustrate, the study of Li et al. (2013) who also worked with *Chlorella vulgaris* and municipal wastewater in batch PBR reported similar results with the present study. Reported TAN, TN and PO_4-P results were 9.81 mg/L.d, 10 mg/L.d, and 1.64 mg/L.d, respectively in the study of Li et al. (2013).

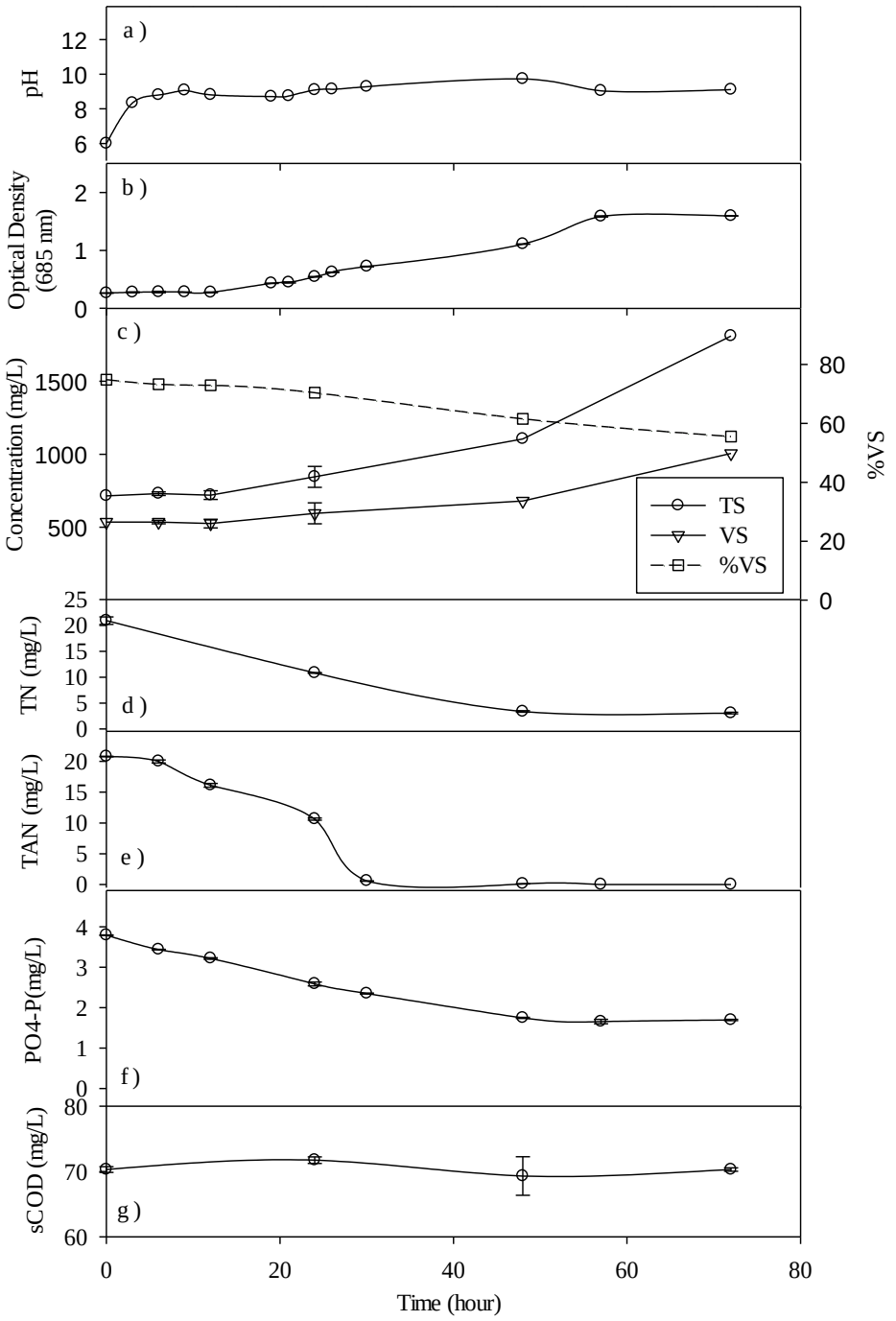

Figure 4-10 The change in parameters a) pH, b) Optical density, c) TS concentration, VS concentration, %VS, d) TN, e) TAN, f) PO_4-P and g) sCOD in XB-2 reactor with respect to time

Unacclimated versus Acclimated Culture

A study by Lau et al. (1996) investigated the differences between acclimated and unacclimated *Chlorella vulgaris* culture in terms of nutrient treatment and algal growth. According to the study, acclimated cells were more active, had more chlorophyll synthesis and took up more nitrogen and phosphorus from primary settled wastewater for their growth and metabolism. Furthermore, the growth rate and nutrient removal rate of acclimated cells are significantly higher than unacclimated ones (Lau et al., 1996). When the results of this study were compared with the results of the batch municipal wastewater study (Section 4.2.1), similar results were concluded. The growth rate of the unacclimated culture, reactor B1-100, is lower than both of the growth rates of XB-1 and XB-2 reactors. The growth rates (calculated based on optical densities) of B1-100, XB-1 and XB-2 reactors are 0.39 1/d, 0.82 1/d and 0.72 1/d, respectively. TAN removal rates of XB-1 and XB-2 reactors (8.6 and 10.4 mg/L.d, respectively) almost doubles the TAN removal rate of B1-100 reactor (4.5 mg/L.d). However, phosphorus removal rate of B1-100 reactor (1.64 mg/L.d) is higher than those of both XB-1 and XB-2 reactor (1.54 and 1.02 mg/L.d) which is an unexpected result. Nevertheless, acclimated culture generally showed much better performance than unacclimated culture. This reveals that acclimation is important for algal biomass growth and nutrient removal (Lau et al., 1996).

4.3. Treatment of Industrial Wastewater via Microalgal Culture

Algal culture acclimated to municipal wastewater was used to treat industrial wastewater. Batch experiments were initially performed to determine the optimum mixing ratio of industrial wastewater with thickener supernatant. Then, optimum HRT(s) were determined for nutrient removal in semi-continuous experiments. The results of the experiments are discussed below.

4.3.1. Determination of the Optimum Nitrogen: Phosphorus Ratio of Wastewater

It was initially aimed to determine the optimum mixing ratio of coke wastewater and thickener supernatant, the latter supplying both dilution effect and phosphorus. Batch reactors were conducted with microalgal culture and mixed wastewaters with N/P ratios of 6, 8, and 10.

The results of CB6 reactor, which was fed with mixed wastewater with N/P ratio of 6, are provided in Figure 4-11. The reactor was operated for 10 days until nutrients were almost depleted. No pH adjustments were made to the CB6, CB8, CB10 reactors, since supplying 4% CO_2-enriched air balanced pH in time. As it can be seen in Figure 4-11.a, pH of the CB6 reactor was balanced to averagely 6.29 ± 0.25, which is favorable for *Chlorell vulgaris* (Powell et al., 2009).

Optical density of C6 reactor was initially 0.58, and at the end it reached to 8.94 (Figure 4-11.b.) which was considered as high with respect to the experiments performed with municipal wastewater and ambient air supply (Average optical density values were 0.99 ± 0.13 for X1 reactor and 1.06 ± 0.15 for X2 reactor, Section 4.2.2). This is attributed to the high nutrient content of the mixed wastewater and high CO_2 supply. Parallel to the increase in optical density, TS concentration increased from 1396 mg/L to 2853 mg/L while VS concentration increased from 790 mg/L to 957 mg/L. For the first four days of the study, % VS was around 60%, and then it decreased to 35% at the end (Figure 4-11.c). The decrease in VS content can be explained with the increase in optical density and biomass growth. Due to high concentration of microalgal biomass, light penetration to the reactor might have been blocked and microalgal biomass could not get the necessary light. Some of the microalgae might have died due to lack of light and broken into inorganic matter after lysis. Dead cells were still measured as TS, not as VS and that explains the decrease in % VS.

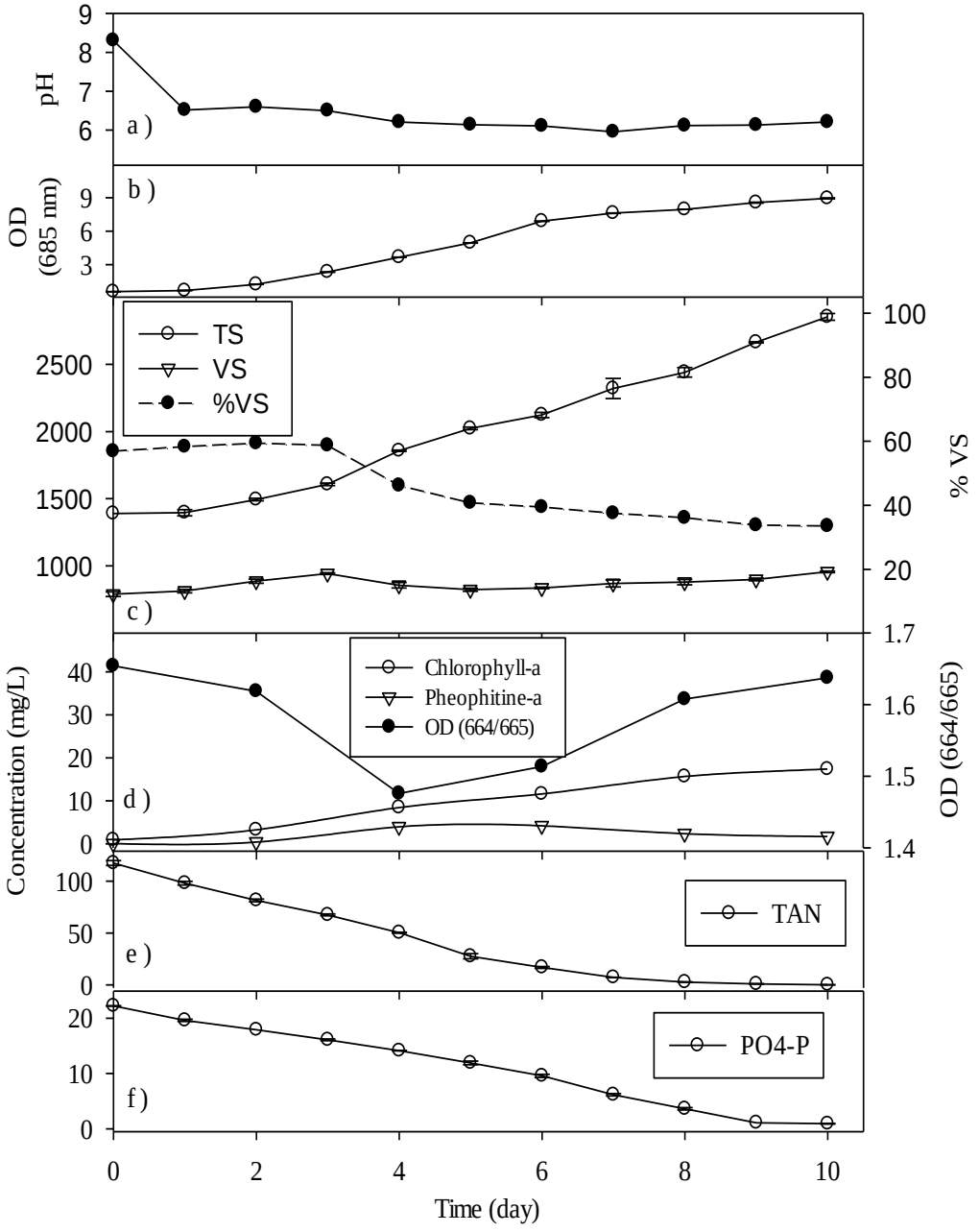

Figure 4-11 Change in a) pH, b) Optical Density (685 nm), c) TS concentration, VS concentration, %VS, d) Chlorophyll-a, Pheophitine-a, OD ($664_b/665_a$), e) TAN, f) PO_4-P parameters of CB6 reactor with respect to time.

Chlorophyll-a concentration of the CB6 reactor was increased from 0.9 to 17.4 mg/L which indicates a healthy growth. Meanwhile, the pheophitine-a concentration increased from 0.1 mg/L to 4.2 mg/L at 6^{th} day and at the 10^{th} day, decreased to 1.7 mg/L. Minimum OD ($664_b/665_a$) ratio was calculated as 1.5, indicating that even though pheophitine-a concentrations peaked, there was no problem with the health of the algal culture (Figure 4-11.d). Also, high nutrient removal rates indicate healthy algal culture (Figure 4-11.e and f). 99.9% of TAN and 95.8% of PO_4-P removal was observed at the end of 10^{th} day. Nutrients were depleted almost at the same time which resulted in high removal rate for each nutrient.

The results of CB8 reactor, which was fed with the mixed wastewater with N/P ratio of 8, are provided in Figure 4-12. The reactor was operated for 12 days until PO_4-P was depleted. pH was balanced averagely at 6.66±0.15 with the help of 4% CO_2 enriched air supply (Figure 4-12.a.). High algal growth was observed like it was observed in CB6 reactor. Optical density increased to 9.62 at the 12^{th} day from 0.59. In a parallel way, TS concentration increased from 1000 mg/L to 2707 mg/L while VS concentration increased from 587 mg/L to 937 mg/L.

At the first day of operation, an increase was observed in TS and VS concentrations (Figure 4-12.c). However, other growth parameters such as chlorophyll-a and optical density did not increase at that day of operation (Figure 4-12.b and d). This increase observed for solids concentration could be a result of an experimental error such as taking non-homogeneous sampling.

Initially, all growth parameters (optical density, TS, VS, chlorophyll-a) sligtly decreased. However, at the 6^{th} day of operation, chlorophyll-a started to increase. The reason of the decrease at first 4 day of operation might be microalgal biomass to acclimate the new type of wastewater. This acclimation period can be observed from the increase in pheophitine-a concentration at first 4 days (Figure 4-12.d). After the acclimation was achieved, pheophitine-a concentration decreased and OD ($664_b/665_a$) ratio increased.

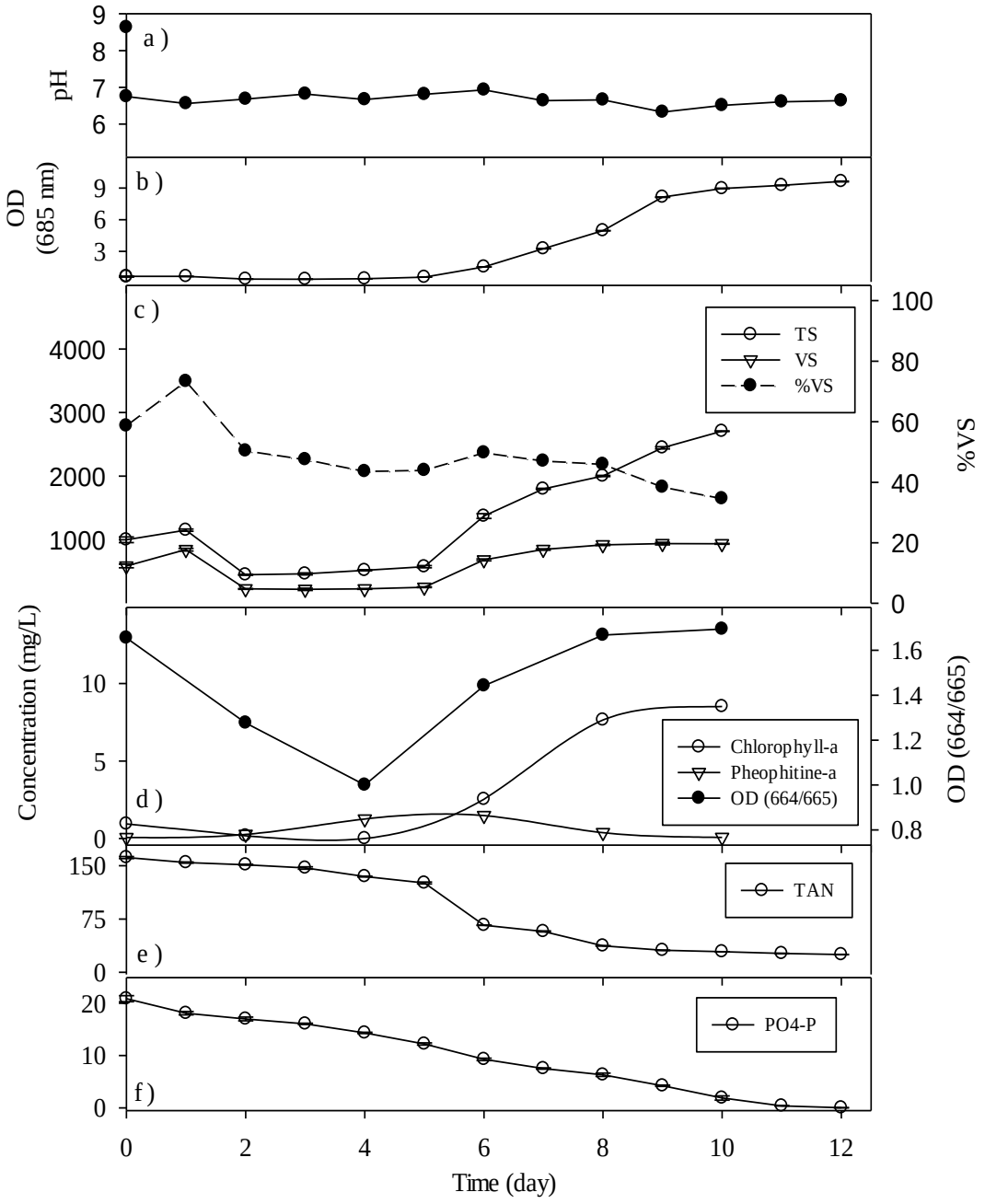

Figure 4-12 Change in a) pH, b) Optical Density (685 nm), c) TS concentration, VS concentration, %VS, d) Chlorophyll-a, Pheophitine-a, OD ($664_b/665_a$), e) TAN, f) PO_4-P Parameters of CB8 Reactor with respect to time

As can be seen in Figure 4-12.e, no significant TAN removal observed during the acclimation period of the first 6 days of operation. After that period, with the increase in microalgal growth, TAN removal started to increase. 85% TAN removal efficiency was achieved at the end of the operation. PO_4-P removal percentage was 99.8%. This results in 25 mg/L of TAN left untreated since not enough PO_4-P was left for removal of TAN by microalgal culture. This revealed that mixed wastewater with N/P ratio of 8 caused a P-limiting system.

