COUPLED WASTEWATER TREATMENT AND CO_2 MITIGATION BY MICROALGAL (*CHLORELLA VULGARIS*) CULTURES

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Submitted by **DİRENİŞ ÇAYLI** in partial fulfillment of the requirements for the degree of **Master of Science in Earth System Science Department, Middle East Technical University** by,

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

Prof. Dr. Ayşen Yılmaz _____
Head of Department, **Earth System Science**

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

Assoc. Prof. Dr. Tuba H. Ergüder Bayramoğlu _____
Co-Supervisor, **Environmental Engineering Dept., METU**

Examining Committee Members:

Prof. Dr. Ayşen Yılmaz _____
Institute of Marine Science, METU

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

Prof. Dr. Hami Alpas _____
Food Engineering Dept., METU

Assist Prof. Dr. Derya Dursun Balcı _____
Environmental Engineering Dept., METU

Assist Prof. Dr. Evren Doruk Engin _____
Basic Biotechnology Dept., Ankara University

Date: *30.03.2017*

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 materials and results that are not original to this work.

Name, Last Name: Direniş Çaylı

Signature:

ABSTRACT

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Çaylı, Direniş
M.Sc., Department of Earth System Science
Supervisor: Prof. Dr. Göksel N. Demirer
Co-Supervisor: Assoc. Prof. Dr. Tuba H. Ergüder Bayramoğlu

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Eutrophication, ecosystem damage and poor water quality predominantly are among the major problems which are brought about by excess nitrogen and phosphorus discharged to receiving environments by different wastewaters. Nutrient (mainly nitrogen and phosphorus) removal from wastewaters is still an unsolved problem in many countries, including Turkey. For example, the ratio of wastewaters which are subjected to tertiary (advanced) wastewater treatment is around 38.3% (Turkish Statistical Institute, 2012). Therefore, there has been an increasing interest for seeking better and more feasible nutrient removal techniques. Microalgal cultures have been effectively used in nutrient removal from wastewaters for a long time.

Global warming has reached to an alarming level because of the elevated CO_2 levels in the atmosphere. United Nations promoted Kyoto Protocol which is an international commitment to reduce carbon emission of more than 170 countries. Like nutrient removal, there are different physical, chemical, and biological technologies to mitigate CO_2. One of the biological methods for CO_2 mitigation is the algae based CO_2 uptake.

This research has focused on investigating the possibility of parallel nutrient removal from domestic and industrial wastewaters and CO_2 mitigation from industrial flue gas. The aim of this study is to understand (i) the tolerance of high CO_2 levels by microalgae, (ii) removal rate of nutrients from wastewater, (iii) the amount of CO_2 biotransformed by microalgae, and (iv) the performance of microalgae cultivation in reactors fed by real flue gas collected from iron and steel industry.

In this study, batch and fed-batch reactors were operated for investigation of nutrient removal efficiency of *Chlorella vulgaris*. Maximum N and P removal efficiencies were achieved in these reactors between 82% and 99% at the end of experiment.

CO_2 biofixation rates were normalized dry biomass of microalgae. The results indicated that CO_2 biofixation performance of these reactors were in the range of 6.44 and 0.32 g CO_2/TS.

The results of this study indicated that microalgal biotechnology is a feasible alternative for integrated nutrient removal and CO_2 biofixation applications.

Keywords: *Chlorella vulgaris*, Photobioreactor, CO_2 mitigation, Flue gas, Global Warming, Nutrient removal

ÖZ

MİKROALGAL (CHLORELLA VULGARİS) KÜLTÜRLERİ İLE ENTEGRE BESİYER MADDE GİDERİMİ VE CO_2 MİTİGASYONU

Çaylı, Direniş
Yüksek Lisans, Yer Sistem Bilimleri Bölümü
Tez Yürütücüsü: Prof. Dr. Göksel N. Demirer
Yrd. Tez Yürütücüsü: Doç. Dr. Tuba H. Ergüder Bayramoğlu

Mart 2017, 124 sayfa

Farklı atık sulardan alıcı sucul ortamlara atılan fazla azot ve fosfor, ötrifikasyon, ekosistem hasarı ve düşük su kalitesi gibi başlıca problemlere sebep olmaktadır. Atık sulardan çoğunlukla azot ve fosfor gibi besiyer maddelerin uzaklaştırılması, Türkiye de dâhil olmak üzere birçok ülkede hâlâ çözülemeyen bir sorundur. Örneğin, Türkiye'de üçüncül (ileri) atık su arıtımına tabi olan atık suların oranı %38.3 civarındadır (Türkiye İstatistik Kurumu, 2012). Bu yüzden daha iyi ve daha uygulanabilir besiyer madde giderimi teknikleri arayışları devam etmektedir. Mikroalgal kültürler uzunca bir süredir atık sulardan besiyer maddelerin uzaklaştırılmasında etkili bir şekilde kullanılmaktadır.

Atmosferdeki CO_2 seviyesinin yükselmesinden kaynaklanan küresel ısınma, endişe verici boyutlara ulaşmaktadır. Birleşmiş Milletler İklim Değişikliği Çerçeve Sözleşmesi içinde imzalanmış olan Kyoto Protokolü, 170'den fazla ülkenin karbon salınımını azaltmaya yönelik uluslararası bir sözleşmedir. Besiyer madde giderimi gibi, CO_2'yi de gidermek için farklı fiziksel, kimyasal ve biyolojik teknolojiler vardır. CO_2 giderimi için biyolojik yöntemlerden biri de mikroalg esaslı CO_2 giderimi yöntemidir.

Bu çalışmada, evsel ve endüstriyel atık sulardan entegre besiyer maddelerinin uzaklaştırılması ve endüstriyel baca gazı kaynaklı CO_2 gideriminin araştırılması üzerine yoğunlaşılmıştır.

Bu çalışmanın amacı, (i) yüksek CO_2 seviyelerinin mikroalgler tarafından toleransını, (ii) mikroalglerin atıksudaki besiyer madde giderim oranlarını, (iii) mikroalgler tarafından biyo-transforme edilen CO_2 miktarını ve (iv) mikroalglerin demir çelik endüstrisinden toplanan gerçek baca gazı ile beslenen reaktörlerdeki performansını anlamaktır.

Bu çalışmada, *Chlorella vulgaris*'in besiyer giderimi veriminin araştırılması amacıyla yarı-kesikli ve sürekli fotobiyoreaktör işletilmiştir. En yüksek azot ve fosfor giderimleri %82 ve %99 aralığında kaydedilmiştir.

Mikroalglerin CO_2 giderim miktarları kuru kütleleri ile normalize edilmiştir. Sonuçlar, bu reaktörlerin CO_2 giderim performansının 6.44 ve 0.32 g CO_2 / g TKM aralığında olduğunu göstermiştir.

Bu çalışmanın sonuçları, mikroalg biyoteknolojisi ile entegre besiyer madde giderimi ve CO_2 giderimi uygulamalarının gerçekleştirilebilir olduğunu göstermektedir.

Anahtar Kelimeler: *Chlorella vulgaris*, Fotobiyoreaktör, CO_2 giderimi, Baca gazı, Küresel ısınma, Besiyer madde giderimi

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ABBREVIATIONS

μ	:	Specific Growth Rate, (d^{-1})
ATP	:	Adenosine Triphosphate
BM	:	Basal Medium
BOD	:	Biochemical Oxygen Demand
C	:	Carbon
C_c	:	Average Carbon Content
CH_4	:	Methane
CO_2	:	Carbon Dioxide
CO_3^{2-}	:	Carbonate Ion
COD	:	Chemical Oxygen Demand
DNA	:	Deoxyribonucleic Acid
H_2CO_3	:	Carbonic Acid
H_2O	:	Water
HCO_3^-	:	Bicarbonate Ion
HRT	:	Hydraulic Residence Time
KH_2PO_4	:	Monopotassium Phosphate
N	:	Nitrogen
NADPH	:	Nicotinamide Adenine Dinucleotide Phosphate
NH_3	:	Free Ammonia
NH_4^+	:	Ammonium Ion
NH_4^+-N	:	Ammonium - Nitrogen
NO_3^-	:	Nitrate Ion
NO_3^--N	:	Nitrate - Nitrogen
NO_x	:	Nitrogen Oxide
M_C	:	Elemental Carbon
M_{CO2}	:	Molecular Weight of CO_2
O_2	:	Oxygen
OD	:	Optical Density
O-PO_4^{3-}-P	:	Orthophosphate- Phosphorus
P	:	Phosphorus
p	:	Productivity, (mg/(L.d))
PAR	:	Photosynthetically Active Radiation, (nm)
PO_4^{3-}-P	:	Phosphate Ion
R_{CO2}	:	CO_2 Fixation Rate
sCOD	:	Soluble Chemical Oxygen Demand
SO_x	:	Sulphur Oxide
TAN	:	Total Ammonifiable Nitrogen
tCOD	:	Total Chemical Oxygen Demand
TKN	:	Total Kjeldahl Nitrogen
TN	:	Total Nitrogen
TP	:	Total Phosphorus
TS	:	Total Solids

TSS	:	Total Suspended Solids
X	:	Biomass Concentration, (mg/L)
VS	:	Volatile Solids
VSS	:	Volatile Suspended Solids
vvm	:	Volume Gas per Volume of Broth per Minute

CHAPTER 1

INTRODUCTION

According to Intergovernmental Panel on Climate Change (IPCC, 2001), Climate Change stands for an increase in the average temperature in the lower atmosphere that causes global warming. Human activities are the main cause of unprecedented greenhouse gas (GHG) emissions. Huntley and Redalje (2007) pointed out that since the beginning of the Industrial Revolution GHG emissions are the main cause of current alteration in climatic patterns across the World. Compounds that cause Greenhouse Effect are water vapor, carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), ozone, and chlorofluorocarbons (CFCs). IPCC (2007) presented evidence which indicates that carbon dioxide is the most important anthropogenic greenhouse gas. Due to industrialization and urbanization, fossil fuel consumption is the main actor for carbon dioxide emissions (IPCC, 2007). The 2009 United Nations Climate Change Conference in Copenhagen declared that there is a necessity for preventing more greenhouse gas accumulation in the atmosphere. In order to stabilize greenhouse gas composition in the atmosphere, inevitable solution was stated as "deep cuts in global emissions" (UNFCC, 2010).

The Kyoto Protocol, which aim was to reduce the GHGs produced by signatory countries by 5.2%, was promoted in 1997 by the United Nations. More than 170 countries confirmed the protocol. Following Kyoto Protocol, the Copenhagen Accord (CA) was accepted in 2009. Targets of this accord are both reduction in GHG emissions and reduction in effects of climate change, first 2°C and then 1.5°C by 2015 (CA, 2009). Moreover, in 2015 Conference of Parties (COP) met in Paris and

this meeting known as COP21 or the 2015 Paris Climate Conference. The aim of this conference is to achieve a legally binding and universal agreement on climate and to keep global warming below 2°C (Sutter, 2015).

Besides the CO_2 emission based climate change, another problem for human well-being and environment is the nutrient pollution in water bodies (Berhe et al., 2005). Nitrogen, phosphorous and potassium are the main nutrients required for terrestrial and aquatic life. Nutrient pollution creates risk for not only public health but also environmental quality and economy by affecting atmosphere, biosphere and hydrosphere. Eutrophication, ecosystem damage and poor water quality predominantly are among the major problems which are brought about by excess nitrogen and phosphorus discharged to receiving environments by different wastewaters (Nixon, 1995).

Microalgal cultures have been effectively used in nutrient removal from wastewaters for a long time. They can use variable wastes as nutrient source such as agricultural run-off, animal feed wastes, industrial and domestic wastewaters etc. (Brennan and Owende, 2010; Cuellar-Bermudez et al., 2015). By utilizing these wastes, they not only supply their nitrogen and phosphorous requirement but also contribute to wastewater treatment. Microalgae are autotrophic organisms which can convert CO_2 into organic matter, fixing carbon in their biomass. Lardon et al. (2009) demonstrated that approximately 1.8 gram of CO_2 can be fixed in every 1 gram of microalgal biomass production. This CO_2 may be provided from atmosphere or flue gas from different industrial facilities. Thus, microalgae can be used not only to treat wastewater but also sequester CO_2 at the same time.

The produced microalgal biomass can be used for different purposes such as nutrient recovery for animal feed, bio-product production like fertilizer, human food, pharmaceutical products, fine chemicals and bulk products such as fatty acid, oils, sugars, pigments, etc. In addition to these consumer products, microalgal biomass can be used for biofuel production (Cuellar-Bermudez et al., 2015).

According to Rahaman et al. (2011), CO_2 is readily available in the atmosphere in concentration of 0.03-0.06 % (v/v). On the other hand, concentration of CO_2 is originated from flue gases may reach to 6-15 % (v/v). High concentration of CO_2 improves the photosynthetic efficiency of microalgae to reproduce within a shorter time which means that higher biomass yields can be obtained. In spite of that, Ramanan et al. (2010) and Zhao and Su (2014) demonstrated that CO_2 concentration above 5% (v/v) is considered as toxic to microalgal growth. Lee et al. (2000) pointed out that continuous injection of increasing CO_2-containing flue gas concentration inhibits microalgal growth. It was found that this flue gas inhibition is mainly occurred because of the presence of toxic pollutants such as NO_x and SO_x, which caused acidification of cultivation medium (Ho et al., 2013; Lee et al., 2000; Zhao and Su, 2014).

On the basis of these facts, this study was designed to undertake coupled nutrient removal from wastewater and CO_2 uptake from flue gas by using microalgal cultures. To this purpose, the nutrient removal rate of green alga, *C. vulgaris*, from primary effluents of municipal wastewater and steel-making industry wastewater and CO_2 mitigation from the flue gas of steel-making industry were investigated.

Specific objectives were:

1. Investigation of nutrient removal rates from wastewater by operating of fed-batch microalgal photobioreactors fed with both municipal and industrial wastewaters.
2. Determination of CO_2 bioconversion efficiency of *C. vulgaris*
3. Determination of tolerance of high CO_2 levels of microalgae by using artificial CO_2 and air mixture.
4. Investigation of performance of microalgae cultivation reactors fed by flue gas.