The results of CB10 reactor, which was fed with the mixed wastewater with N/P ratio of 10, are provided in Figure 4-13. The reactor was operated for 10 days until TAN was depleted. Average pH was measured as 6.78 ±0.1 with the help of 4% CO_2 - enriched air supply (Figure 4-13.a).

During the operation period, each growth parameter increased in value. Optical density of C10 reactor was initially 0.62 and increased to 7.87 at the end of operation period (Figure 4-13.b). TS and VS concentrations were initially 1930 mg/L and 817 mg/L, respectively, while at the end of 10^{th} day they reached 2930 mg/L and 903 mg/L, respectively (Figure 4-13.c). Moreover, chlorophyll–a concentration increased from 0.94 mg/L to 1.23 mg/L. . However, at the first 4 days of operation, due to acclimation period as it was explained for CB8 reactor, slight decrease or insignificant increase was observed. For instance, chlorophyll-a concentration decreased to 0.36 mg/L at the 2^{nd} day of operation. Although pheophitine-a concentration continued to increase until Day 6, average concentrations of chlorophyll-a and OD ($664_b/665_a$) ratio were 0.97±1.2 mg/L and 1.54±0.12, which indicates that culture remained healthy (Figure 4-13.d).

After the acclimation period (4^{th} day of operation) , TAN removal increased and it reached to 98% at the end of operation. However, PO_4-P removal was stopped at 44% which means that mixed wastewater with N/P ratio of 10 caused an N-limiting system, which is the opposite of the CB8 reactor's situation.

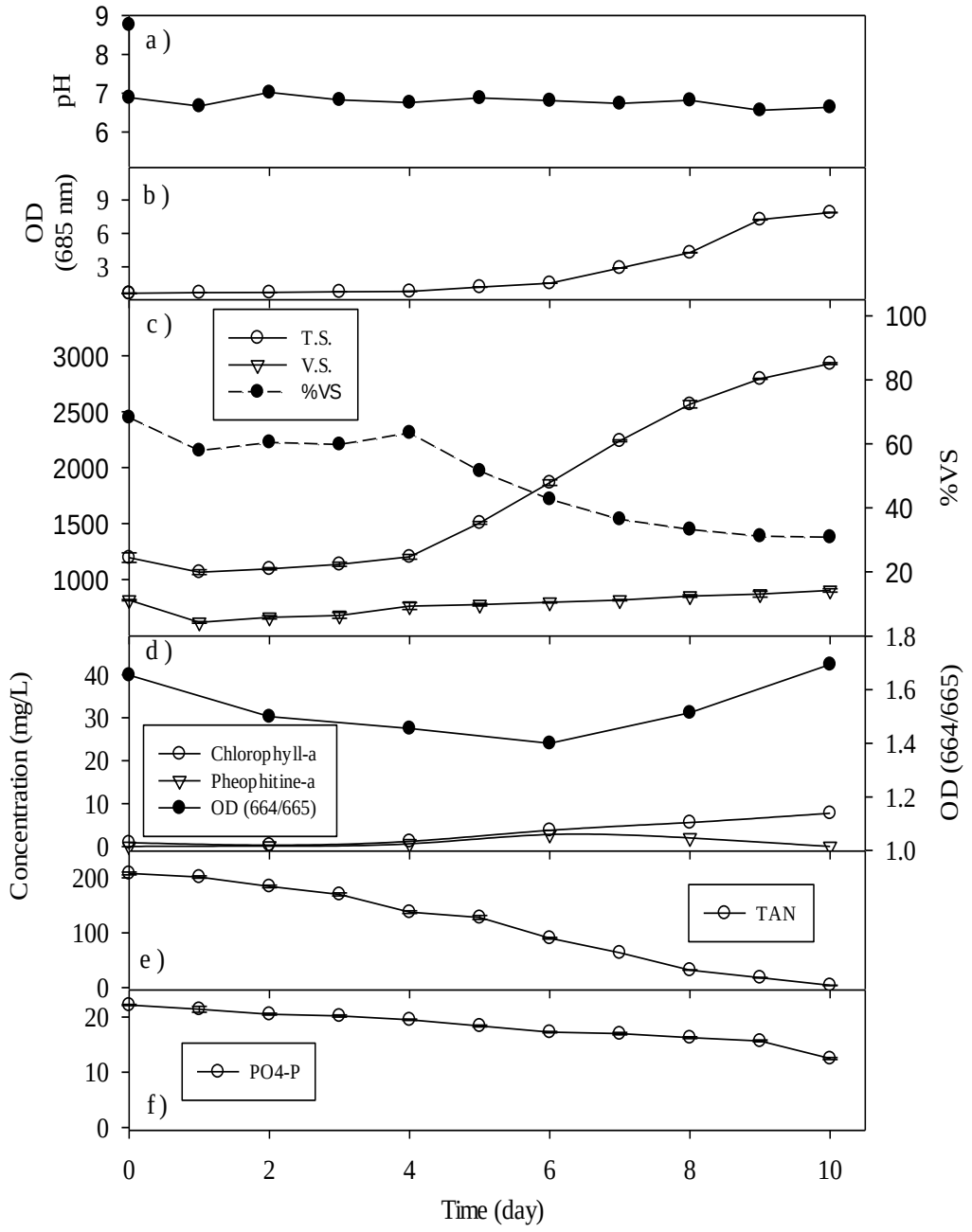

Figure 4-13 Change in a) pH, b) Optical Density (685 nm), c.) TS concentration, VS concentration, %TVS, d) Chlorophyll-a, Pheophitine-a, OD ($664_b/665_a$), e) TAN, f) PO4-P parameters of CB10 Reactors with respect to time

When the results of CB6, CB8 and CB10 reactors were evaluated together, CB6 reactor was determined as the most efficient one as TAN and PO_4-P were treated almost completely. Biomass concentration obtained at the end of operation period in CB8 reactor was higher than CB6 reactor. Since the goal of our study was to determine the most efficient N/P ratio and, thus, mixing ratio of two wastewaters where TAN and PO_4-P can be treated together, mixed wastewater with N/P ratio 6 was chosen to be used for further semi-continuous experiments.

4.3.2. Determination of the Optimum HRT Leading To Maximum Nutrient Removal in Semi-continuous PBRs

The purpose of this study was to determine the optimum HRT(s) that the highest nutrient removal and biomass growth rate can be achieved from a mixture of coke wastewater and thickener supernatant with an N/P ratio of 6 (Table G-1, Appendix-G). It was also aimed to investigate carbon dioxide sequestration.

Reactor C5, which is operated at 5-day HRT, was runned for 28 days. Average pH value of the reactor during operation was 6.62±0.3 (Figure 4-14.a). pH 6-7 is optimum for microalgae to grow (Mayo, 1997). Change in pH values through the operation was below 5% owing to buffering effect of 4% CO_2-enriched air supply.

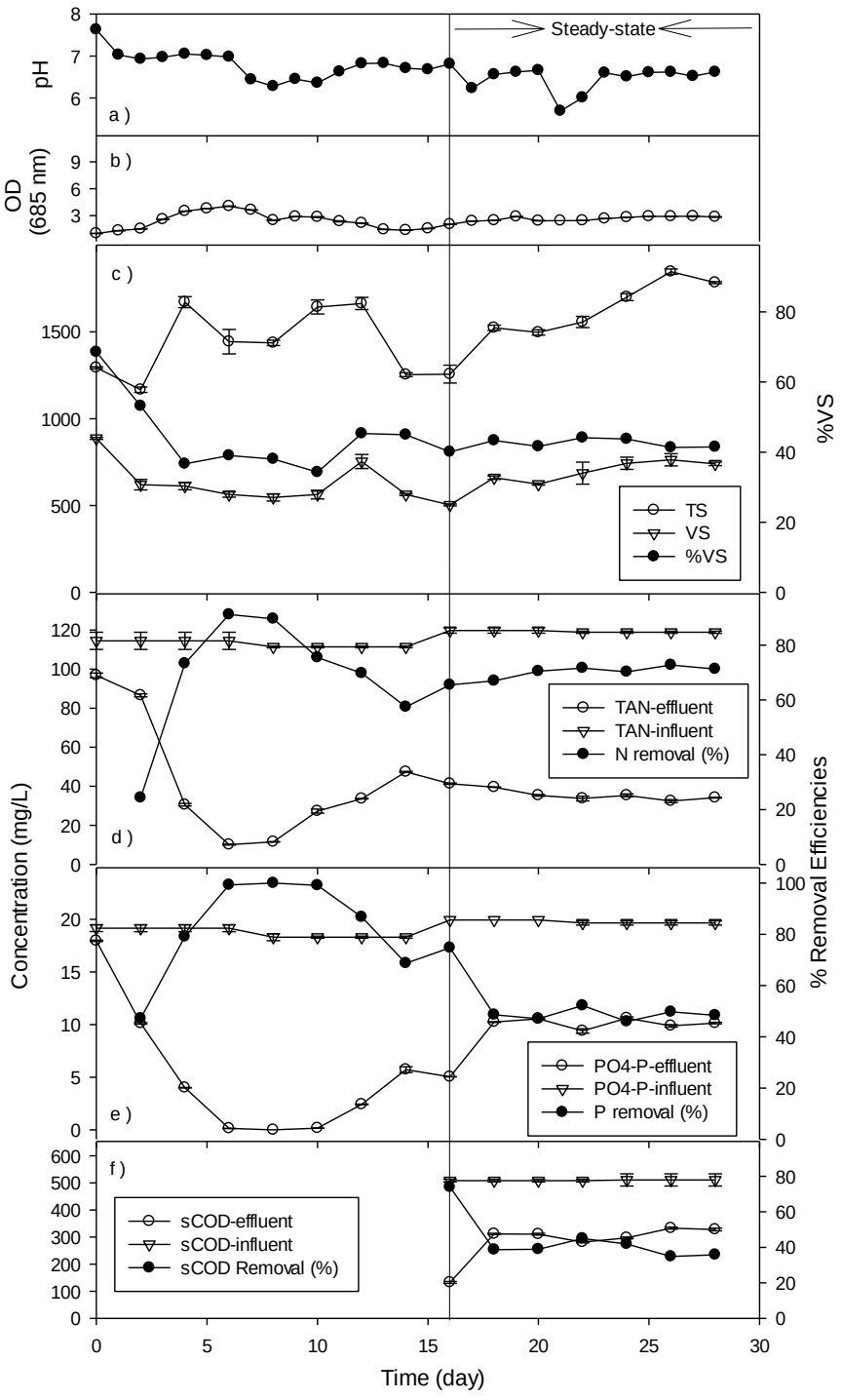

Figure 4-14 Change in parameters of C5 reactor with respect to time; a) pH, b) optical density at 685 nm, c) TS concentration, VS concentration, %VS, d)TAN, e) PO_4-P, f) sCOD.

Steady-state was achieved after the 16th day of operation. Before steady-state was achieved, some fluctiations was observed at the optical density, TS and VS parameters (Figure 4-14.b and c), similar to the X1 and X2 reactors operated with municipal wastewater (Section 4.2.2) Optical density of the culture increased rapidly to 4.052 by 6th day of operation, then started to decrese (Figure 4-14.b). At the 6th day, TAN and PO_4-P removal peaked to 91% and 100%, respectively. TAN and PO_4-P removal efficiencies were between 24-91% and 47-100% before steady-state, respectively.

After the achievement of steady-state conditions, removal efficiencies ranged between 66-73% for TAN and 46-52% for PO_4-P (Figure 4-14.d and e). As pH was below 7 all the times, ammonia removal cold not be associated with ammonia stripping (Molinuevo-Salces et al., 2016). Average optical density, TS concentration and VS concentration were 2.67 ± 0.2, 1651±147 mg/L and 703 ± 55 mg/L, respectively for C5 Reactor (Figure 4-14.c). After the 16th day of operation, sCOD was started to be measured. Removal efficiencies for sCOD were between 35-74% and 39±3.8% on average (Figure 4-14.f).

CO_2 effluent concentrations were measured at 24th, 26th, and 28th operational days. These concentrations were 3.85%, 3.8% and 3.82% for 24th, 26th, and 28th operational days, respectively for 3.99±0.00% influent CO_2 concentration. Average CO_2 removal was 4.1±0.6%.

Reactor C8 was operated for 52 days at 8-day HRT. System was run at steady-state conditions between 18th and 43th days of operation with respect to optical density (Figure 4-15). Average pH value of the reactor during operation was 6.35±0.3 until 43th day of operation which was optimum for microalgal growth (Mayo, 1997). After the 43th day of operation, pH adjustments were made to increase CO_2 removal rate. Stability of the pH values was owed to the buffering effect of 4% CO_2-enriched air supply (Figure 4-15.a).

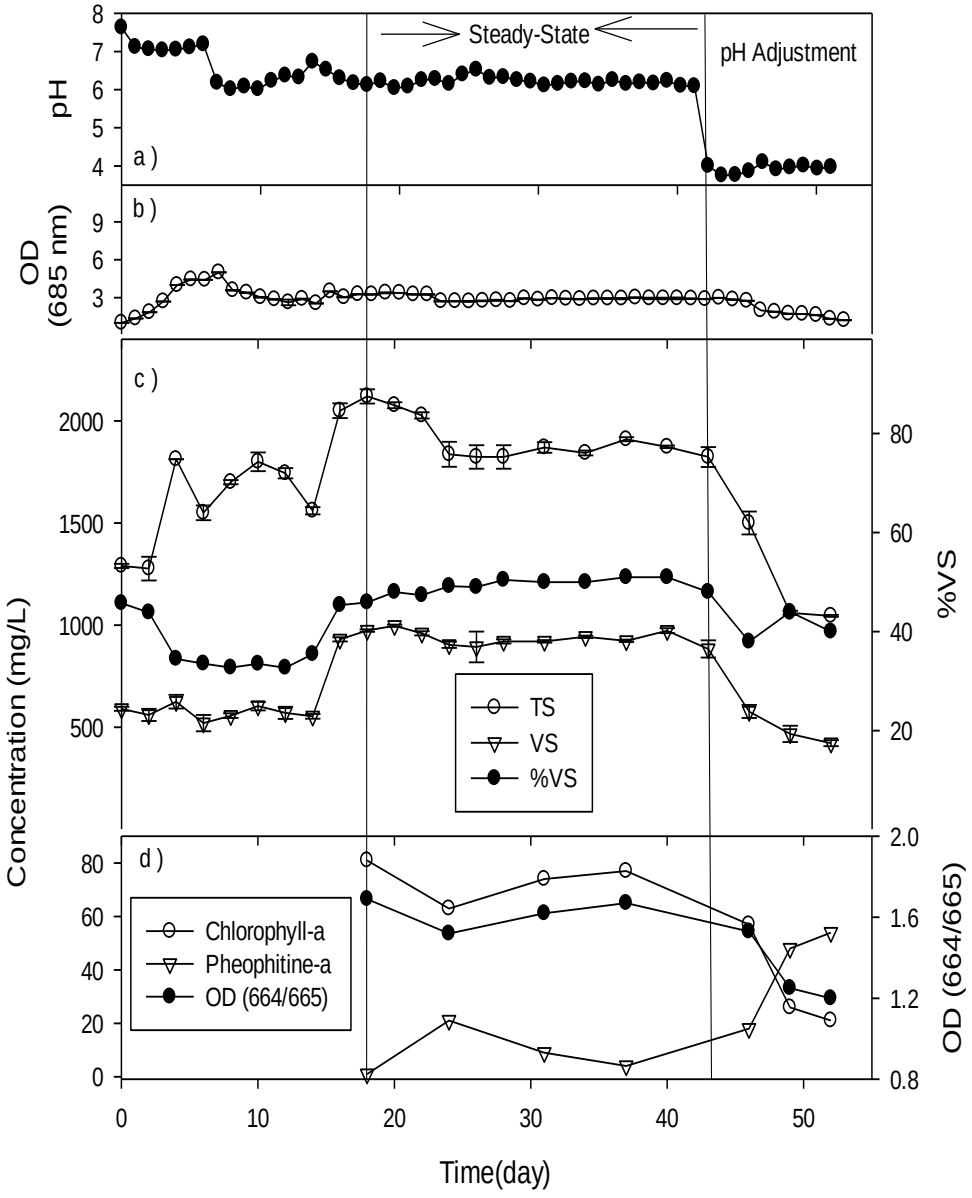

Figure 4-15 Change in parameters of C8 reactor with respect to time; a) pH, b) optical density at 685 nm, c) TS concentration, VS concentration, %VS, d) Chlorophyll-a, Pheophitine-a, OD ($664_b/665_a$).