CHAPTER 2

LITERATURE REVIEW

2.1. Air Pollution

Air pollution is the contamination of Earth's atmosphere by harmful substances such as chemical, physical or biological agents, gaseous, liquid, or solid wastes and particles. Introduction of these pollutants and contaminants into the air can cause drastic climate change (EPA, 2016).

2.1.1. Greenhouse Gases and Global Warming

The drastic increase in anthropogenic greenhouse gases (GHG), of which carbon dioxide (CO_2) constitutes 68% of total emission (Maeda et al., 1995), is the major cause for global warming and climate change (Stewart and Hessami, 2005). According to World Meteorological Organization (WMO) report, the atmospheric concentration of CO_2 has gradually increased from 280 ppm in 1750 to 390.9 ppm in 2011 and is currently increasing at the rate of 2 ppm/year in the past 10 years (WMO, 2012). Futhermore, it was reported that the highest-ever daily average CO_2 emission reached 409.56 ppm in March, 2017.

Global warming is defined as a rise in the average temperature of Earth's climate system due to the greenhouse gases. Kyoto Protocol, which is a legally binding agreement, was promoted by The United Nations (1997) with the aim of lowering overall emissions of six greenhouse gases - carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), hydrofluorocarbons (HFC), perfluorocarbons (PFC) and sulphur hexafluoride (SF_6), by 5.2% on the basis of emissions in 1990 and more than 170

countries have signed the protocol (Gutiérrez et al., 2008). Moreover, the introduction of a carbon credit system was also proposed by the United Nation in 2010, with an estimated unit price of US $270/ton (Stewart and Hessami, 2005). Finally, developed countries promised to provide long-term finance of a further US$100 billion a year for greenhouse mitigation by 2020 at the Copenhagen Climate Change Conference in 2009 (Kintisch, 2010). Furthermore, COP21, in other words, 2015 Paris Climate Conference is a legally binding global agreement on the reduction of climate change. The aim of this agreement is to limit global warming to less than 2 ^{0}C compared to pre-industrial levels (Sutter, 2015).

It was reported that atmospheric concentrations of these six important greenhouse gases have increased by approximately 20 percent since beginning of industrialization (EPA, 2017). There is a significant difference between heat absorbing abilities of greenhouse gases in the atmosphere. For example, HFCs and PFCs have the most heat absorbent capability. According to Allen and Rosselot (1997), methane traps up to 21 times more heat in the atmosphere than carbon dioxide and nitrous oxide absorbs 270 times more heat in the atmosphere than carbon dioxide.

Major sources of global CO_2 emissions in the world are flue gases from electric power plants and steel plants (Gielen, 2003; Kadam, 2002). For example, CO_2 emission from coal-fired thermoelectric power plants is estimated up to %7 of total world CO_2 emissions (de Morais and Costa, 2007). Currently, many research and attempts have been made to reduce CO_2 emissions. For the purpose of reducing atmospheric concentration of CO_2, various CO_2 mitigation strategies have been evaluated such as physical, chemical and biological methods (Farrelly et al., 2013).

2.1.2. Composition of Flue Gases

Besides fossil fuel combustion, cement and iron-steel industries are the largest contributors to high CO_2 emissions (Farrelly et al., 2013). Furthermore, other industries such as paper, sugar, inorganic chemicals, aluminum, fertilizer and mining are responsible for significant amount CO_2 emissions (Farrelly et al., 2013). Flue

gases refer to gases discharged through a flue or stacks. Generally, CO_2 concentration in flue gas is between 3% and 15% depending on the fuel source and plant design (Packer, 2009). However, it was reported that CO_2 concentration in flue gas from cement industry can reach up to 25% (Alie et al., 2005). Besides CO_2, CO, SO_x (SO_2 and SO_3), H_2, H_2O, N_2, NO_x (NO and NO_2), unburned carbohydrates (C_xH_y), particulate matter, halogen acids and heavy metals also include in flue gas (Van Den Hende et al., 2012). Moreover, Simoneita et al. (2001) reported that there is up to 142 different compounds in flue gas.

According to Rao and Rao (1996), approximately 80% of flue gas sulfur found in the form of SO_2 and SO_3 and their concentration change between 0.05 – 0.25%. On the other hand, approximately 90% of the NO_x is found in the form of NO.

It was stated that CO_2 content in flue gas is available for microalgae at little or no cost. Furthermore, the direct utilizing of flue gas is beneficial for reducing the cost of separating CO_2 gas (Maroto-Valer, 2010).

Although power plant flue gas can be seen as a source of CO_2 for microalgae, inhibitory compounds such as NO_x and SO_x cause extreme conditions for microalgae (Lee et al., 2000). Therefore, it is important to select NO_x, SO_x and high level of CO_2 tolerant microalgae species for algae studies (Lee et al., 2000).

2.2. Soil and Water Pollution by Nutrients

Over-fertilization and irrigation for crop production are very common, which resulted in nutrient accumulation in soil and led to soil salinity (Chen et al. 2004). Nitrogen and phosphorus cycles and their negative feedback are essential for environmental management. Nutrients, especially phosphorus is often limiting to ecosystems and crops. Large amounts of phosphorus are required to produce good yield so fertilizer is used for modern agriculture. However, much of this fertilizer from a field is carried into a stream by surface runoff and cause eutrophication or become unavailable for recycling (Elser, 2012). Finally, these pollutions cause environmental damage and create risk for human health and economy.

There are many strategies to reduce nutrient pollution of soil and water such as fertilizer management, planting cover crops, managing livestock wastes, efforts for improving water quality, drainage water management, bioreactors and saturated buffers (Bryant and Goldman-Carter, 2016).

2.2.1. Groundwater Pollution by Nutrients

If excess nitrogen and phosphorus enter the environment due to human activities such as agriculture or point sources such as intentional or accidental spills of sewage from septic tanks, water can become polluted. Moreover, atmospheric deposition and the spreading of sewage sludge and manure to land can be counted as pollutant for groundwater (Rivett et al., 2008).

Nutrient pollution reaches many aquatic systems such as streams, rivers, lakes, bays and coastal waters and brings about risky conditions for environment, public health and economy. For example, contaminated ground water affects public drinking water source and causes severe problems for public health. Also excess nitrogen effects plant growth and health of forest severely (EPA, 2009).

It was reported that organic pollutants found in groundwater can be classified as carcinogens, mutagens and promoters (Rao and Rao, 1997)

According to a report by Food and Agriculture Organization of the United Nations and United Nations Economic Commission for Europe (FAO/ECE, 1991), groundwater is polluted mainly by nitrates. It was stated that nitrate contamination of groundwater has become a significant environmental problem in many parts of the world (Rivett et al., 2008).

Nitrate contamination in groundwater causes methemoglobinemia which is a disease related with oxygen–carrying capacity of blood. Methemoglobinemia causes death in infants under 6 months of age (Johnson et al., 1987).