Before steady-state was achieved in C8 reactor, fluctuations in optical density, TS and VS parameters were observed, similar to C5 reactor. Optical density value peaked at 7th day of operation, then started to decrease. After the steady-state was achieved at 16th operation day, average steady-state optical density was 2.97 ± 0.2 (Figure 4-15.b). 1920±112 mg/L and 941 ± 34 mg/L was average steady-state concentrations of TS and VS, respectively (Figure 4-15.c). During the steady-state, chlorophyll-a content of the culture was also monitored once a week. Chlorophyll-a values were close to each other until the pH adjustments. Average chlorophyll-a concentration was 74±7.4 mg/L. Pheophitine-a concentrations (average 8.88±8 mg/L) indicated that dead microalgal cells were removed from the reactor efficiently. Average OD($664_b/665_a$) ratio of 1.63±0.07 showed that microalgal culture was healthy as the ratio is close to 1.7 (Figure 4-15.d).

TAN and PO_4-P concentrations peaked before the achievement of steady-state conditions (defined with respect to optical density) (Figure 4-16.c and d). At 6th-8th operation days' removal efficiencies peaked at 98-99% for TAN and 98-100% for PO_4-P. This corresponds to the peak value of optical density at the 7th operation day (Figure 4-16.b). During the steady-state, removal efficiencies ranged between 87-93% for TAN and 94-99% for PO_4-P. Average TAN and PO_4-P removal rates were 90%±1.5 and 95±1.4%, respectively (Figure 4-16.c and d). TN and sCOD were started to be measured following the achievement of steady-state conditions. TN removal efficiencies were similar to TAN removal efficiencies. 89-91% TN removal was observed in C8 reactor until pH adjustment (Figure 4-16.b). Removal efficiencies for sCOD were between 51-63% and 59±4.0% on average (Figure 4-16.f).

Average inlet and outlet CO_2 percentages of C8 reactor were 4.00±0.005% and 3.30±0.014%, respectively. Average steady-state CO_2 removal rate was 17.4±0.3% (Figure 4-16.a). This removal efficiency is much higher than that of C5 reactor. However, in order to improve CO_2 removal performance of the reactor more, pH adjustment was tried after the 43rd day of operation to increase solubility of CO_2. pH

was adjusted after each feeding until the 52nd day of operation. For the first 3 days, pH was adjusted to 4. Hereby, free CO_2 would transfer to aqueous phase and become more available for microalgal usage (Hulatt and Thomas, 2011). The adjusted pH was not in the range of optimum pH for *Chlorella vulgaris*; however, it has been known that *Chlorella vulgaris* can survive at pH values as low as 2 (Lustigman et al., 1995). Nevertheless, optical density of the culture started to decrease sharply (Figure 4-15.b). Similarly, removal rate of TAN dropped to 45% (from 92%) at the 3rd day of pH adjustment. Considering the adverse effect of low pH on system, pH was adjusted to 4.5 initially, then to 5. However, optical density continued to decrease sharply as also observed for removal efficiencies (Figure 4-15.b, Figure 4-16.b, c, d and e). Chlorophyll-a concentration of the culture was measured as 21 mg/L at the final day of operation. In addition to chlorophyll-a concentration, pheophitine-a concentration increased to 54 mg/L. Incremental decrease in OD($664_b/665_a$) ratio from 1.65 to 1.2 means culture lost with the time. TN, TAN, PO_4-P and sCOD removal efficiencies decreased to 19%, 13%, 52%, and 42% respectively. Even so, no increase in CO_2 removal was observed. At the 3rd day of pH adjustment, removal efficiency dropped to 6% and then to 4% at Day 52. Therefore, the operation was terminated at 52nd day of operation (i.e. 9th day of pH adjustment) due to worsen performance. It can be concluded that adjusting pH to a lower value to increase CO_2 solubility and availability was not suitable for microlgal culture as pH of 4-5 is not appropriate for culture to survive.

The results of C5 and C8 reactors revealed that the increase in HRT from 5-day to 8-day increased the biomass concentration and removal performances. Optical density values and VS concentrations increased 10% and 33%, respectively. Moreover, 26% TAN, 93% PO_4-P, 51% sCOD and 324% CO_2 increase was determined for the removal efficiencies.

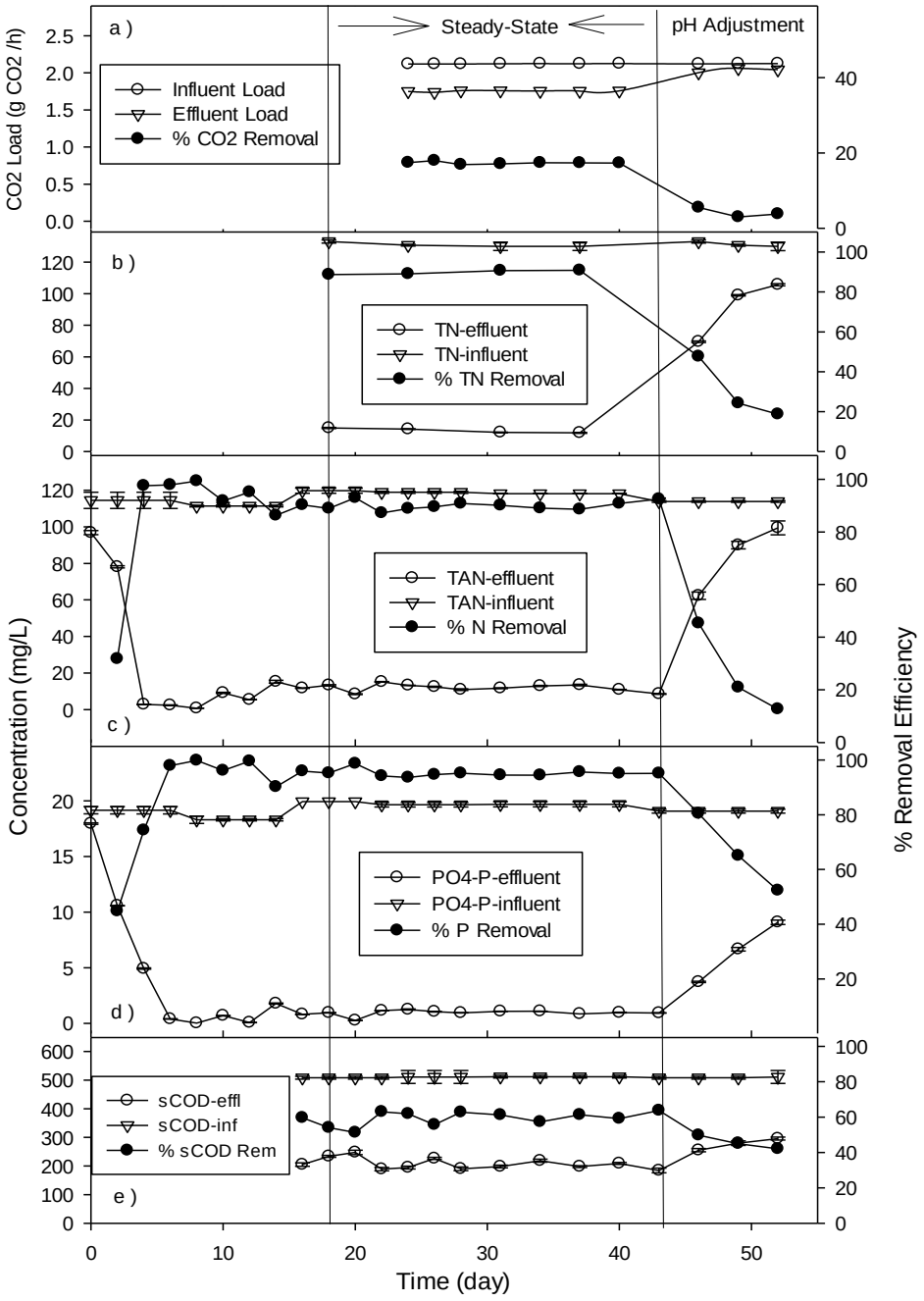

Figure 4-16 Change in parameters of C8 reactor with respect to time; a) CO_2 influent and effluent load, removal, b)TAN influent and effluent concentration, TAN removal c) TN influent and effluent concentration, TN removal, d) PO_4-P influent and effluent concentration, PO_4-P removal, e.) sCOD influent and effluent concentration and sCOD removal.

Reactor C12 was operated for 60 days at 12 day-HRT. System was run at steady-state conditions between 18th and 60th days of operation with respect to optical density. With the help of 4% CO_2-enriched air supply, pH value was 6.17±0.4 on average which is optimum for microalgae (Mayo, 1997) (Figure 4-17.a).

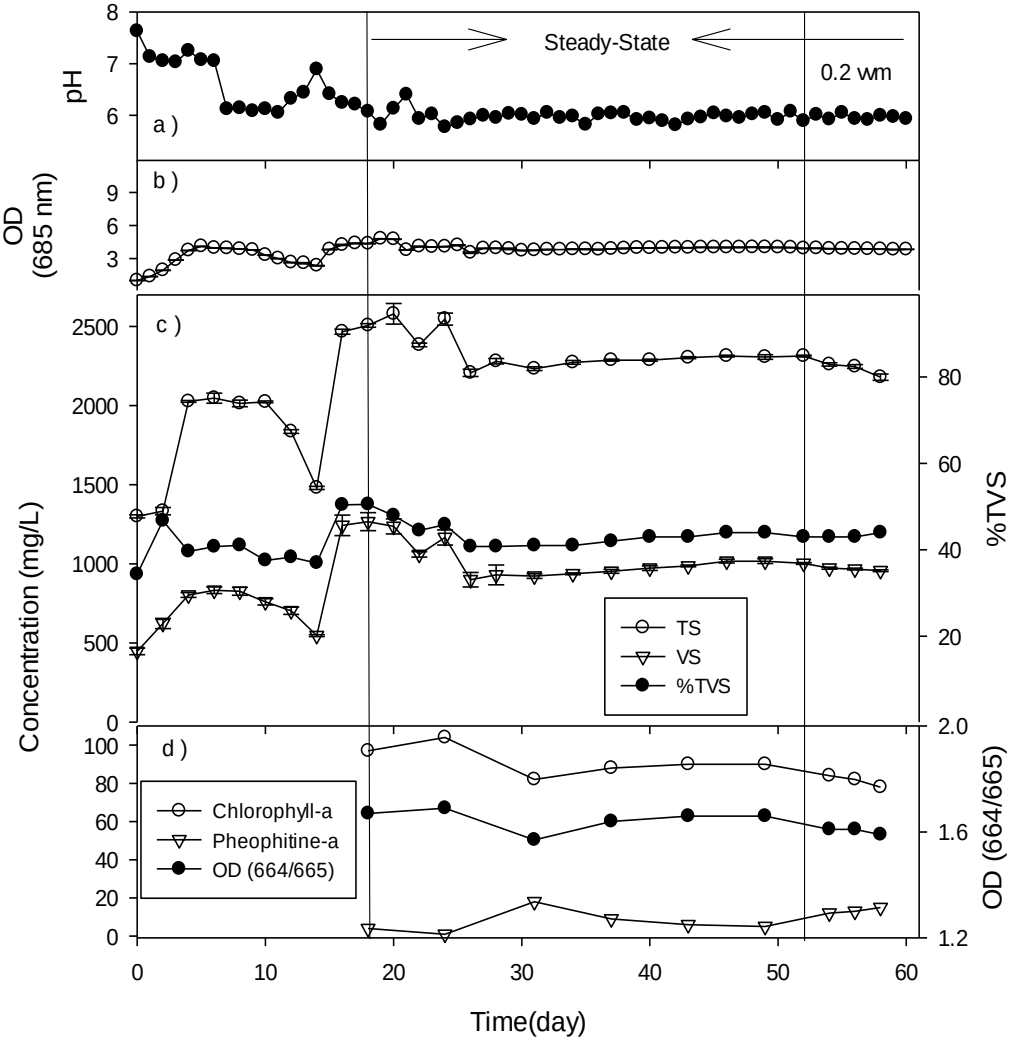

Figure 4-17 Change in parameters of C12 reactor with respect to time; a) pH, b) optical density at 685 nm, c) total solid concentration, volatile solid concentration, %TVS, d) Chlorophyll-a, Pheophitine-a, OD ($664_b/665_a$).

Optical density of C12 reactor increased to 4.103 at 5th day of operation, then started to decrease. However, unlike C5 and C8 reactor, this increase and decrease in the optical density values were not sharp (Figure 4-17.b). Average optical density at steady-state was 4.0±0.25. Average TS and VS concentrations at steady-state were 2347±121 mg/L and 1028 ± 121 mg/L, respectively (Figure 4-17.c). Moreover, C12 reactor had a healthy culture during steady-state conditions (Figure 4-17.d). OD ($664_b/665_a$) ratio was 1.65±0.04 on average which is close to 1.7 indicating healthy culture. Chlorophyll-a concentrations of the reactor were high, between 82-104 mg/L.

TAN removal efficiencies ranged between 27-99% and 94-100% before and during the steady-state conditions, respectively (Figure 4-18.c). Steady-state PO_4-P removal efficiencies were high as TAN removal. Between 42-98% and 96-100% PO_4-P removal efficiencies were observed before and during the steady-state conditions, respectively (Figure 4-18.d). Average 96.5±2.04% TN removal was achieved during steady-state (Figure 4-18.b). Also, sCOD removal efficiencies changed between 54-66% during steady-state conditions (Figure 4-18.e).

Compared to C8 reactor, 7.2% TN, 8.3% TAN, 2.1% PO_4-P and 5% sCOD increase in removal efficiencies were determined in C12. Moreover, 35% increase in optical density, 9.4% increase in VS concentrations and 24% increase in chlorophyll-a content were determined with respect to C8 reactor. However, it should be noted that the increase in the biomass concentration and removal efficiency parameters observed with the HRT increase from 5-day to 8-day is higher than that observed for the increase from 8-day to 12-day HRT.

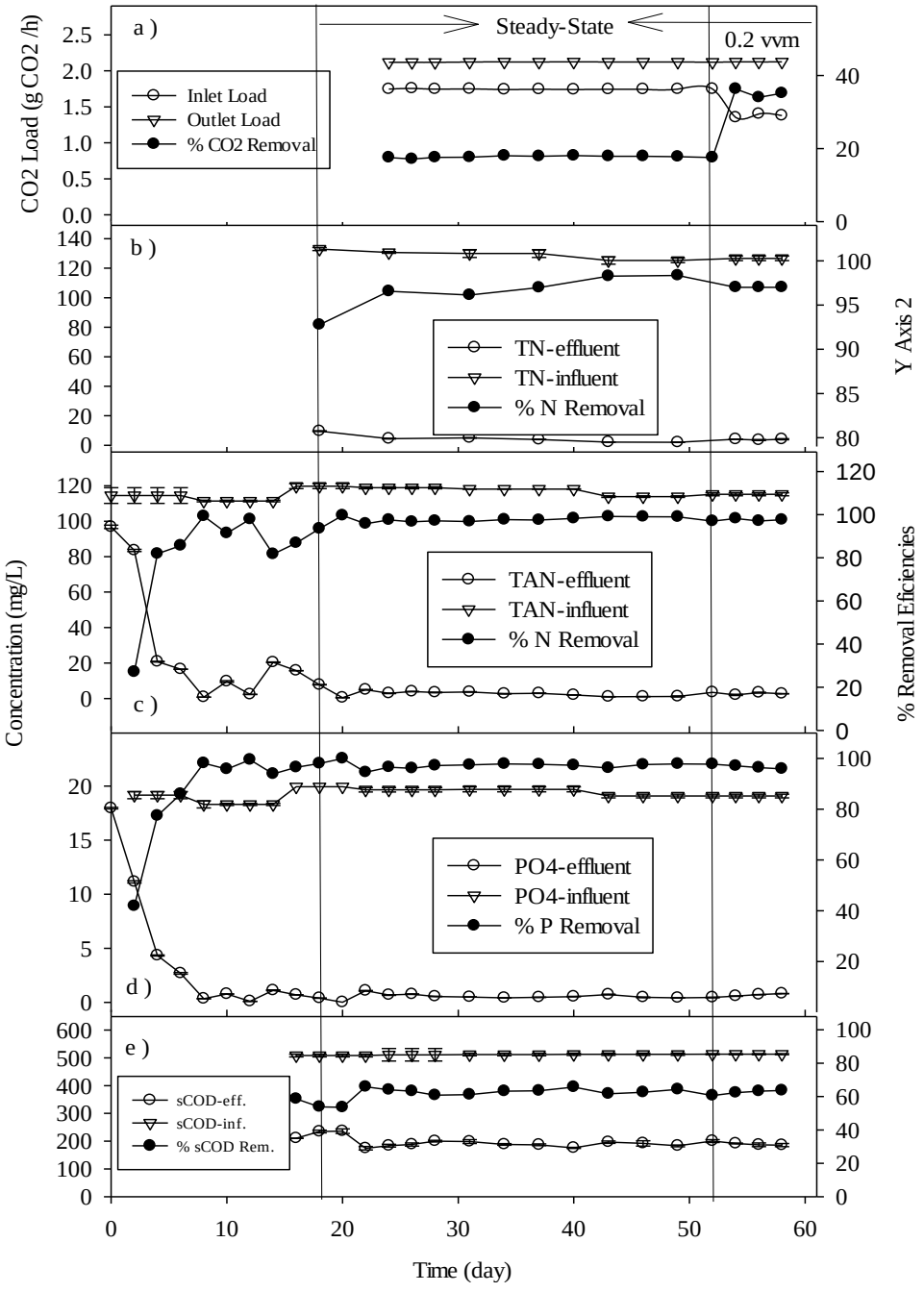

Figure 4-18 Change in parameters of reactor C12 with respect to time; a) CO_2 influent and effluent load, removal, b) TAN influent and effluent concentration, TAN removal c) TN influent and effluent concentration, TN removal, d) PO_4-P influent and effluent concentration, PO_4-P removal, e.) sCOD influent and effluent concentrations, sCOD removal.

Average inlet and outlet CO_2 percentages of C12 reactor were 4.00±0.004% and 3.29±0.253%, respectively. Average steady-state CO_2 removal rate was 17.7±0.3% (Figure 4-18.a). Compared to C8 reactor, 2% increase in CO_2 removal was observed in C12 reactor. In order to to increase CO_2 removal more, it was decided to decrease the air flowrate. Generelly, growth rate increases with the air flowrate since solubility of CO_2 is affected from flowrate and bubbling (Singh et al., 2015). However, if the solubility of CO_2 is enough for microalgae, unnecessary high air flowrate may disrupt the cell structure of microalgal cells while valuable CO_2 content would be lost to air (Cheng et al., 2006). Moreover, lower air flowrate may increase the residence time of the air bubbles and improve the solubility of CO_2. In the study of Singh et al. (2015), 0.2 vvm resulted in higher growth rate than 0.4 vvm. Therefore, 0.5 vvm was lowered to 0.2 vvm in C12 reactor after 52th day of operation. Almost no change in parameters were observed (Figure 4-17, Figure 4-18). Steady-state of the reactor was not disrupted after lowering the air flowrate. Slight decrease in the chlorophyll-a value was observed (84 mg/L to 78 mg/L); however, OD ($664_b/665_a$) ratio was still close to 1.7 indicating a healthy, functioning culture (Figure 4-17.d). CO_2 removal efficiency of the reactor increased incrementally to 21.52% at the 56th day. At the last day of operation, removal of CO_2 was slightly decreased to 20.95%. To conclude, reducing the inflow rate of CO_2-enriched air can increase the removal of CO_2 by increasing its solubility. In addition, 0.2 vvm was found to be appropriate for C12 reactor.

Comparison of Reactors' Performances

The evaluation of reactors C5, C8 and C12 were presented in Table 4-4. It is seen that the mixed (coke wastewater and thickener supernatant) wastewater could be treated with microalgal culture in semi-continuous PBRs at all HRTs studied with 4% CO_2-enriched air. TAN removal ranges were between 66-73% for C5 reactor, 87-93% for C8 reactor and 87-100% for C12 reactor at steady-state. It should be noted that TAN removal observed could not be related to nitrification or stripping. The nitirification could not have performed because operational conditions such as

continuous illumination, optimum pH and temperature favor microalgal growth and inhibit bacterial growth inheret to wastewater (thickener supernatant). Moreover, No NO_x could be detected from the NO_2^- and NO_3^- measurements done regularly (operation days of 0, 18, 31, 43, 52). Removal of TAN via stripping mechanisim was insignificant when the pH was lower than 8. Highest TAN that could be stripped was calculated as 0.44 mg/L, 0.32 mg/L and 0.20 mg/L for C5, C8 and C12 reactors, respectively considering the corresponding temperature, pH conditions and influent TAN concentration at steady-state (Equation 4-1 and 4-2) (Anthonisen, et al., 1976). This correnponds to 0.17%, 0.27% and 0.37% of the influent TAN concentration for C5, C8 and C12 reactors, respectively indicating negligible ammonia stripping.

PO_4-P removal ranges were between 46-52% for C5 reactor, 94-99% for C8 reactor and 95-100% for C12 reactor at steady-state. As mentioned in Section 4.2.2, phosphorus removal can be achieved with biological removal and chemical precipitation. Similar to the municipal wastewater study, lack of anaerobic zones in the system prevents luxury phosphorus uptake from the system by PAOs (Morse, et al., 1998). Phosphorus can be chemically precipitated with the additional dosage of metal (iron, aluminum and calcium) salts into the system (Morse et al., 1998). Precipitation of phosphorus occurs at elevated pHs (9-11) (Cai et al., 2013). In the present study, pH range between 5.7-6.66; therefore, phosphorus precipitation was not expected.

Table 4-4 Average steady-state values of parameters in C5, C8 and C12 reactors

Parameter	Reactor Name		
	C5	C8	C12
Optical Density	2.68±0.2	2.97±0.2	4.001±0.25
TS (mg/L)	1651±146	1920±112	2347±121
VS (mg/L)	703±55	941±34	1029±121
Chlorophyll-a (mg/L)	-	74±7.5	92±7.7
OD ($664_b/665_a$)	-	1.63±0.03	1.65±0.04
TN Removal (%)	-	90±1	96.5±2.03
TN-effluent (mg/L)	-	13±1.6	4.55±2.7
TAN Removal (%)	71±2	90±1.5	97.5±1.94
TAN-effluent (mg/L)	35±2	12.3±2.1	2.91±1.97
PO_4-P Removal (%)	49±2.1	95±1.3	97±1.26
PO_4-P effluent (mg/L)	10±0.45	0.96±0.26	0.54±0.24
sCOD Removal (%)	39±3.7	59±4	62±3.9
sCOD effluent (mg/L)	310±19	210±19	195±19
CO_2 Removal (%)	4.1±0.6	17.4±0.32	17.7±0.28

As expected, higher biomass concentration and removal performances were observed in the reactor operated with higher HRT (Table 4-4) (Larsdotter, 2006; Tang et al., 2012). For example, optical density value of C12 reactor is 49% and 35% higher than optical density values of C5 and C8 reactor, respectively. When all reactors were considered, it can be stated that fluctuations in optical density, TS, VS, TAN and PO_4-P were observed for all of the reactors before steady-state was achieved. To summarize general trend, optical density once peaked and decreased, then became steady. This was a result of self-shading effect. Since nutrient concentration in the mixed wastewater (Appendix–G) was abundant for microalgal culture, an immediate increase was observed initially. After a peak was reached at biomass density, optical density started to decrease as a result of self-shading effect. Overly populated microalgal cells inhibited the light penetration through the reactors (Sforza et al.,

2014). Then, a steady-state was reached for optical density where culture could sustain itself with the minimized self-shading effect. Even though this pattern was observed for each reactor, increase or decrease in the optical density values and the treatment performances became sharper as HRT shortened. Namely, in C5 reactor, fluctuations were significant because 5-day HRT (i.e. 5-day SRT) caused high nutrient replenishment rates and high nutrient load to the reactor; thus, might have resulted in shorter adaptation period and more vulnurable culture. In C12 reactor, on the other hand, microalgal culture adapted more easily and became more stable due to lower nutrient load and replenishment rate of the system.

As mentioned before, the removal efficiencies of C5 reactor was relatively lower than the other reactors (C8 and C12). Because of lower biomass concentration and removal performances, C5 reactor was terminated at the 28th day of operation. This lower performance could be a result of short HRT of the reactor. Similar studies reported higher removal efficiencies in continuous reactors operated at 5-day or lower HRTs. In the study of Woertz et al. (2010), municipal wastewater was treated in semi-continuous reactors with *Chlorella vulgaris*. 98% TAN and 93% PO_4-P removal was reported in reactors aerated with 2% CO_2-enriched air and operated at 2-day HRT. Influent concentrations of TAN and PO_4-P were 39 mg TAN/L and 2.1 mg P/L. However, 100% TAN and PO_4-P removal was reported when reactor was operated at 3-day HRT. As there were still N and P left to remove, the system was not N or P-limiting. Low HRT caused the decrease in removal rates. Similarly, in the study of Wang et al. (2010), digested dairy manure with 80-90 mg/L TAN and 5.5-6.5 mg/L TP influent concentrations were treated in semi-continuous reactors with *Chlorella vulgaris.* Around 50% TAN and 30% TP was removed at 5-day HRT. When HRT was increased to 10 days, TAN and TP removal efficiencies increased to 58% and 82%, respectively. Only after 20-day of HRT, 100% TAN and 90% TP could be removed. Higher HRTs are needed when treating high strength wastewaters. That is the reason, in the study of Woertz et al. (2010), why the nutrients were removed from low strength wastewater (39 mg TAN/L and 2.1 mg PO_4-P/L) almost completely at a shorter HRT (3 days). However, it is needed 20-day HRT to remove

100% TAN from digested dairy manure (Wang et al., 2010). Similar to those studies, in present study, only 71% TAN, 49% PO_4-P, 39% sCOD and 4.1% CO_2 could be removed at C5 reactor. Removal efficiencies showed that the system was not N, P or C-limiting. Influent concentration of TAN and PO_4-P in mixed wastewater were around 115-120 mg/L and 20 mg/L, respectively (Table G-1, Appendix-G). Influent concentrations showed that mixed wastewater was a high strenght wastewater as TAN and PO_4-P concentrations were higher than 75 mg/L and 15 mg/L, respectively (Henze and Morgens, 2008). Therefore HRT of the system should be higher to treat more nutrients. That is the reason of high removal efficiencies and biomass concentrations of C8 and C12 reactors. In the study of Wang et al. (2010), removal efficiencies at 10-day HRT were lower than C8 and C12 reactors even though influent concentrations were lower than those in the present study. The reason could be related with lower CO_2 content (2%) of the airflow in the study of Wang et al. (2010) than the present study (4%). In the present study, better efficiencies were obtained with lower HRTs with respect to the study of Wang et al. (2010). Better results were obtained in the present study with respect to similar studies (Boonchai et al., 2012; Kapdan and Aslan, 2008; Li et al., 2013; Woertz et al., 2010); however, influent nutrient concentrations and HRTs of these studies are too small for the present study to make a proper comparison.

The related regulation for the discharge of coke wastewater is Table 9.2 of water pollution control regulation (MoFWA, 2004). However, no discharge criteria for nitrogen or phosphorus were determined for coke wastewater in the mentioned regulation. As mixed wastewater also contains thickener supernatant, discharge criteria for urban wastewater may apply. According to the table 2 of urban wastewater treatment regulation, total nitrogen and total phosphorus discharge concentrations should be below 10 and 1 mg/L or removal rates of the parameters should be 80% and 70-80% or more, respectively. Average TN and PO_4-P effluent concentrations of C12, which were 4.55 mg/L N and 0.54 mg/L P, respectively, complied with the discharge criteria. Average PO_4-P effluent concentration of C8 reactor, 0.96 mg/L P, is below discharge criteria for P. C8 reactor complies with the criteria for nitrogen,

since its average TN removal was 90% which is more than nitrogen removal criteria (80%), even though average TN concentration of C8 reactor, 13 mg/L N, is beyond discharge criteria for N (10 mg/L). Nevertheless, C12 reactor performed best and its effluent concentrations complied with the regulations. Effluents of C5 reactor, on the other hand, do not comply due to its low removal rates and high effluent concentrations of nitrogen and phosphorus.

According to the growth patterns shown in Figure 4-14, Figure 4-15, Figure 4-17, it can be said that toxic effects of heavy metal and phenol content (Table 3-3) of the coke wastewater were overcame by microalgal culture. 950 mg/L phenol was present in the coke wastewater. Dilution with thickener supernatant reduced the phenol value to 19 mg/L and thus probably help microalgal culture to adapt. Scragg (2006) reported that growth pattern of *Chlorella vulgaris* culture in phenol-free conditions resemles to the growth pattern of the culture below 300 mg/L phenol.

After dilution with the thickener supernatant, cadmium (Cd) concentration of the coke wastewater became 1.4 x 10^{-9} mg/L. Bajguz (2000) reported that chorophyll content of Chlorella vulgaris cells grew in 10^{-6} mg/L Cd and heavy-metal free conditions were close to each other. Total chromium and iron concentration of the mixed wastewater in present study was 0.156 µg/L and 0.18 mg/L, respectively. Abou-Shanab et al. (2013) was able to grow polycultural microalgae (including Chlorella vulgaris) with piggery wastewater containing 30 µg/L Chromium and 0.22 mg/L iron. 50 µg/cm3 Arsenic (As) was reported not to affect the growth of Chlorella vulgaris (Suhendrayatna et al., 1999). As mixed wastewater only contains 15 µg/L, it has no inhibitory effect on microalgal culture. To conclude, as a result of the dilution with another wastewater, possible inhibitory effects of phenol and heavy metals were overcame and microalgae could be grown efficiently.

On average, 0.37 g CO_2/h in C8 reactor and 0.38 g CO_2/h in C12 reactor were removed at steady-state. This corresponds to very close CO_2 removal efficiencies of 17.4% and 17.7% for C8 and C12 reactors, respectively. Likewise, the CO_2

biofixation rate of both reactors, which was calculated with algal production rate and carbon content of algal cells, were similar; 424 mg/L/d for C12 and 418 mg/L/d for C8 reactor. Similar CO_2 removal efficiencies such as 16% (Chiu et al., 2008) and 17% (de Morais and Costa, 2007) were achieved in bubble column PBRs in literature similar to the PBRs used in the present study (Table 4-5). However, some of the studies from literature achieved higher CO_2 removal rates (Chiu et al., 2008; Costa et al., 2015; Keffer and Kleinheinz, 2002). 27% CO_2 removal efficiency was reported by Chlorella sp. in semi-continuous reactor aerated with 5% CO_2-enriched air (Chiu et al., 2008). Optical density (682 nm) of the culture was reported to be between 3.68-4.22. Optical density values, airflow's CO_2 percentages, operational mode of the present study and the study of Chiu et al. (2008) were similar to each other. However, HRT, wastewater type (or basal medium) and airflow rate of the systems differ. In the study of Chiu et al. (2008), the reactor was illuminated with the light intensity of 300 μmol. $m^{-2}.s^{-1}$, aerated with 0.25 vvm and operated with 2-day HRT. 2-day HRT would be too short for the wastewater of present study and 300 μmol. $m^{-2}.s^{-1}$ may cause photoinhibition (Carvalho et al., 2011). Lower airflow rate (0.25 vvm), that is, higher CO_2 solubility could explain the difference between CO_2 removal rates of the present study and the study of Chiu et al. (2008) On the other hand, using basal medium instead of real wastewater could be another factor that affected CO_2 removal efficiencies. Modified Zarrouk medium, modified f/2 medium and double strength mineral medium were used in the studies of Chiu et al. (2008), Costa et al. (2015), Keffer and Kleinheinz (2002), respectively. Basal medium has advantages over real wastewater because of no turbidity of impurities in wastewater, no competition between micoalgae and other organisms coming from wastewater. Additionally, content of basal mediums is generally determined to favor microalgal growth; however, some of the ingredients (phenol, chromium, cadmium, arsenic, cyanide, etc.) in the wastewater may obstruct microalgal growth.