2.2.2. Eutrophication

Eutrophication is defined as excessive plant and algal growth because of an increase in the rate of one or more limiting factors required for photosynthesis (Schindler, 2006) namely, sunlight, CO_2, or nutrient fertilizers (Chislock et al., 2013). It was reported by Carpenter et al. (1998) that human activities bring about dramatic increase in nutrient loading such as nitrogen and phosphorus into aquatic systems and this speed up the rate of eutrophication severely.

Excess amount of nitrogen and phosphorus due to the anthropogenic activities such as agriculture, industry, and sewage disposal cause algae to grow faster than ecosystems can handle. Excessive algal growth reduces water clarity and harms water quality. Also, it affects food resources and habitats, and decreases the oxygen concentration and limits light penetration which are required for survival of fish and other aquatic life. Significant growths of algae are defined as algal blooms and they can bring about fish kills and loss of plant beds, coral reef and other species. Moreover, some algal blooms create risk for public health by producing noxious toxins (EPA, 2009). Finally, the water sources cannot be used for agricultural, recreational, industrial and drinking purposes anymore (Carpenter et al., 1998).

Eutrophication is one of the most common environmental problems in many parts of world from Europe to North America (WHO, 1999). Unfortunately, it was reported by UNEP (2006) that occurrences of harmful algal blooms over the last few decades have increased in size and number.

2.3. Microalgae

Algae divide into two basic groups such as prokaryotic and eukaryotic (Lee, 2008). Microalgae are made up of eukaryotic cells and are classified as Kingdom Protista. Microalgae cells include cell wall, plasmatic membrane, cytoplasm, nucleus and organelles, such as mitochondria, lysosomes and golgi (Taher et al., 2011). Microalgae have also chlorophyll and like most other photosynthetic organisms, microalgae convert the sun's energy into chemical energy. Microalgae can either be autotrophic or heterotrophic. Some photosynthetic algae are mixotrophic, that is, they

have ability to both drive phototrophy and utilize exogenous organic nutrients (Lee, 2008). Microalgae produce biomass during daylight and use this biomass during the night due to respiration (Chisti, 2007). Although microalgae are mostly aquatic systems, they can be found in a wide habitat range and symbiotic systems over a broad range of salinities, temperatures, and pH ranges. An average composition for microalgae biomass contains three main components: proteins, carbohydrates and lipids and these component portions can be variable depend on the environment and species.

2.3.1. Algal Physiology and Specification

Algae are a very diverse family of organisms. 72.500 different algal species are estimated in nature (Guiry, 2012). Depending on the species, they can vary in size from a few micrometers to several meters. Systematic classification of algae based on pigment component. According to their pigment component, algae can be classified into nine main groups. Chlorophyceae (green algae), Phaeophyceae (brown algae), Pyrrophyceae (dinoflagellates), Chrysophyceae (golden-brown algae), Bacillariophyceae (diatoms) and Rhodophyceae (red algae) are the largest classes of algae (Harwood and Guschina, 2009).

Definite fossil records stated that the cyanobacteria are the oldest group of algae dating back 2700 million years (Lee, 2008). Algae are seen as primitive plant like organisms since they have a similar cell structure with basic plant cells. Although they do not have roots, stems or leaves, they contain chlorophyll pigment like plants (Lee, 2008). Algae can be defined as prokaryotic cells (cyanobacteria) or eukaryotic cells (Olaizola, 2003). They were classified as prokaryotes since they do not have membrane bound organelles such as golgi, mitochondria, nuclei, plastids etc. and are more similar to bacteria compared to algae. On the other hand, eukaryotic cells have these organelles that control and organize the functions of the cells (Brennan & Owende, 2010).

It is known that microalgae chemical composition is not an intrinsic factor but varies over a range (Becker, 1994). Some of microalgae can modify their biochemical and

fatty acid composition in response to culture age, growth phases of the algae and change in environmental conditions (Fernandez-Reiriz et al., 1989).

2.3.2. Algal Photosynthesis

Photosynthetic life on earth has existed more than 3.5 billion years ago, providing the foundation for all aerobic forms of life. Photosynthesis is the process in which plants and photosynthetic microorganisms (including microalgae and cyanobacteria) fix CO_2 into sugar with the aid of light and water as energy and electron source, respectively (Kumar et al., 2011). The following simplified Equation 2-1 describes overall reaction for photosynthesis:

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

Photosynthesis consists of two steps. First one is light dependent reaction, which only takes place in the presence of light, and energy carrier molecules namely ATP and NADPH are produced in this step. Second step is light independent reaction, which takes place under both the absence and presence of light.

The light dependent step is catabolic and light energy is absorbed and then converted into chemical energy in this step. This energy is stored in organic form in the second step. Proper cycling of two successive reactions is necessary for fixing carbon. Excess exposure to light and oxygen rich environments inevitably lead to photo inhibition and photo oxidative damage, respectively, which reduce the efficiency of the photosynthetic process.

The Calvin cycle, also known as the Calvin Benson cycle, is the key step of the mechanism that leads to the conversion of carbon dioxide into sugars by plants, algae, photosynthetic bacteria, and certain other bacteria (Whitmarsh and Govindjee, 1999).

2.3.3. Growth Kinetics

Commonly, growth of unicellular algae can be measured in terms of cell number in a given culture or cell biomass. "Cell concentration" term defined as the number of individual cells per unit volume and "cell density" defined as biomass of cells per unit volume. The growth of microalgae in the batch culture can be modeled with several different phases: adaption (lag phase), accelerating growth phase, exponential growth (log phase), decreasing log growth (linear growth), stationary phase, accelerated death, log death. These growth phases are given in Figure 2-1 (Shuler and Kargi, 2002).

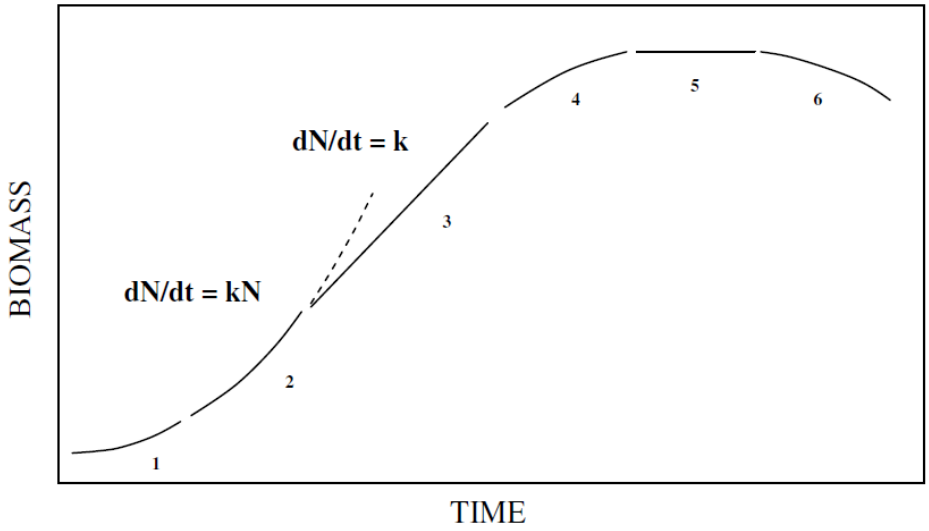

Figure 2-1. Growth phases of microalgal cultures (Shuler and Kargi, 2002)

Growth phases of microalgal cultures are not always seen as clear as Figure 2-1. Actual shape and slope may vary in respect of magnitude, length or height. In addition, the transition mode from one phase to another phase can be varied differently. According to Shuler and Kargi (2002), a growth phase is based on the culture type, nutrient concentration and culture conditions such as light, temperature, pH etc.