Table 4-5 Comparison of some microalgal studies with the present study

Microalgae species	**Wastewater**	**PBR**	**CO_2 content of feeding gas**	**Airflow rate (vvm)**	**CO_2 removal efficiency**	**REF**
Chlorella sp.	Basal medium	Single semi-continuous PBR	2-15%	0.25	16-27%	Chiu et al. (2008)
Spirulina sp.	Basal medium	Raceway-type PBR	102 g/L (Flue gas)	0.3	24%	Costa et al. (2015)
Scenedesmus obliquus					13%	
Spirulina sp.	Basal medium	Bubble-column PBRs	0-12%	0.3	6-17%	de Morais and Costa, (2007)
Scenedesmus obliquus					4-8%	
Chlorella vulgaris	Basal medium	Tubular PBR	0.2%	0.5	56%	Keffer and Kleinheinz (2002)
Chlorella vulgaris	Coke wastewater	Bubble-column PBR	4%	0.5	17.7%	Present study
				0.2	21%	

Therefore, two methods were tried to improve CO_2 removal performance of the reactors. Firstly, pH was decreased to lower levels to improve CO_2 solubility and availability (C8 reactor). Yet, adjusting pH to a lower value was not suitable for microlgal culture as 4-5 pH is not appropriate for culture to survive. Secondly, the air flowrate was decreased to improve CO_2 solubility and availability (C12 reactor). Secondly, decreasing the inflow rate of CO_2-enriched air improved the removal of CO_2 by increasing its solubility. In addition, 0.2 vvm was found to be appropriate for C12 reactor. The improved CO2 removal efficiency was 21%, which is comparable to the 17.7% CO_2 removal efficiency.

sCOD removal efficiencies of the reactors (C5, C8 and C12) operated with industrial wastewater were higher than the semi-continuous reactors (X1 and X2) operated with municipal wastewater (Section 4.2.2). 9% and 19% sCOD were removed from X1 (2-day HRT) and X2 (4-day HRT) reactors, respectively. During the present study, 39-62% average sCOD removal efficiencies were achieved. *Chlorella vulgaris* is a mixotrophic organism. It can utilize carbon (C) from CO_2 through photosynthesis and organic-C heterotrophically (Heredia-Arroyo et al., 2011). In municipal wastewater study (Section 4.2.2), low nutrient inflow and continuous illumination might have led microalgae to autotrophy (Markou and Georgakakis, 2011); therefore, low sCOD was consumed. However, in the present study, nutrient concentration was much higher than that of municipal wastewater. Moreover, even though self-shading effect was minimized by the microalgal system by adjusting its cell density, high biomass concentration was probably causing the shading effects and creating photoperiods (Sforza et al., 2014). This might have resulted in in higher consumption of sCOD heterotrophically by the culture. In addition to that, higher C-consumption whether by utilizing organic-C from wastewater (sCOD) or capturing C from airflow, enhances the elemental composition of the microalgae culture (Mandalam and Palsson, 1998). C-content of the culture was analyzed (Appendix-H). The analyses indicated that C-content of microalgal culture increased in the present study, compared to those cultivated with 3N BBM+V (Section 4.1.2) and with municipal wastewater (Section 4.2.2) where the cultures were aerated only with air. These results also verify that nutrient-rich environments allow more the C uptake.

Outcomes of the study were summarized as follows;

- The mixed wastewater could be treated with microalgal culture in semi-continuous reactors with 4% CO_2-enriched air at 5, 8 and 12-day HRTs.
- The highest biomass concentrations and removal rates were observed in C12 reactor (12-day HRT). The performance of C8 reactor (8-day HRT) was close to that of C12 reactor; however, the performance of C5 reactor was significantly lower than others.

- 4.1%, 17.4% and 17.7% CO_2 could be removed from the air flow with C5, C8 and C12 reactors, respectively. CO_2 removal efficiencies of C8 and C12 reactors were more or less the same even though there is 4-day difference between their HRTs.
- Adjusting pH to 4-5 at C8 reactor caused no increase in CO_2 as a matter of fact caused sharped decrease due to culture lost.
- Lowering the inflow rate to 0.2 vvm from 0.5 vvm at C12 reactor resulted in increase in CO_2 removal to around 21% without disrupting steady-state of the reactor.

CHAPTER 5

CONCLUSION

The results of the experiments presented in this thesis study lead us to a better understanding of growth, nutrient removal and carbon dioxide capture behavior of *Chlorella vulgaris* while working with different wastewaters as well as basal medium.

Growth phases and semi-continuous cultivation of axenic *Chlorella vulgaris* culture were investigated. The results revealed that;

- *Chlorella vulgaris* culture can be grown with 3N BBM+V in batch PBRs with a specific growth rate of 0.71 d^{-1}. After 12 days, culture reached stationary phase.
- pH control at feeding procedure and temperature regulation with ventilation are requirement for semi-continuous cultivation at steady-state.
- Gas transfer of PBR for the semi-continuous *Chlorella vulgaris* cultivation is appropriate to avoid oxygen accumulation.
- Continuous illumination was better than photoperiods for microalgal growth.
- A steady-state *Chlorella vulgaris* growth could be obtained in semi-continuous cultivation PBRs.
- Nutrient uptake rate by microalgae increases at steady-state conditions.

Semi-continuous, and unacclimated and acclimated batch PBRs were operated with microalgal culture to treat municipal wastewater. It was found out that;

- *Chlorella vulgaris* culture was able to remove nutrients from municipal wastewater in semi-continuous PBRs with 2, 4 and 8-day HRTs.

- Good nutrient removal rates were achieved with 2-day HRT such as 83-91% TAN, 85-90% TN and 68-75% PO_4-P removal.
- High nutrient removal rates were achieved with 4-day HRT such as 98-100% TAN, 93-98% TN and 85-99% PO_4-P removal.
- Even though 99-100% TAN and 64-91 PO_4-P removal efficiencies were achieved at 8-day HRT, microalgal biomass was determined to be lost with respect to chlorophyll-a content of the culture. Therefore, 4-day HRT was determined to be the optimum HRT for nutrient removal from municipal wastewater in semi-continuous PBRs aerated with air.
- Acclimated microalgal culture can grow faster and remove TAN faster (10.4 mg/L.d) than unacclimated one (4.5 mg/L.d).
- Unlike unacclimated culture, no lag phase was observed in batch PBRs for acclimated culture.

- Both 1/10 and 1/20 seeding ratios did not cause any self-shading effect on the growth of microalgal culture in batch PBRs. Removal rates of 1/10 seeding PBR (4 mg/L.d for TAN and 1.64 mg/L.d for PO4-P) was better than 1/20 seeding PBR (4.5 mg/L.d for TAN and 1.22 mg/L.d for PO4-P).

It was aimed to investigate the treatment of coke factory wastewater (industrial wastewater) via microalgal culture. Since coke factory wastewater contains high amounts of heavy metals and no phosphorus, it would be impossible to treat this wastewater without dilution and phosphorus addition. To solve these problems, another problematic wastewater, thickener supernatant, was used to dilute the coke factory wastewater and supply phosphorus. Initally, the optimum N/P ratio for *Chlorella vulgaris* was determined in batch PBRs. Semi-continuous PBR study was performed with the properly mixed coke factory wastewater and thickener supernatant to investigate nutrient removal and also to observe CO_2 sequestration.

- N/P ratio 6 was determined to be the optimum N/P ratio for microalgal culture to treat nitrogen and phosphorus completely. N/P ratio 8 turned out to be P-limiting while N/P ratio 10 was N-limiting for *Chlorella vulgaris*.

- The mixed wastewater could be treated with microalgal culture in semi-continuous PBRs with 4% CO_2-enriched air at 5, 8 and 12-day HRTs.
- The highest biomass concentrations and removal rates were observed at 12-day HRT. The effect of 8-day HRT was close to that of 12-day HRT; however, 5-day HRT led to significantly low treatment performance compared to others. On average, 97.5% TAN, 97% PO_4-P and 17.7 % CO_2 removal efficiencies were achieved at 12-day HRT.
- 4.1%, 17.4% and 17.7% CO_2 could be removed from air flow at 5, 8 and 12-day HRTs, respectively.
- Adjusting pH to 4-5 at 8-day HRT caused no increase in CO_2 removal as a matter of fact caused sharped decrease due to culture lost.
- Lowering the airflow rate improved CO_2 removal efficiency to 21% without disrupting steady-state at 12-day HRT.
- Two problematic wastewaters, thickener supernatant and coke factory wastewater, could be treated together economically without causing inhibitory effect on microalgae culture at an N/P ratio of 6.

The results of the experiments showed that *Chlorella vulgaris* can treat nutrients from municipal wastewater to problematic high strength wastewaters such as thickener supernatant and coke factory wastewater. Microalgae are easily adaptive, fast growing microorganisms. The optimum operational parameters such as illumination, air flow, CO_2 content of air and temperature should be investigated to get the best efficiency from them.

Future Recommendations

This study with microalgae focused on wastewater treatability of microalgae. Comparison of unacclimated and acclimated culture could be investigated in detail for each wastewater and basal medium. Investigation of CO_2 removal could be expended to varying air supply rates. Moreover, microalgae can be further used for treating flue gas, bioenergy production (biodiesel, bioethanol, biohydrogen and

biomethane), producing fertilizers, pharmaceuticals, pigments, and medical supplements. This thesis study is a part of TÜBİTAK project (111Y205). Within the context of this project, CO_2 capture feature and cultivation of microalgae were investigated with synthetic and real flue gas. Biomethane, biohydrogen and fertilizer potential of algal biomass harvested during reactor studies was also investigated. Furthermore, effect of NOx and SOx in flue gas on microalgae can be examined in the future studies. Lipid content of the harvested biomass for biodiesel production are important issues for bioenergy production to be investigated in the future researches.

REFERENCES

Abdel-Raouf, N., Al-Homaidan, A. A., and Ibraheem, I. B. M. (2012). Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences*, *19*(3), 257–275. http://doi.org/10.1016/j.sjbs.2012.04.005

Abou-Shanab, R. A. I., Ji, M. K., Kim, H. C., Paeng, K. J., and Jeon, B. H. (2013). Microalgal species growing on piggery wastewater as a valuable candidate for nutrient removal and biodiesel production. *Journal of Environmental Management*, *115*, 257–264. http://doi.org/10.1016/j.jenvman.2012.11.022

Acuner, E., and Dilek, F. B. (2004). Treatment of tectilon yellow 2G by Chlorella vulgaris. *Process Biochemistry*, *39*(5), 623–631. http://doi.org/10.1016/S0032-9592(03)00138-9

Al-lwayzy, S. H., Yusaf, T., and Al-Juboori, R. a. (2014). Biofuels from the fresh water microalgae chlorella vulgaris (FWM-CV) for diesel engines. *Energies*, *7*(3), 1829–1851. http://doi.org/10.3390/en7031829

Ambient Water Quality Criteria for Dissolved Oxygen. (2014). Retrieved April 21, 2012, from http://www.env.gov.bc.ca/wat/wq/BCguidelines/do/do-01.htm

Andersen, R. A. (2005). *Algal Culturing Techniques*. San Diego: Cambridge: Academic Press, Elsevier Science.

Anjos, M., Fernandes, B. D., Vicente, A. A., Teixeira, J. A., and Dragone, G. (2013). Optimization of CO_2 bio-mitigation by Chlorella vulgaris. *Bioresource Technology*, *139*, 149–54. http://doi.org/10.1016/j.biortech.2013.04.032

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., Stinath, E. G. (1976). Inhibition of nitrification by ammonia and nitrous-acid. *J. Water Pollut. Control Fed.*, *48*(5), 1976.

Aslan, S., and Kapdan, I. K. (2006). Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecological Engineering*, *28*(1), 64–70. http://doi.org/10.1016/j.ecoleng.2006.04.003

Azeez, R. A. (2009). A Study on The Effect Of Temperature on The Treatment of Industrial Wastewater Using Chlorella Vulgaris Alga. *Eng. and Tech. Journal*, *28*(4), 785–792.

Azov, Y., and Goldman, J. C. (1982). Free ammonia inhibition of algal photosynthesis in intensive cultures. *Applied and Environmental Microbiology*, *43*(4), 735–739.

Bajguz, A. (2000). Blockade of heavy metals accumulation in Chlorella vulgaris cells by 24-epibrassinolide. *Plant Physiology and Biochemistry*, *38*(10), 797–801. http://doi.org/10.1016/S0981-9428(00)01185-2

Benemann, J. R. (2000). Hydrogen production by microalgae. *Journal of Applied Phycology*, *12*, 291–300.

Benemann, J. R. (2009). Microalgal Biofuels: A Brief Introduction. Retrieved October 24, 2015, from http://advancedbiofuelsusa.info/wp-content/uploads/2009/03/microalgae-biofuels-an-introduction-july23-2009-benemann.pdf

Bich, N. N., Yaziz, M. I., and Bakti, N. A. K. (1999). Combination of Chlorella vulgaris and Eichhornia crassipes for wastewater nitrogen removal. *Water Research*, *33*(10), 2357–2362. http://doi.org/10.1016/S0043-1354(98)00439-4

Blair, M. F., Kokabian, B., and Gude, V. G. (2014). Light and growth medium effect on Chlorella vulgaris biomass production. *Journal of Environmental Chemical Engineering*, *2*(1), 665–674. http://doi.org/10.1016/j.jece.2013.11.005

Boonchai, R., Seo, G. T., Park, D. R., and Seong, C. Y. (2012). Microalgae Photobioreactor for Nitrogen and Phosphorus Removal from Wastewater of Sewage Treatment Plant. *International Journal of Bioscience, Biochemistry and Bioinformatics*, *2*(6), 407–410. http://doi.org/10.7763/IJBBB.2012.V2.143

Borowitzka, M. a. (1999). Commercial production of microalgae: ponds, tanks, tubes and fermenters. *Journal of Biotechnology*, *70*(1–3), 313–321. http://doi.org/10.1016/S0168-1656(99)00083-8

Borowitzka, M. A. (2012). Algae Mass Cultivation Systems. Retrieved from http://www.dbs.nus.edu.sg/biofuel2012/presentation/22 Borowitzka (ok).pdf

Brennan, L., and 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. http://doi.org/10.1016/j.rser.2009.10.009

Brown, M. R. (1997). Nutritional properties of microalgae for mariculture. *Aquaculture*, *151*, 315–331. http://doi.org/10.1.1.459.6284

Bryant, D. a, and Frigaard, N.-U. (2006). Prokaryotic photosynthesis and phototrophy illuminated. *Trends in Microbiology*, *14*(11), 488–96. http://doi.org/10.1016/j.tim.2006.09.001

Cai, T., Park, S. Y., and Li, Y. (2013). Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renewable and Sustainable Energy Reviews*, *19*, 360–369. http://doi.org/10.1016/j.rser.2012.11.030

Carvalho, A. P., Meireles, L. a., and Malcata, F. X. (2006). Microalgal reactors: A review of enclosed system designs and performances. *Biotechnology Progress*, *22*(6), 1490–1506. http://doi.org/10.1021/bp060065r

Carvalho, A. P., Silva, S. O., Baptista, J. M., and Malcata, F. X. (2011). Light requirements in microalgal photobioreactors: an overview of biophotonic aspects. *Applied Microbiology and Biotechnology*, *89*(5), 1275–88. http://doi.org/10.1007/s00253-010-3047-8

Cassidy, K. O. (2011). *Evaluating Algal Growth at Different Temperatures*. University of Kentucky. Retrieved from http://uknowledge.uky.edu/bae_etds/3

CCAP. (2013). Strain Information:211/11B. Retrieved January 1, 2012, from https://www.ccap.ac.uk/strain_info.php?Strain_No=211/11B

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

Cheng, L., Zhang, L., Chen, H., and Gao, C. (2006). Carbon dioxide removal from air by microalgae cultured in a membrane-photobioreactor. *Separation and Purification Technology*, *50*(3), 324–329.

Chinnasamy, S., Ramakrishnan, B., Bhatnagar, A., and Das, K. C. (2009). Biomass production potential of a wastewater alga Chlorella vulgaris ARC 1 under elevated levels of CO_2 and temperature. *International Journal of Molecular Sciences*, *10*(2), 518–32. http://doi.org/10.3390/ijms10020518

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances*, *25*(3), 294–306. http://doi.org/10.1016/j.biotechadv.2007.02.001

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

Chiu, S.-Y., Kao, C.-Y., Chen, C.-H., Kuan, T.-C., Ong, S.-C., and Lin, C.-S. (2008). Reduction of CO2 by a high-density culture of Chlorella sp. in a semicontinuous photobioreactor. *Bioresource Technology*, *99*(9), 3389–96.

Christenson, L., and Sims, R. (2011). Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advances*, *29*(6), 686–702. http://doi.org/10.1016/j.biotechadv.2011.05.015

Costa, J. A. V., Morais, M. G., Radmann, E. M., Santana, F. B., Camerini, F. V., Souza, M. da R. A. Z., … L uacute cio, B. (2015). Biofixation of carbon dioxide from coal station flue gas using Spirulina sp. LEB 18 and Scenedesmus obliquus LEB 22. *African Journal of Microbiology Research*, *9*(44), 2202–2208. http://doi.org/10.5897/AJMR2015.7640

Craggs, R. (2009). Bio-oil from wastewater algae. http://doi.org/10.1353/mis.0.0042

de-Bashan, L. E., Moreno, M., Hernandez, J.-P., and Bashan, Y. (2002). Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae Chlorella vulgaris coimmobilized in alginate beads with the microalgae growth-promoting bacterium Azospirillum brasilense. *Water Research*, *36*(12), 2941–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12171390

de Morais, M. G., and Costa, J. A. V. (2007). Biofixation of carbon dioxide by Spirulina sp. and Scenedesmus obliquus cultivated in a three-stage serial tubular photobioreactor. *Journal of Biotechnology*, *129*(3), 439–445.

Degen, J., Uebele, a, Retze, a, Schmid-Staiger, U., and Trösch, W. (2001, December 28). A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect. *Journal of Biotechnology*. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11640980

Demirbas, A. (2011). Biodiesel from oilgae, biofixation of carbon dioxide by microalgae: A solution to pollution problems. *Applied Energy*, *88*(10), 3541–3547. http://doi.org/10.1016/j.apenergy.2010.12.050

Eriksen, N. T., Poulsen, B. R., and Iversen, J. J. L. (1998). Dual sparging laboratory-scale photobioreactor for continuous production of microalgae. *Journal of Applied Phycology*, *10*(1), 377–382.

Fan, L.-H., Zhang, Y.-T., Cheng, L.-H., Zhang, L., Tang, D.-S., and Chen, H.-L. (2007). Optimization of Carbon Dioxide Fixation byChlorella vulgaris Cultivated in a Membrane-Photobioreactor. *Chemical Engineering & Technology*, *30*(8), 1094–1099. http://doi.org/10.1002/ceat.200700141

Feng, Y., Li, C., and Zhang, D. (2011). Lipid production of Chlorella vulgaris cultured in artificial wastewater medium. *Bioresource Technology*, *102*(1), 101–5. http://doi.org/10.1016/j.biortech.2010.06.016

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

Gonzalez, L. E. (1997). Efficiency of ammonia and phosphorus removal from a colombian agroindustrial wastewater by the microalgae Chlorella vulgaris and Scenedesmus dimorphus, *60*, 259–262.

Guillard, R., and Ryther, J. (1962). Studies of marine planktonic diatoms . I . Cyclotella nana Hustedt and Detonula confervaceae (Cleve) Gran. *Can. J. Microbiol.*, *8*, 229–239.

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

He, Q., Yang, H., and Hu, C. (2015). Optimizing light regimes on growth and lipid accumulation in Ankistrodesmus fusiformis H1 for biodiesel production. *Bioresource Technology*, *198*, 876–883.

Henze, and Morgens. (2008). Wastewater Characterization. In *Biological Wastewater Treatment: Principles, Modelling and Design* (pp. 33–52). London:IWA Publishing.

Heredia-Arroyo, T., Wei, W., Ruan, R., and Hu, B. (2011). Mixotrophic cultivation of Chlorella vulgaris and its potential application for the oil accumulation from non-sugar materials. *Biomass and Bioenergy*, *35*(5), 2245–2253. http://doi.org/10.1016/j.biombioe.2011.02.036

Holland, H. D. (1984). *The Chemical Evolution of the Atmosphere and Oceans*. Princeton: New Jersey: Princeton University.

Hu, Q. (2004). Environmental Effects on Cell Composition. In *Handbook of Microalgal Culture: Biotechnology and Applied Phycology* (pp. 83–93). Oxford: UK: Blackwell. http://doi.org/10.1111/j.1094-348X.2007.00163.x

Hulatt, C. J., and Thomas, D. N. (2011). Productivity, carbon dioxide uptake and net energy return of microalgal bubble column photobioreactors. *Bioresource Technology*, *102*(10), 5775–5787.

IEA. (2007). *World Energy Outlook - China and India Insignt*. Paris.

Iersel, S. van, Gamba, L., Rossi, A., Alberici, S., Dehue, B., Staaji, J. van de, and Flammini, A. (2009). *Algae-based biofuels: A Review of Challenges and Opportunities for Ceveloping Countries*. Retrieved from http://www.fao.org/3/a-ak333e.pdf

Jacob-Lopes, E., Scoparo, C. H. G., Lacerda, L. M. C. F., and Franco, T. T. (2009). Effect of light cycles (night/day) on CO2 fixation and biomass production by microalgae in photobioreactors. *Chemical Engineering and Processing: Process Intensification*, *48*(1), 306–310.

Johnson, M. B. (2009). *Microalgal Biodiesel Production through a Novel Attached Culture System and Conversion Parameters*. Virginia Polytechnic Institute and State University.

Johnson, W. Z. and M. B. (2009). Microalgae as a Feedstock for Biofuel Production. *New Biotechnology*, *25*, 442–886. http://doi.org/10.1016/j.nbt.2009.06.630

Juneja, A., Ceballos, R., and Murthy, G. (2013). Effects of Environmental Factors and Nutrient Availability on the Biochemical Composition of Algae for Biofuels Production: A Review. *Energies*, *6*(9), 4607–4638.

Kapdan, I. K., and Aslan, S. (2008). Application of the Stover – Kincannon kinetic model to nitrogen removal by Chlorella vulgaris in a continuously operated immobilized photobioreactor system. *Chem Technol Biotechnol*, *83*, 998–1005. http://doi.org/10.1002/jctb

Keffer, J. E., and Kleinheinz, G. T. (2002). Use of Chlorella vulgaris for CO(2) mitigation in a photobioreactor. *Journal of Industrial Microbiology & Biotechnology*, *29*(5), 275–80. http://doi.org/10.1038/sj.jim.7000313

Khalili, A., Najafpour, G. D., Amini, G., and Samkhaniyani, F. (2015). Influence of Nutrients and LED Light Intensities on Biomass Production of Microalgae Chlorella vulgaris. *Biotechnology and Bioprocess Engineering*, 284–290.

Kim, J., Lingaraju, B. P., Rheaume, R., Lee, J.-Y., and Siddiqui, K. F. (2010). Removal of Ammonia from Wastewater Effluent by Chlorella Vulgaris. *Tsinghua Science & Technology*, *15*(4), 391–396. http://doi.org/10.1016/S1007-0214(10)70078-X

Kitaya, Y., Azuma, H., and Kiyota, M. (2005). Effects of temperature, CO2/O2 concentrations and light intensity on cellular multiplication of microalgae, Euglena gracilis. *Advances in Space Research*, *35*, 1584–1588. http://doi.org/10.1016/j.asr.2005.03.039

Kligerman, D. C., and Bouwer, E. J. (2015). Prospects for biodiesel production from algae-based wastewater treatment in Brazil: A review. *Renewable and Sustainable Energy Reviews*, *52*, 1834–1846.

Kong, Q. X., Li, L., Martinez, B., Chen, P., and Ruan, R. (2010). Culture of microalgae chlamydomonas reinhardtii in wastewater for biomass feedstock production. *Applied Biochemistry and Biotechnology*, *160*(1), 9–18. http://doi.org/10.1007/s12010-009-8670-4

Kumar, A., Ergas, S., Yuan, X., Sahu, A., Zhang, Q., Dewulf, J., … van Langenhove, H. (2010). Enhanced CO(2) fixation and biofuel production via microalgae: recent developments and future directions. *Trends in Biotechnology*, *28*(7), 371–80. http://doi.org/10.1016/j.tibtech.2010.04.004

Kumar, K., and Das, D. (2012). Bioresource Technology Growth characteristics of Chlorella sorokiniana in airlift and bubble column photobioreactors. *Bioresource Technology*, *116*, 307–313.

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

Lam, M. K., and Lee, K. T. (2012a). Microalgae biofuels: A critical review of issues, problems and the way forward. *Biotechnology Advances*, *30*(3), 673–90. http://doi.org/10.1016/j.biotechadv.2011.11.008

Lam, M. K., and Lee, K. T. (2012b). Potential of using organic fertilizer to cultivate Chlorella vulgaris for biodiesel production. *Applied Energy*, *94*, 303–308. http://doi.org/10.1016/j.apenergy.2012.01.075

Larkum, A. W. D., Ross, I. L., Kruse, O., and Hankamer, B. (2012). Selection, breeding and engineering of microalgae for bioenergy and biofuel production. *Trends in Biotechnology*, *30*(4), 198–205.

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

Larsdotter, K., Jansen, J. L. C., and Dalhammar, G. (2010). Phosphorus removal from wastewater by microalgae in Sweden--a year-round perspective. *Environmental Technology*, *31*(2), 117–23. http://doi.org/10.1080/09593330903382815

Lau, A. S., Tam, N. F. Y., and Wong, Y. S. (1996). Wastewater Nutrients Removal by Chlorella Vulgaris: Optimization Through Acclimation. *Environmental Technology*, 183–189.

Lee, K., and Lee, C.-G. (2001). Effect of Light/dark Cycles on Wastewater Treatments by Microalgae. *Biotechnology and Bioprocess Engineering*, *6*, 194–199. http://doi.org/10.1007/BF02932550

Lee, R. E. (2008). *Phycology*. New York: Cambridge University Press.

Li, C., Yang, H., Li, Y., Cheng, L., Zhang, M., Zhang, L., and Wang, W. (2013). Novel bioconversions of municipal effluent and CO2 into protein riched Chlorella vulgaris biomass. *Bioresource Technology*, *132*, 171–7. http://doi.org/10.1016/j.biortech.2012.12.017

Li, Y., Zhou, W., Hu, B., Min, M., Chen, P., and Ruan, R. R. (2012). Effect of light intensity on algal biomass accumulation and biodiesel production for mixotrophic strains Chlorella kessleri and Chlorella protothecoide cultivated in highly concentrated municipal wastewater. *Biotechnology & Bioengineering*, 2222–2229.

Liao, Q., Li, L., Chen, R., and Zhu, X. (2014). A novel photobioreactor generating the light/dark cycle to improve microalgae cultivation. *Bioresource Technology*, *161*, 186–191. http://doi.org/10.1016/j.biortech.2014.02.119

Lim, S.-L., Chu, W.-L., and Phang, S.-M. (2010). Use of Chlorella vulgaris for bioremediation of textile wastewater. *Bioresource Technology*, *101*(19), 7314–22. http://doi.org/10.1016/j.biortech.2010.04.092

Luo, H.-P., and 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. http://doi.org/10.1002/bit.10831

Lustigman, B., Lee, L. H., and Khalil, A. (1995). Effects of Nickel and pH on the growth of Chlorella vulgaris. *Bulletin of Environmental Contamination and Toxicology*, 73–80.

Mandalam, R. K., and Palsson, B. O. (1998). Elemental balancing of biomass and medium composition enhances growth capacity in high-density Chlorella vulgaris cultures. *Biotehnology & Bioengineering*, 605–611. http://doi.org/10.1111/j.1094-348X.2007.00163.x

Markou, G., and Georgakakis, D. (2011). Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review. *Applied Energy*, *88*(10), 3389–3401. http://doi.org/10.1016/j.apenergy.2010.12.042

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

Mayo, A. W. (1997). Effects of temperature and pH on the kinetic growth of unialga Chlorella vulgaris cultures containing bacteria. *Water Environ. Res.*, 64–72. http://doi.org/10.3998/3336451.0014.102

McGriff, E. C., and McKinney, R. E. (1972). The removal of nutrients and organics by activated algae. *Water Research*, *6*(10), 1155–1164. http://doi.org/10.1016/0043-1354(72)90015-2

Mckendry, P. (2002). Energy production from biomass (part 2): conversion technologies, *83*(July 2001), 47–54. http://doi.org/10.1016/S0960-8524(01)00119-5

Merchuk, J., Ronen, M., Giris, S., and Arad, S. (1998). Light/dark cycles in the growth of the red microalga porphyridium sp. *Biotechnology and Bioengineering*, *59*(6), 705–13. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10099391

MoFWA. Water Pollution Control Regulation Vol 25687 (2004). Turkish Ministry of Forestry and Water Affairs, Official Gazette.

MoFWA. Urban Wasewater Treatment Regulation Vol 26047 (2006). Turkish Ministry of Forestry and Water Affairs, Official Gazette.

Mohammed, S. A. M., and Shanshool, H. A. (2009). Phosphorus Removal from Water and Waste Water by Chemical Precipitation Using Alum and Calcium Chloride. *Iraqi Journal of Chemical and Petroleum Engineering*, *10*(2), 35–42.

Molinuevo-Salces, B., Mahdy, A., Ballesteros, M., and González-Fernández, C. (2016). From piggery wastewater nutrients to biogas: Microalgae biomass revalorization through anaerobic digestion. *Renewable Energy*, *96*, 1103–1110. http://doi.org/10.1016/j.renene.2016.01.090

Monkonsit, S., Powtongsook, S., and Pavasant, P. (2011). Comparison between Airlift Photobioreactor and Bubble Column for Skeletonema Costatum Cultivation. *Engineering Journal*, *15*(4), 53–64. http://doi.org/10.4186/ej.2011.15.4.53

Morais, M. G., Radmann, E. M., and Costa, J. A. V. (2011). Biofixation of CO2 from synthetic combustion gas using cultivated microalgae in three-stage serial tubular photobioreactors. *Zeitschrift Fur Naturforschung - Section C Journal of Biosciences*, *66 C*(5–6), 313–318. http://doi.org/10.5560/ZNC.2011.66c0313

Morse, G. K., Brett, S. W., Guy, J. A., and Lester, J. N. U. (1998). Review : Phosphorus removal and recovery technologies. *The Science of the Total Environment*, *212*, 69–81.

Moser, A. (1992). Tubular Bioreactors : Case Study of Production and Scientific Research. *Biotechnology and Bioengineering*, *37*(7695), 1054–1065.

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

Myers, J. (1953). Growth kinetics of algae in relation to the problemsof mass culture. In *In Algal Cultures from Laboratory to Pilot Plant* (pp. 34–54). Washington: D.C.: Carnigie Inst. of Wash.

Octavio, Escalante, De-Bashan, and Bashan. (2011). Heterotrophic cultures of microalgae: metabolism and potential products. *Water Research*, *45*(1), 11–36. http://doi.org/10.1016/j.watres.2010.08.037

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

Olaizola, M., Bridges, T., Flores, S., Griswold, L., Morency, J., and Nakamura T. (2004). Microalgal removal of CO2 from flue gases: CO2 capture from a coal combustor. *Mera Pharmaceuticals, Inc., Kailua-Kona, Physical Sciences Inc., Andover.*

Park, J., Jin, H.-F., Lim, B.-R., Park, K.-Y., and Lee, K. (2010). Ammonia removal from anaerobic digestion effluent of livestock waste using green alga Scenedesmus sp. *Bioresource Technology*, *101*(22), 8649–57. http://doi.org/10.1016/j.biortech.2010.06.142

Park, K.-H., and Lee, C.-G. (2001). Effectiveness of flashing light for increasing photosynthetic efficiency of microalgal cultures over a critical cell density. *Biotechnology and Bioprocess Engineering*, *6*(3), 189–193. http://doi.org/10.1007/BF02932549

Pawlowski, A., Mendoza, J. L., Guzmán, J. L., Berenguel, M., Acién, F. G., and Dormido, S. (2014). Effective utilization of flue gases in raceway reactor with event-based pH control for microalgae culture. *Bioresource Technology*, *170*, 1–9. http://doi.org/10.1016/j.biortech.2014.07.088

Paxinos, R., and Mitchell, J. G. (2000). A rapid Utermöhl method for estimating algal numbers. *Journal of Plankton Research*, *22*(12), 2255–2262. http://doi.org/10.1093/plankt/22.12.2255

Perez-Garcia, O., Bashan, Y., and Esther Puente, M. (2011). Organic Carbon Supplementation of Sterilized Municipal Wastewater Is Essential for Heterotrophic Growth and Removing Ammonium By the Microalga Chlorella Vulgaris. *Journal of Phycology*, *47*(1), 190–199. http://doi.org/10.1111/j.1529-8817.2010.00934.x

Popa, P., Timofti, M., Voiculescu, M., Dragan, S., Trif, C., and Georgescu, L. P. (2012). The cientific WorldJOURNAL Study of Physico-Chemical Characteristics of Wastewater in an Urban Agglomeration in Romania, *2012*. http://doi.org/10.1100/2012/549028

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

Putman, W. M., Ott, L., Darmenov, A., and daSilva, A. (2016). A Global Perspective of Atmospheric Carbon Dioxide Concentrations. *Parallel Computing*, *55*, 2–8. http://doi.org/10.1016/j.parco.2016.03.001

Rashid, N., Rehman, M. S. U., Memon, S., Ur Rahman, Z., Lee, K., and Han, J. I. (2013). Current status, barriers and developments in biohydrogen production by microalgae. *Renewable and Sustainable Energy Reviews*, *22*, 571–579. http://doi.org/10.1016/j.rser.2013.01.051

Renuka, N., Sood, a., Ratha, S. K., Prasanna, R., and Ahluwalia, a. S. (2013). Nutrient Sequestration, Biomass Production By Microalgae and Phytoremediation of Sewage Water. *International Journal of Phytoremediation*, *15*(8), 789–800. http://doi.org/10.1080/15226514.2012.736436

Ruiz-Marin, A., Mendoza-Espinosa, L. G., and 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. http://doi.org/10.1016/j.biortech.2009.02.076

Ruiz-Martinez, a, Martin Garcia, N., Romero, I., Seco, a, and Ferrer, J. (2012). Microalgae cultivation in wastewater: nutrient removal from anaerobic membrane bioreactor effluent. *Bioresource Technology*, *126*, 247–53. http://doi.org/10.1016/j.biortech.2012.09.022

Ruiz, J., Álvarez-Díaz, P. D., Arbib, Z., Garrido-Pérez, C., Barragán, J., and Perales, J. a. (2013). Performance of a flat panel reactor in the continuous culture of microalgae in urban wastewater: prediction from a batch experiment. *Bioresource Technology*, *127*, 456–63.