After inoculation, the culture needs time for adaptation to new environment and new conditions. Culture tries to acclimatize to new environment before start growing. During this phase, culture is more sensitive to changing conditions such as temperature or pH. After adaptation, exponential growth of the culture starts. Since neither light nor nutrient limitation does not occur during exponential growth phase, the increase in algal biomass per time is proportional to the biomass in the population at any given time. According to Zhao et al. (2011), the overall biomass productivity, *p*, can be obtained using Equation 2-2.

$$p\left(\frac{\mathrm{mg}}{\mathrm{L}*\mathrm{d}}\right)=\frac{\mathrm{X_1}-\mathrm{X_0}}{\mathrm{t_1}-\mathrm{t_0}} \tag{2-2}$$

where X_0 and X_1 are the biomass concentration (mg/L) at times t_0 and t_1, respectively. Specific growth rate (μ) is a measure of number of generations (the number of doublings) that occur per unit of time in an exponentially growing culture. The exponential (straight line) phase of growth is carefully determined and slope of this line is equal to the specific growth rate, μ, and can be obtained using Equation 2-3 (Zhao et al., 2011)

$$\mu\left(\mathrm{d}^{-1}\right)=\frac{\ln\left(\frac{\mathrm{X_1}}{\mathrm{X_0}}\right)}{\mathrm{t_1}-\mathrm{t_0}} \tag{2-3}$$

where X_0 and X_1 are the biomass concentration (g/L) at times t_0 and t_1, respectively. Since the cell density reaches the critical point and nutrients get depleted, logarithmic growth declines. Light supply becomes limited factor for algal growth. The increase in algal biomass becomes almost linear.

The log phase of algal growth is followed by stationary phase, in which the size of a population of algae remains constant. Maximum biomass concentration can be reached in this stage.

Finally, due to unfavorable conditions such as nutrient depletion, light limitation, waste accumulation or infection by other organisms, algal cells enter to death phase (Becker, 1994).

2.3.4. Nutrients

In order to grow and reproduce, algae need some additional nutrients. Nutrients correspond between 45% and 60% of dry weight of microalgae (Muñoz and Guieysse, 2006). Since carbon, nitrogen, phosphorous and silicon are required in high amounts, they are the most critical elements to produce biomass (Mehlitz, 2009). Generally, under optimum temperature and pH conditions algal growth rate is proportional to the uptake rate of the most limiting nutrient (Tilman, 1977)

Nutrient concentration has immense effect on not only growth rate but also final biomass concentration of microalgae. Microalgae generally are known to grow more abundantly under nutrient rich waters (eutrophic) and mostly cause algal blooms (Schenk et al., 2008).

Stoichiometric ratio of algal metabolite composition and content which is developed by Redfield et al. (1934), is C:N:P = 106:16:1. Anderson, (1995) developed this ratio and found indicated that N/P ratio of algal mass equals to 7.2 g N/g P on average.

In addition to main component, trace elements also essential for algal growth and reproduction since they often act as catalysts and so, regulate and accelerate the process significantly.

2.3.4.1. Carbon

Carbon is one of the main elements that must be supplied for microalgal growth and metabolism. Algae require an inorganic carbon source to conduct photosynthesis like other photosynthetic organisms. Carbon can be utilized not only in the form of CO_2, but also carbonate ($CO3^{2-}$), or bicarbonate (HCO^{3-}) for autotrophic growth and in form of acetate or glucose for heterotrophic growth. Microalgae prefer to uptake bicarbonate, over to CO_2 (Carvalho et al., 2006).

Since atmospheric CO_2 concentration (0.03%) is very low to maintain optimal growth and high productivity, freshwater algae must be supplied with additional carbon source. Generally, CO_2 enriched air is provided to the systems. In water, CO_2 present in different forms, depending upon the pH, temperature and the nutrient content (Becker, 1994):

$$CO_2 + H_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO^{3-} \leftrightarrow 2H^+ + CO_3^{2-} \qquad (2\text{-}4)$$

According to Equation 2-4, it can be concluded that it is important to maintain pH within an adequate range to avoid the loss of CO_2 present in the media. The fixation of CO_2 by the growing microalgae brings about a shift on the equilibrium shown in Equation 2-4 which causes pH level increase because of the excretion of OH^- by the algae into the media.

Kumar et al. (2011) stated that carbon dioxide concentrations above a certain level are detrimental for microalgal growth since high level of CO_2 reduces CO_2 fixation capacity of microalgae and causes decrease in culture pH because of the formation of high amount of bicarbonate buffer.

2.3.4.2. Nitrogen

Nitrogen is not only one of the most important elements for growth and metabolism of algal cells but also main element for protein and nucleic acid synthesis (Juneja et al., 2013). Usually, the nitrogen source exists in the several forms such as urea, nitrate (NO^{3-}), nitrite (NO^{2-}) and ammonia salt, mostly ammonium (NH^{4+}) in the culture medium. Some fast growing microalgal species generally prefer ammonium over to nitrate as the primary nitrogen source (Zhang, 2015). If growth medium lacks of nitrates, additional nitrogen sources will enhance microalgal growth. However, high concentration of ammonia can inhibit growth of algal cell. The cells only utilize ammonia forms so nitrate and nitrite must be converted to ammonia by an enzyme as shown in Equation 2-5. (Darley, 1982):

$$NO_3 \rightarrow NO_2 \rightarrow NH_4^+ \quad (2\text{-}5)$$

According to Becker (1994), typically nitrogen content of many green algae is approximately between 5% and 10% of the dry weight. Nitrogen deficiency causes not only algal growth limitation but also lipid accumulation in cells (Illman et al., 2000). Also, nitrogen content reduction causes drastic decrease in oxygen evolution, carbon dioxide uptake, chlorophyll content, and tissue production (Richardson et al., 1969). Round (1984), found that nitrogen limitation can change enzymatic reaction pathways, bringing about increase in fat synthesis and reduction in chlorophyll synthesis resulting in excess carotenoids in the cells.

2.3.4.3. Phosphorous

Phosphorus is another significant nutrient for algal growth, many biochemical processes and development of algal cells. Phosphate is an integral part of essential molecules such as ATP, DNA and RNA. Also phosphorous is an important component of phospholipids. Generally, microalgae can take inorganic phosphorus sources such as $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-} (Becker, 1994). Organic forms of phosphorus can be used but lesser amount since it is more difficult and slow to achieve. Orthophosphate (PO_4^{3-}) is the most preferable phosphorus sources for microalgae, because it can be part of several organic molecules by reacting with various phosphorylation reactions easily. It was reported that phosphorus is the main limiting macromolecule for algae compared with nitrogen (Larned, 1998). It has been shown that phosphorus depletion causes astaxanthin accumulation and overall decrease in algal cell growth (Kobayashi et al., 1993).