Sankar, V., Daniel, D. K., Krastanov, A., Division, C. E., and Nadu, T. (2011). Carbon Dioxide Fixation by Chlorella Minutissima Batch Cultures in a Stirred Tank. *Biotechnology & Biotechnological Equipment*, *25*(3), 2468–2476. http://doi.org/10.5504/bbeq.2011.0058

Schneider, S. H. (1989). The greenhouse effect: science and policy. *Science (New York, N.Y.)*, *243*(4892), 771–781. http://doi.org/10.1126/science.243.4892.771

Schuler, M., and Kargi, F. (2002). *Bioprocess Engineering*. Upper Saddle River, NJ: USA: Prentice Hall PTR.

Scragg, A. H. (2006). The effect of phenol on the growth of Chlorella vulgaris and Chlorella VT-1. *Enzyme and Microbial Technology*, *39*(4), 796–799. http://doi.org/10.1016/j.enzmictec.2005.12.018

Sforza, E., Gris, B., De Farias Silva, C. E., Morosinotto, T., and Bertucco, A. (2014). Effects of light on cultivation of scenedesmus obliquus in batch and continuous flat plate photobioreactor. *Chemical Engineering Transactions*, *38*, 211–216. http://doi.org/10.3303/CET1438036

Siddiqui, S., Rameshaiah, G. N., and Kavya, G. (2015). Development of Photobioreactors for Improvement of Algal Biomass Production. *International Journal of Scientific Research*, *4*(2277), 220–226.

Singh, D., Yadav, K., and Singh, R. S. (2015). Biofixation of carbon dioxide using mixed culture of microalgae. *Indian Journal of Biotecnology*, *14*(April), 228–232.

Singh, G., and Thomas, P. B. (2012). Nutrient removal from membrane bioreactor permeate using microalgae and in a microalgae membrane photoreactor. *Bioresource Technology*, *117*, 80–5.

Singh, R. N., and Sharma, S. (2012). Development of suitable photobioreactor for algae production – A review. *Renewable and Sustainable Energy Reviews*, *16*(4), 2347–2353. http://doi.org/10.1016/j.rser.2012.01.026

Sirin, S., and Sillanpaa, M. (2015). Cultivating and harvesting of marine alga Nannochloropsis oculata in local municipal wastewater for biodiesel. *Bioresource Technology*, *191*, 79–87.

Sriram, S., and Seenivasan, R. (2012). Microalgae Cultivation in Wastewater for Nutrient Removal. *Journal of Algal Biomass*, *3*(2), 9–13.

Sterner, C. (2013). *Top Five Things You Should Know About Algae*. *Office of Energy Efficiency and Renewable Energy*. Retrieved from http://www.energy.gov/eere/articles/top-five-things-you-should-know-about-algae

Stewart, C., and 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. http://doi.org/10.1016/j.enconman.2004.03.009

Suh, I. S., and Lee, C.-G. (2003). Photobioreactor Engineering Design and Performance.Pdf. *Biotechnology and Bioprocess Engineering*, *8*(6), 313–321.

Suhendrayatna, Ohki, A., Kuroiwa, T., and Maeda, S. (1999). Arsenic compounds in the freshwater green microalgaChlorella vulgaris after exposure to arsenite. *Applied Organometallic Chemistry*, *13*(2), 127–133.

Sung, K. D., Lee, J. S., Shin, C. S., Park, S. C., and Choi, M. J. (1999). CO2 fixation by Chlorella sp. KR-1 and its cultural characteristics. *Bioresource Technology*, *68*(3), 269–273. http://doi.org/10.1016/S0960-8524(98)00152-7

Takawira, S., Hoko, Z., and Nhapi, I. (2014). Characterisation of raw sewage and performance assessment of primary settling tanks at Firle Sewage Treatment Works , Harare , Zimbabwe. *Physics and Chemistry of the Earth*, *67–69*, 226–235. http://doi.org/10.1016/j.pce.2013.10.004

Tam, N. F. Y., and Wong, Y. S. (1989). Wastewater nutrient removal by Chlorella pyrenoidosa and Scenedesmus sp. *Environmental Pollution*, *58*(1), 19–34. http://doi.org/10.1016/0269-7491(89)90234-0

Tam, N. F. Y., and Wong, Y. S. (1996). Effect of ammonia concentrations on growth of Chlorella vulgaris and nitrogen removal from media. *Bioresource Technology*, *57*, 45–50.

Tang, H., Chen, M., Ng, K. Y. S., and Salley, S. O. (2012). Continuous microalgae cultivation in a photobioreactor. *Biotechnology and Bioengineering*, *109*(10), 2468–74. http://doi.org/10.1002/bit.24516

Tarlan, E., Dilek, F. B., and Yetis, U. (2002). Effectiveness of algae in the treatment of a wood-based pulp and paper industry wastewater. *Bioresource Technology*, *84*(1), 1–5.

Tchobanoglous, G., Burton, F. L., and Stensel, H. D. (2003). *Wastewater Engineering: Treatment, Disposal, and Reuse.* (Metcalf and Eddy, Eds.) (4th ed.). New York: McGraw-Hill Inc.

Travieso, L., Benítez, F., Sánchez, E., Borja, R., Martín, a., and Colmenarejo, M. F. (2006). Batch mixed culture of Chlorella vulgaris using settled and diluted piggery waste. *Ecological Engineering*, *28*(2), 158–165. http://doi.org/10.1016/j.ecoleng.2006.06.001

TÜİK. (2015). *TÜİK Haber Bülteni*. *Belediye Atıksu İstatikleri-2014*.

Uduman, N., Qi, Y., Danquah, M. K., Forde, G. M., and Hoadley, A. (2010). Dewatering of Microalgal Cultures: a Major Bottleneck to Algae-Based Fuels. *Journal of Renewable and Sustainable Energy*, *2*(1), 15. http://doi.org/10.1063/1.3294480

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

Valderrama, L. T., Del Campo, C. M., Rodriguez, C. M., De- Bashan, L. E., and Bashan, Y. (2002). Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalga Chlorella vulgaris and the macrophyte Lemna minuscula. *Water Research*, *36*(17), 4185–4192. http://doi.org/10.1016/S0043-1354(02)00143-4

VK Bincy, GS Dasog, BH. Prasannakumar, CR Patil, Hebbara, M. (2015). Chemical and Microbial Composition of Municipal Sewage water around Hubli-Dharwad in Karnataka. *Res J. Chem. Environ. Sci. Vol*, *3*(June), 1–5.

Wang, B., Li, Y., Wu, N., and Lan, C. Q. (2008). CO(2) bio-mitigation using microalgae. *Applied Microbiology and Biotechnology*, *79*(5), 707–18. http://doi.org/10.1007/s00253-008-1518-y

Wang, L., Li, Y., Chen, P., Min, M., Chen, Y., Zhu, J., and Ruan, R. R. (2010). Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. *Bioresource Technology*, *101*(8), 2623–8. http://doi.org/10.1016/j.biortech.2009.10.062

Wang, L., Wang, Y., Chen, P., and Ruan, R. (2010). Semi-continuous cultivation of Chlorella vulgaris for treating undigested and digested dairy manures. *Applied Biochemistry and Biotechnology*, *162*(8), 2324–32.

Wang, Lan, and Horsman, M. (2012). Closed photobioreactors for production of microalgal biomasses. *Biotechnology Advances*, *30*(4), 904–912. http://doi.org/10.1016/j.biotechadv.2012.01.019

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

Widjaja, A., Chien, C.-C., and Ju, Y.-H. (2009). Study of increasing lipid production from fresh water microalgae Chlorella vulgaris. *Journal of the Taiwan Institute of Chemical Engineers*, *40*(1), 13–20. http://doi.org/10.1016/j.jtice.2008.07.007

Woertz, I., Feffer, A., Lundquist, T., and Nelson, Y. (2010). Algae Grown on Dairy and Municipal Wastewater for Biofuel Feedstock, *135*(11).

Woertz, I., Feffer, a., Lundquist, T., and Nelson, Y. (2009). Algae Grown on Dairy and Municipal Wastewater for Simultaneous Nutrient Removal and Lipid Production for Biofuel Feedstock. *Journal of Environmental Engineering*, *135*(11), 1115–1122. http://doi.org/10.1061/(ASCE)EE.1943-7870.0000129

Yeh, K.-L., and Chang, J.-S. (2012). Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga Chlorella vulgaris ESP-31. *Bioresource Technology*, *105*, 120–7. http://doi.org/10.1016/j.biortech.2011.11.103

Yun, Y.-S., Lee, S. B., Park, J. M., Lee, C.-I., and Yang, J.-W. (1997). Carbon Dioxide Fixation by Algal Cultivation Using Wastewater Nutrients. *Journal of Chemical Technology & Biotechnology*, *69*(4), 451–455.

Zarrouk, C. (1966). *Contribution a l'etude d'une cyanobacterie: influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de Spirulina maxima (Setchell et Gardner) Geitler*. University of Paris, France.

Znad, H., Naderi, G., Ang, H. M., and Tade, M. O. (2012). CO2 Biomitigation and Biofuel Production Using Microalgae : Photobioreactors Developments and Future Directions. In Z. Nawaz (Ed.), *Advances in Chemical Engineering*. Intech.

APPENDIX A

IMAGES OF MICROSCOPIC ANALYSES OF MICROALGAL CULTURE

Images from microbial analyses which were conducted using Automated Inverted Microscope for Life Science Research (Leica, DMI4000 B) were provided in Figure A-1, Figure A-2, Figure A-3 and Figure A-4.

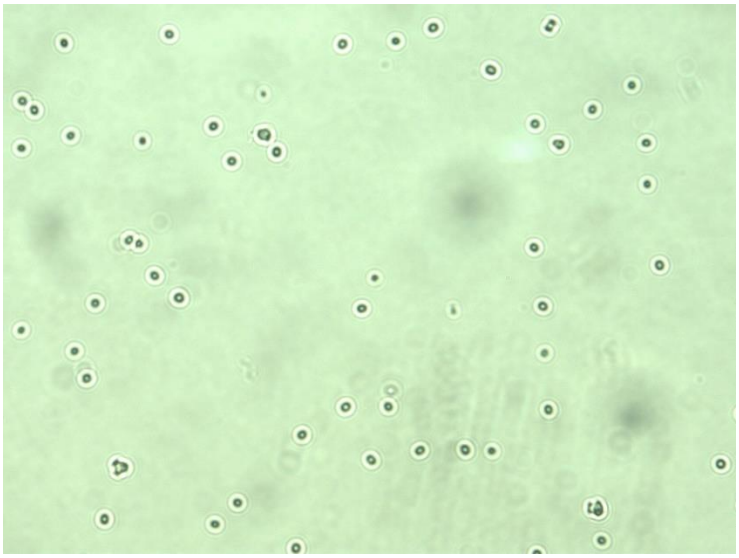

Figure A-1 Image of microscopic analyses of a sample from the cultivation of *Chlorella vulgaris* culture in batch PBR study (Section 4.1.1).

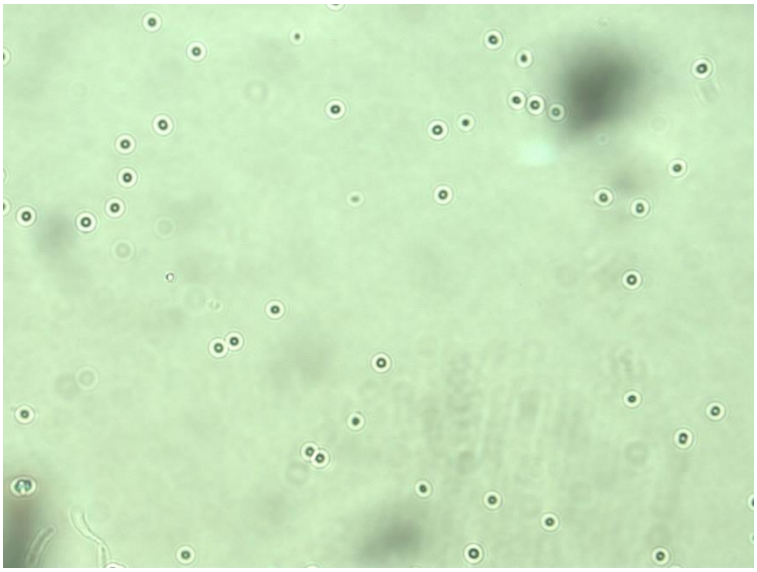

Figure A-2 Image of microscopic analyses of a sample from the cultivation of *Chlorella vulgaris* culture in semi-continuous PBRs study (Section 4.1.2).

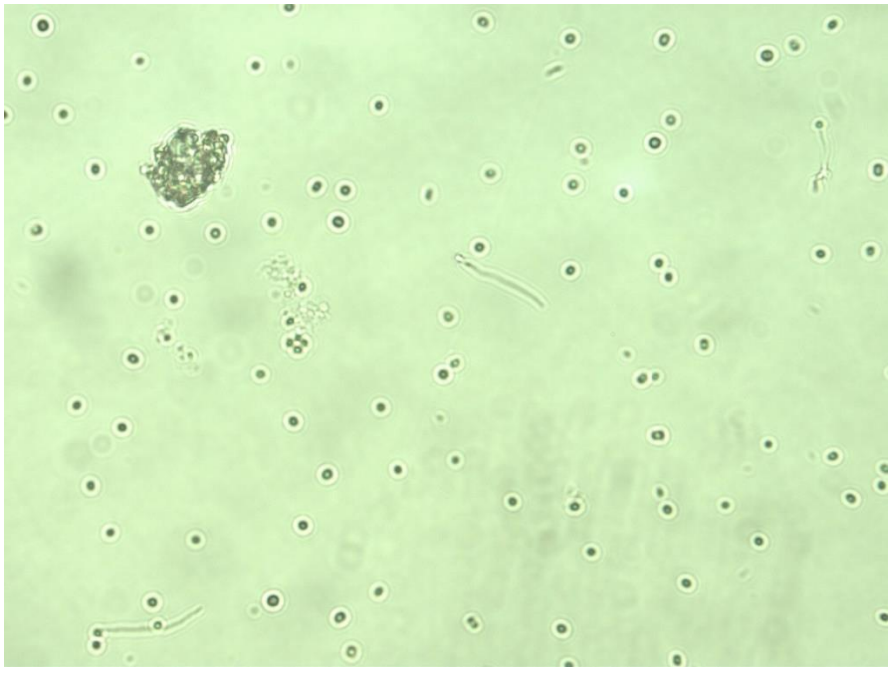

Figure A-3 Image of microscopic analyses of a sample from the nutrient (N and P) removal from municipal wastewater in semi-continuous PBRs study (Section 4.2.2).

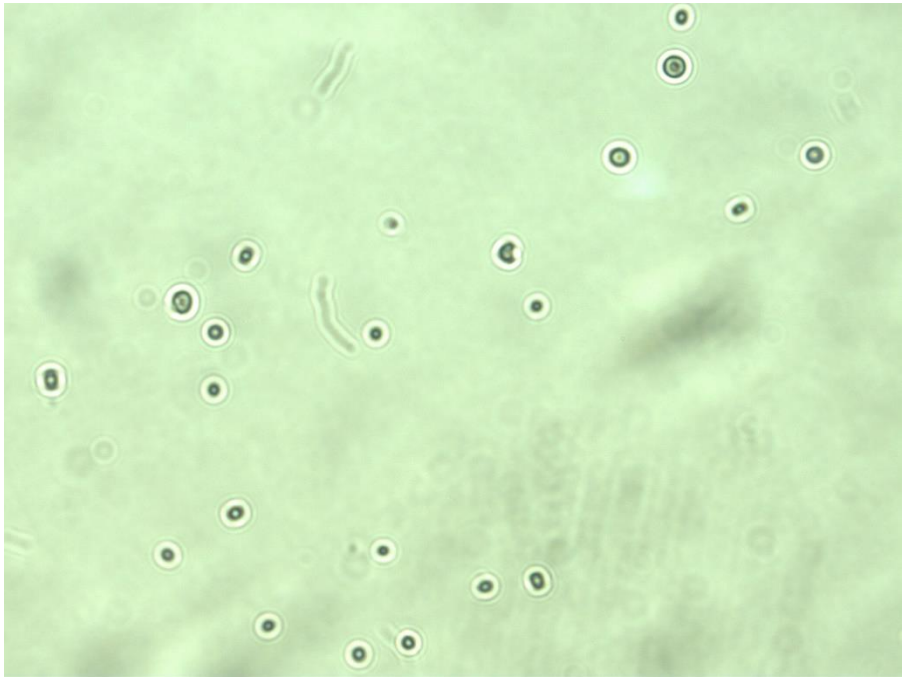

Figure A-4 Image of microscopic analyses of a sample from the nutrient (N and P) removal from industrial wastewater study (Section 4.3.2).

APPENDIX B

CALIBRATION CURVE FOR CO_2 MEASUREMENTS

Calibration curve for CO_2 measurements with GC Agilent 6890N was presented in Figure B-1.

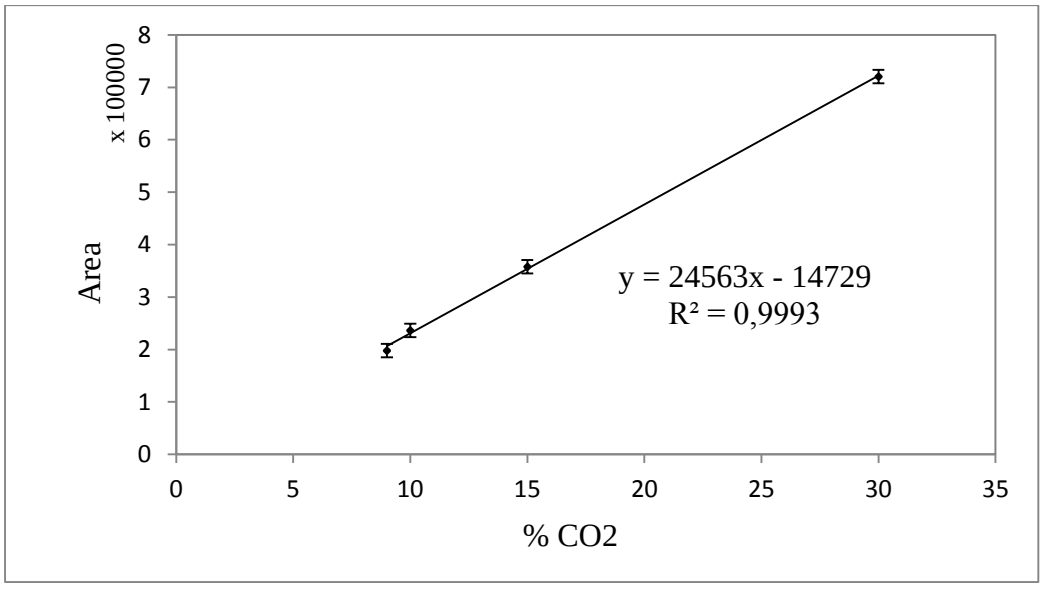

Figure B-1 Calibration curve and equation for CO_2

APPENDIX C

GROWTH CALCULATIONS

Logarithmic growth phase can be defined as steady increase in growth parameter. Logarithmic growth rate (the net specific growth rate) (μ) is calculated according to Equation C-1 while biomass production rate (P) is calculated according to the Equation C-2 (Sankar et al., 2011). X and t represent microalgal concentration and time, respectively.

$$\mu = \frac{(\ln X2 - \ln X1)}{(t2 - t1)} \qquad \text{Equation (C-1).}$$

$$P = \frac{(Xt - X0)}{(t2 - t1)} \qquad \text{Equation (C-2).}$$

APPENDIX D

CALIBRATION CURVE FOR *CHLORELLA VULGARIS* CULTURE

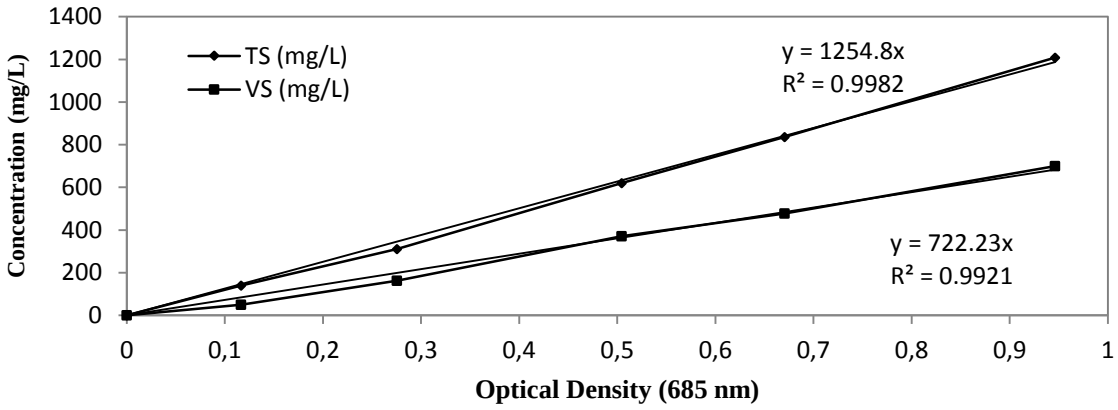

Figure D-1 Calibration curve and equation of optical density at 685 nm to TS and VS concentration of *Chlorella vulgaris* culture

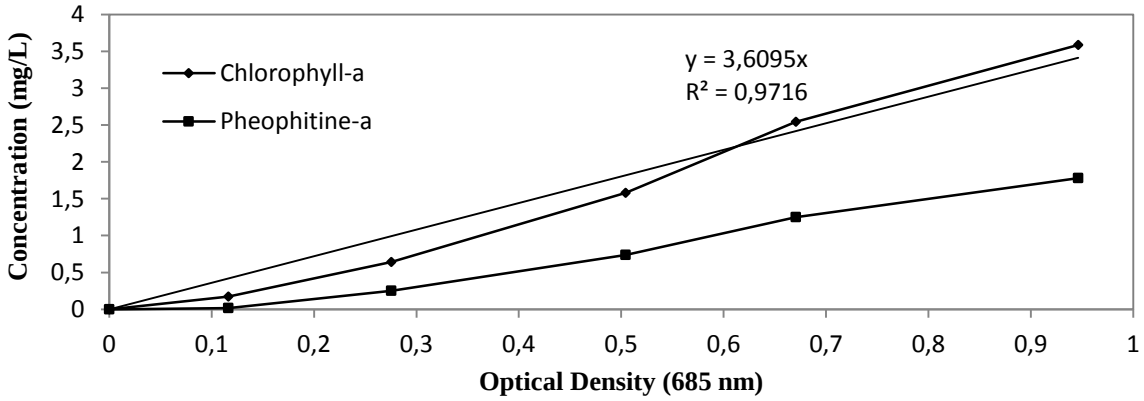

Figure D-2 Calibration curve and equation of optical density at 685 nm to chlorophyll-a concentration of *Chlorella vulgaris* culture

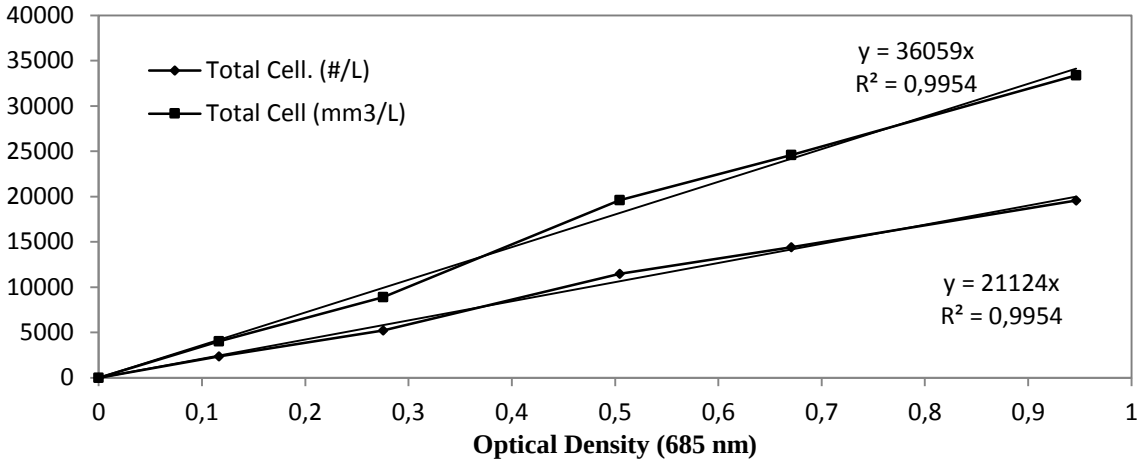

Figure D-3 Calibration curve and equation of optical density at 685 nm to total cell number and volume of *Chlorella vulgaris* culture

APPENDIX E

ABSORBANCE CURVE OF *CHLORELLA VULGARIS*

Figure E-1 presents the light absorbance of two *Chlorella vulgaris* solutions screened between 600 and 750 nm. 625 and 685 nm are the two peak points observed. The highest absorbance was obtained at 685 nm which means that it is the most sensitive wavelength to quantify *Chlorella vulgaris* samples. Therefore, all optical density readings were done at this wavelength.

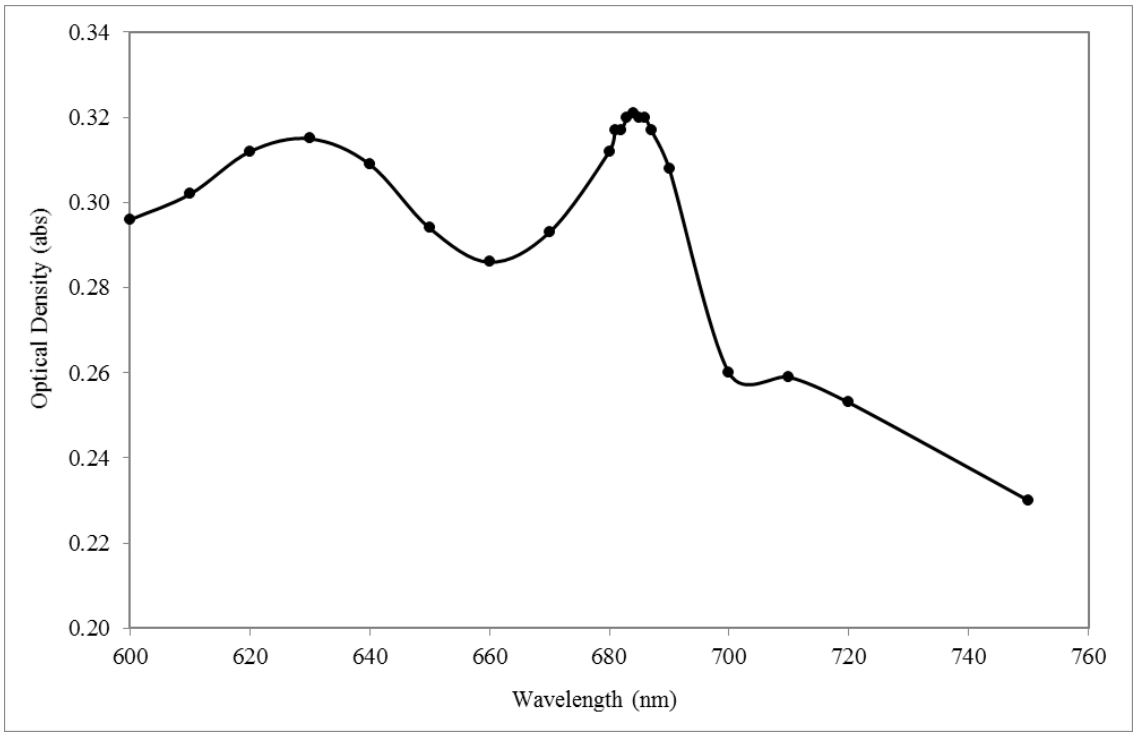

Figure E-1 Absorbance curve for *Chlorella vulgaris*

APPENDIX F

MASS BALANCE for NITROGEN

$$\Delta \dot{M} = \dot{M}_{in} - \dot{M}_{out} \pm \dot{M}_{rxn}$$

$\dot{M}$: Mass flux (mg/d)

- $\Delta \dot{M} = dS/dt \cdot V$ (Change in mass flux)

V: Volume of the PBR (L) = 1 L

- $\dot{M}_{in} = Q. S_0$ (Influent mass flux)

Q : Flowrate (L/d)

Flowrate for X_2 reactor: Q = 0.25 L/1d = 0.25 L/d (4-day HRT)

S_0 : Influent substrate (TAN) (mg/L) = 30.5 mg/L

- $\dot{M}_{out} = Q.S + Q. X.\%N + \dot{M}_{stripped}$ (Effluent mass flux)

S : Effluent substrate (TAN) (mg/L)

X: Biomass concentration (mg/L)

%N : Percent nitrogen composition of microalgal biomass = 10.8% = 0.108 (Appendix H)

$\dot{M}_{stripped}$: Mass flux of stripped ammonia to air (mg/d)

- $\dot{M}_{rxn} = \mu.X.\%N. V$

μ : Net specific growth rate (1/d) (Kumar and Das, 2012; Liao, et al., 2014) was calculated according to the results of kinetic study (Section 4.2.3):

$$\mu = \ln (X_f / X_0) / \Delta t$$

$$\mu = 0.12\ 1/d$$

X = 613 mg/L (Average biomass concentration of X_2 reactor based on VS)

$$M_{rxn} = 0.12\ (1/d) * 613\ (mg/L) * 0.108 * 1(L) = 7.95\ mg/d$$

Assumptions:

- The system was run at steady-state ($dS/dt = 0$) .
- Average steady-state microalgal concentrations were used for X_1 and X_2 reactors while calculating $\dot{M}_{rxn}$ since coefficient of variance for data were below 10%.

The analyses indicated that neither NO_2 nor NO_3 was detected in the influent and effluent of PBR.

$dS/dt \,.V = 0 = Q.S_0 - Q.S - Q.\, X.\%N - \dot{M}_{stripped} + \mu.X.\%N.V$

$\dot{M}_{stripped} = Q.\,(30.5 - S - X.\,(0.108)) + M_{rxn}$

Sample calculation for X_2 reactor, Day 13:

$\dot{M}_{stripped} = 0.25 * (30.5 - 0.0 - 613*0.108) + 7.95 = 0.095$ mg/d

%Ammonia stripped = $(0.095 / (30.5*0.25))*100 = 1.25$ %

APPENDIX G

COMPOSITION OF MIXED WASTEWATER

Coke plant wastewater was mixed with thickener supernatant for phosphorus addition and to overcome the effect of toxic substances via dilution. The composition of the mixed wastewater prepared at different times was presented in Table G-1.

Table G-1 Composition of mixed wastewater

Operation days	TAN (mgL/)	PO4-P (mg/L)	N/P	TN (mg/L)	sCOD (mg/L)
0	114.51	19.16	5.98		
7	111.39	18.30	6.09		
14	119.84	19.93	6.01	133.00	508.50
20	118.93	19.65	6.05	130.60	511.00
31	118.20	19.69	6.00	130.00	512.00
43	113.85	19.07	5.97	125.30	513.00
52	115.16	19.21	6.00	126.60	513.50

APPENDIX H

ELEMENTAL ANALYSIS OF MICROALGAL CULTURE

The samples from R1, X2 and C12 reactors were analyzed to determine the elemental composition of the culture at steady-state. The results are shown in Table H-1.

Table H-1 Elemental composition of microalgal cultures.

Reactor Name [a]	%C	%H	%N	%S
R1	47.13	6.7	9.86	0.48
X2	47.48	7.04	10.80	0.65
C12	50.71	7.17	8.17	0.7

R1: The semi-continuous PBR from the study of cultivation of *Chlorella vulgaris* culture (Section 4.1.2)

X2: The semi-continuous PBR from the study of nutrient (N and P) removal from municipal wastewater (Section 4.2.2), HRT of 4 days.

C12: The semi-continuous PBR from the study of nutrient (N and P) removal from industrial wastewater (Section 4.3.2), HRT of 12 days.