2.3.4.4. Trace Elements

Extremely small quantities of trace elements are needed for optimal growth of microalgae. Iron (Fe), manganese (Mn), cobalt (Co), zinc (Zn), copper (Cu) and nickel (Ni) are the six main trace metals required by essential metabolic functions and algal growth (Bruland et al., 1991). Not only deficiency in trace metal, but also excesses and high metal concentration can inhibit algal growth and damage the cell

membrane. Becker (1994) reported that trace elements have direct and positive physiological effect on algal growth.

Iron is one of the most essential trace metals which are used in normal algal growth, photosynthesis and respiration pathways, N_2 fixation, nitrate, nitrite and sulfate reduction in the cell (Sunda et al., 2005). Iron works in electron transport reactions in photosynthesis. Iron deficiency reduces electron transfer efficiency in photosynthesis and causes decrease in NADPH formation (Terry, 1986).

Also other essential elements such as copper (Cu), zinc (Zn) are required by algae and taken small quantities since their excesses cause toxicity for algae (Campanella et al., 2001).

If Cu, Ni and Fe are metals which are taken above the toxicity threshold, they are commonly observed to be toxic to the algae. Especially Cu is the most toxic metal among them. Toxic metals are harmful for algae since they do not only inhibit carbon fixation but also delay nutrient uptake (Juneja et al., 2013).

2.3.4.5. Vitamins

Sometimes, algae need additional vitamins for optimal growth and metabolic activity. Provasoli (1974) stated that are B12, thiamine, and biotin are the most common vitamins and their concentration must be range from 1/10 to 1/100 ng/L.

2.3.5. Light

Light is the major energy source for microalgae and organisms use this energy source to convert carbon dioxide to sugar. Generally, the amount of light energy utilized by the cells directly affects the carbon fixation capacity, consequently determining the cell growth rate and the microalgae productivity (Al-Qasmi et al., 2012).

Photosynthetic algae require light with a wavelength between 400 and 700 nm which is termed as photosynthetically active radiation (PAR) (Pecegueiro do Amaral, 2012). Duration and light intensity effects growth of algae directly through its impact on photosynthesis (Al-Qasmi et al., 2012).

As shown in Figure 2-2, growth rate of algae is maximum at "saturation intensity" and rate of growth decrease above or below this saturating limit (Sorokin and Krauss, 1958; Ogbonna and Tanaka, 2000).

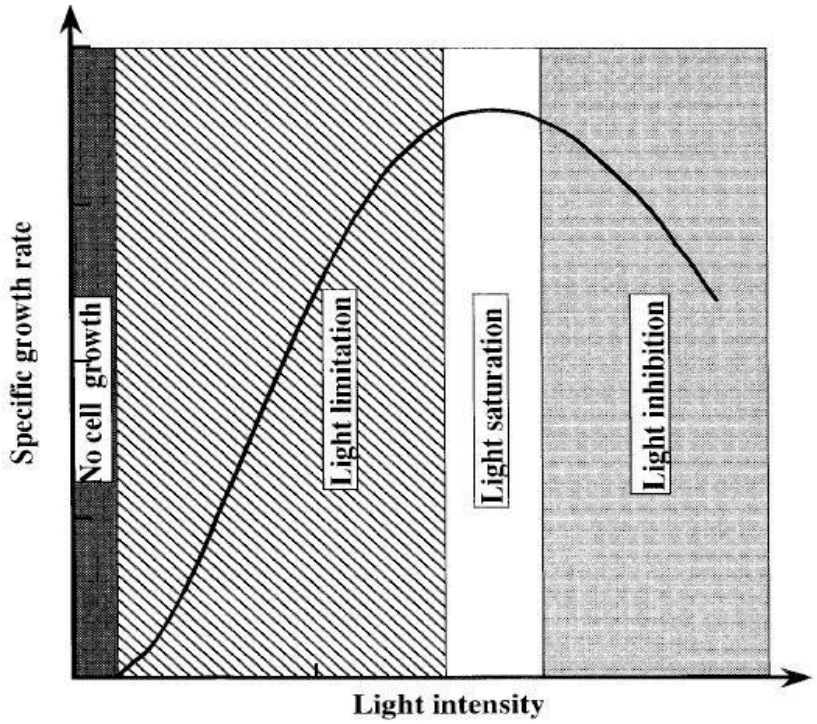

Figure 2-2. Effect of light intensity on growth of microalgae under phototrophic cultivation (Ogbonna and Tanaka, 2000)

Strong light intensity induces the production of reactive oxygen species (ROS) and inhibits the photosystem II in photosynthesis which is termed as photoinhibition (Murata et al., 2007). Moreover, degradation of chlorophyll or cellular damages may be caused from excessive amount of light or the increase in light intensity (Béchet et al., 2013).

Typically utilized light intensities range of 100-210 $\mu E/m^2/s$ and the saturation light intensity varies from 140 to 210 $\mu E/m^2/s$ for green algae (Kumar et al., 2011).

It was stated that the higher the microalgal density, the smaller the light path, brings about limitation in light penetration and cell shading (Cheah et al., 2014). Light intensity is reduced by light absorption and self-shading due to vertical diffusion, algal settling or high cell concentration where biomass concentration above 0.1 g/L (Chiu et al., 2011). Studies show that there is a correlation between light irradiance and optimum culture density (Qiang et al., 1998).

In addition to light intensity, light dark period cycle may also affect growth of microalgae through photosynthesis. In the natural environment, photosynthesis has light and dark periods which have different mechanisms. In order to find best light and dark photoperiod duration for algal productivity, biomass yield and CO_2 fixation rates, many studies was conducted by using 24:0, 16:8, 12:12 (hour) dark: light cycles. Barbosa et al. (2003) and Khoeyi et al. (2011) used 16:8 h light/dark photoperiod duration; Mata et al. 2012 and Sorokin et al (1958) analyzed 12:12 h light: dark cycle; Jacob-lopes et al. (2009) evaluated algal growth under 24:0 (day: night) respectively. Results of these experiments proved that effect of light cycles on maximum values of microalgal biomass production and CO_2 fixation rates depend on microalgae species. Furthermore, results of these studies indicated that the maximum cell concentration and CO_2 fixation rate were obtained under continuous light regime.

2.3.6. pH

The pH is also an important factor in algal cultivation, since it affects solubility and availability of CO_2 as well as algal metabolism. Most of the microalgae can adapt the pH ranges between 7 and 9 but optimum range is often 8.2 – 8.7 (Bitog et al., 2011).

Generally, pH level change is related with changes in temperature, dissolved oxygen, and algae biomass production (Chen and Durbin, 1994).

Higher pH level causes reduction in algal growth by lowering the affinity of algae to free CO_2 and brings about limitation carbon availability from CO_2 (Chen and Durbin, 1994; Azov 1982). Also increase pH level influence uptake and toxicity of copper and zinc metals in algae (Wilde et al., 2006). High pH level which is around 9–11

also causes N and P removal through NH_3 volatilization and orthophosphate precipitation (Craggs et al., 1996; Garcia et al., 2000; Nurdogan and Oswald, 1995).

Low level of pH also suppresses algal growth. Acidic pH not only alter nutrient uptake (Gensemer et al., 1993) but also induce metal toxicity (Sunda, 1978).

As Muñoz & Guieysse (2006) stated, microalgal CO_2 uptake can cause the pH level up to 10–11. Also consumption of nitrate by algae increases alkalinity and pH by producing OH^- (Goldman and Brewer, 1980). However, uptake of ammonium ions may lead pH to drop down to 3 (Larsdotter, 2006).

2.3.7. Temperature

Temperature is one of the most important limiting factors that influences microalgae growth rate, nature of metabolism, cell size, cell composition and nutrient requirements (Richmond, 2008).

There is an optimum temperature value or range for microalgal growth. Raven and Geider (1988) summarized the optimum temperature range for microalgae is 15-25^{o} C. On the other hand, Ghorbani et al., (2014) and Singh and Singh (2015) reported 25–35°C and 20-30°C, respectively. It is obvious that temperature optima can be vary among microalgal species. For example, temperature effect on four maximum growth rates of different algae strains is shown in Figure 2-3.

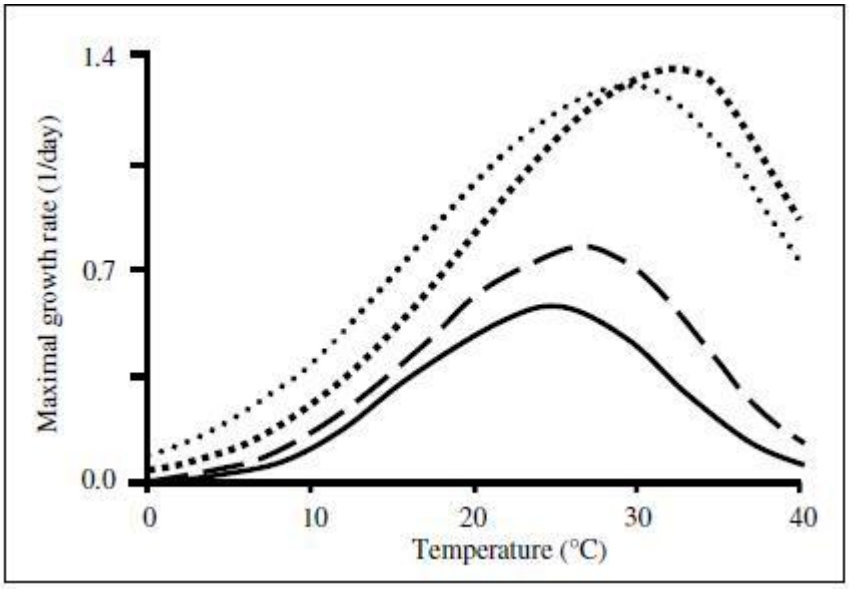

Figure 2-3. Effect of temperature on maximum growth rate of microalgae (Dauta et al., 1990)

Moderately low and high temperature influence carbon fixation ability of microalgae decreasing the ratio of O_2 to CO_2 solubility and causing inhibition of the activity of photosynthetic enzymes (Feller et al., 1998). Also, Zhao and Su (2014) reported that high temperature causes low CO_2 solubility in water.

2.3.8. Oxygen Accumulation

In order to increase efficiency of photosynthesis it is important to feed the microalgae with CO_2 and to remove the produced oxygen from the culture environment (Posten, 2009). The oxygen in the atmosphere is derived from photosynthetic water splitting process. Remained oxygen molecule in the culture causes toxic effects like photo-bleaching and finally limits the photosynthetic efficiency (Kumar et al., 2011). Therefore an efficient degassing system is needed to remove formed O_2 (Kumar et al., 2011), otherwise microalgae inhibiting concentration level which is above 200% of air saturation may occur already after 1 min (Posten, 2009).

2.3.9. Salinity

Generally, salinity means that sodium chloride concentration in the culture. Salinity is used for promoting algal growth and limiting invasion of algal competitors and predators (Bartley et al., 2013). Also salinity is one of the important environmental parameters that can affect biochemical composition of microalga cells via increasing lipid content (Bartley et al., 2013).

2.3.10. Mixing

Turbulent flow and mixing are required hydrodynamic parameters to provide homogeneous distribution of cells, light, heat nutrients, metabolites and toxic effluent. Also enhanced mixing prevents microalgae aggregation and sedimentation. Sobczuk et al. (2006) stated that mixing increases biomass production due to homogenous light regimen. Mixing of the microalgal suspension in photobioreactors forces balanced mix of the light/dark cycle which improves higher microalgal growth rates and photosynthetic efficiency by providing flashing light effect (Abu-Ghosh et al., 2016).

Also, Sobczuk et al. (2006) reported that excessive mixing may damage microalgae since some of them cannot tolerate shear stress.

Mixing also enhances mass transfer by preventing the anaerobic zone formation (Grobbelaar, 2000).

2.4. Optimum Conditions for *C. Vulgaris* Cultivation

C. vulgaris is spherical, eukaryotic, unicellular green algae, with a size of 2-10 μm that grows in fresh water conditions (Safi et al., 2014). It has rapid growth ability during favorable conditions, and it is resistant to invaders and severe environmental conditions (Safi et al., 2014). The algae which is in the water medium, only requires minimal conditions such as light and CO_2 for growth.

In order to achieve rapid growth rates, optimal intensity and wavelength of light, pH, temperature, nutrient availability, mixing speed, the ratio of the concentration of dissolved oxygen and CO_2 in the medium must be provided (Cheah et al., 2014).

2.4.1. Nutrient Composition

C. vulgaris uses glucose, acetate, glycerol and glutamate as carbon sources but maximum specific growth rate is obtained with glucose in heterotrophic culture (Safi et al., 2014). On the other hand, in autotrophic culture system, CO_2 or bicarbonate compounds (such as sodium bicarbonate) are used as the only carbon source.

According to stoichiometric equation of *C. vulgaris* growth reported by Roels (1938), optimum N/P ratio on mass basis for growth of *C. vulgaris* is 7 which is similar to ratio of 7.2 calculated by Redfield (1934). In addition to N/P ratio, C/N ratio is essential to optimize the growth performance of *C. vulgaris* (Hu and Gaol, 2003). The analysis showed that C/N ratio must not be above 17 for obtaining *C. vulgaris* with high specific growth rate (Pagnanelli et al., 2014).

2.4.2. pH

Khalil et al. (2010) reported that *C. vulgaris* can grow in a wide range of pH between 4 and 10 and most biomass productivity is achieved in the alkaline environment (pH= 9 and 10).

According to Edberg (2010), *C. vulgaris* species are not able to grow at all at pH 2. Also final pH (pH=11) causes negative effects on the growth of *C. vulgaris* (Yeh et al., 2010).

2.4.3. Temperature

C. vulgaris has optimum temperature about 30°C, in which the maximum biomass productivity is achieved (Chinnasamy et al., 2009). Converti et al., (2009) reported that *C. vulgaris* growth rate decreases above 30°C. These species are not able to grow at all and cells die at 38°C temperature.

2.4.4. Light

According to Blair et al. (2014), optimum light spectra for *C. vulgaris* cells production are red light (λ=630-665nm) and blue light (λ=430-465) respectively. A study was performed by Wang et al. (2007) showed that *C. vulgaris* cells grow more under blue light, compared to red light.

2.4.5. Carbon dioxide

Maximum growth rate of *Chlorella* species is obtained using 10% CO_2 concentration (Maeda et al., 1995). Also another study showed that *C. vulgaris* is inhibited at 15% (v/v) CO_2 with air (Yun et al., 1997).

Studies showed that addition of $NaHCO_3$ to culture medium increases biomass production by *C. vulgaris* in comparison to the addition of CO_2 alone (Aishvarya et al., 2012).

2.5. Microalgae Cultivation Techniques

There are a number of different cultivation systems for microalgae studied in the literature (Wang et al., 2008; Suali and Sarbatly, 2012; Bahadar and Khan, 2013; Zhao and Su, 2014). Microalgae cultivation techniques can vary with respect to the investment cost, the desired products, the source of nutrients and CO_2 capture (Klinthong et al., 2015). Microalgae cultivation systems are divided into open and closed systems.

2.5.1. Open System

Open ponds are the most commonly used and are the cheapest method for production of large-scale biomass production. Natural waters such as lakes, lagoons, ponds or wastewater or artificial ponds or containers can be categorized into open pond systems. These systems generally built next to municipal water plants, power plants or heavy industry with high CO_2 discharge. Since this system can use CO_2 as carbon source, it ensures not only cost-effective and large scale biomass production but also decrease atmospheric CO_2 pollution. On the other hand, there are several limitations in open pond systems such as poor light and CO_2 utilization, limited mixing, evaporative water losses, contamination risks, temperature and pH fluctuations, requirement for large areas of land and risk of pollution, poor biomass productivity. Some examples of open pond system are shown in Figure 2-4.

Figure 2-4. Photobioreactors used in growing microalgae: (a) raceway pond, (b) flat-plate type (Bitog et al., 2011)

2.5.2. Closed System-Photobioreactors

These microalgae cultivation systems are designed to overcome some of the major problems related with the described open pond systems (See Section 2.5.1). Closed systems can be categorized into the tubular, flat plate, and column photobioreactors. Closed system photobioreactors enable production of single-species of microalgae for prolonged durations with lower contamination risk (Chisti, 2007). Photobioreactors can also be used to produce large quantities of microalgal biomass, (Carvalho et al., 2006).

Although high investment cost and scale up problem, there are also several advantages of microalgae cultivation in closed system photobioreactors such as minimal contamination risk, less CO_2 loss, higher biomass production under excellent control of pH, temperature, light, CO_2 concentration (Bahadar and Bilal Khan, 2013). Some examples of closed system photobioreactors are shown in Figure 2-5.

Figure 2-5. Photobioreactors used in growing microalgae: (a) inclined tubular type, (b) horizontal/continuous type (Bitog et al., 2011).

2.6. Applications of Microalgae

Microalgae species have been used as a food source by humans for thousands of years (Spolaore et al. 2006). However, microalgae culture in modern technology can be considered as new approach which was started to investigate approximately 100 years ago (Borowitzka, 2006).

Currently, there are many useful applications of microalgae such as waste treatment, CO_2 mitigation, biological heavy metal remediation, agricultural fertilizer, human food, animal food and feed, biofuel applications etc.

2.6.1. Wastewater Treatment

Microalgae can be used for wastewater treatment because of their potential to remove chemical and organic contaminants, heavy metals and pathogens from wastewaters generated by different sources (Muñoz and Guieysse, 2006).

Different applications diverse microalgal strains on diverse wastewater such as human sewage, agro-industrial wastes, livestock wastes, industrial wastes have been analyzed in literature (Brennan and Owende, 2010; Cuellar-Bermudez et al., 2014).

It has been shown that *Chlorella*, *Scenedesmus*, *Spirulina*, *Botryocoocus* and *Chlamydomas* are the most widely used microalgal species for nutrient removal (Gonzales et al., 1997; Martinez et al., 2000; Olguin et al., 2003; Palmer, 1974). Furthermore, mixed microalgal cultures and microalgal-bacterial symbiotic cultures were used for nutrient removal from wastewater (Zhou, 2010).

Wastewater treatment with microalgae is not only economic but also ecologic way for nutrient removal compared to physical and chemical processes (Ristenson, 2011).

2.6.1.1. Municipal Wastewater Remediation with Microalgae

Nearly 65 years ago, it was shown that biological treatment of municipal wastewater with algae to remove nutrients like nitrogen and phosphorus was a successful method (Oswald et al., 1953). Several scientists have tried to investigate advanced technologies by using algae's fast growth and high nutrient removal ability (Larsdotter, 2006).

It was stated that domestic wastewater is an ideal source for algal growth as it includes high concentration of required nutrient contents (Larsdotter, 2006). Generally, nitrogen and phosphorus concentrations of municipal wastewater are between 25-45 mg/L and 4-16 mg/L, respectively (Tchobanoglous et al., 2003).

According to Tchobanoglous and Burton (1991), medium strength domestic wastewater values which are measured as 40 mg/L of nitrogen and 8 mg/L of phosphorous, are enough for N and P in each liter to produce 0.6 g of algal biomass. Use of microalgae for wastewater treatment is not only ensuring wastewater treatment but also provide microalgal biomass production.

Microalgae-based wastewater treatment can be accepted as an efficient biological approach for nutrient removal from wastewater. Colak and Kaya (1988) reported 85.7% - 97.8% phosphorus removal from domestic wastewater by algal system. On the other hand, Van-Coillie et al. (1990) observed phosphorus removal changes between 60% and 98%. These studies indicated that microalgal nitrogen removal is more effective than phosphorus removal. For example, Li et al. (1991) reported that

99.3% nitrogen removal and 48.1% phosphorus removal from wastewater. It was also observed that 94% ammonium nitrogen removal in algal pond by Picot el al. (1991). Moreover, Van-Coillie et al. (1990) reported that nitrogen removal is nearly 92.95%.

In order to achieve successful wastewater treatment with microalgae, the most important operational parameters such as depth, turbulence or hydraulic retention time must be arranged precisely (Larsdotter, 2006).

2.6.1.2. Industrial Wastewater Treatment with Microalgae

Since industrialization has spread around the world, toxic wastes discharged into water bodies. Industrial wastewater can contain one or more impurities of industries such as poultry, dairy, iron-steel making, textile, leather, breweries, foods, paper pulp, rubber, petrochemicals, pharmaceuticals, chemicals, pollutants from acid mine drainage and metal working, heavy metal contaminants, toxic chemicals, phenolic components.

Microalgae have been used in industrial wastewater treatment and recycling. Microalgae-based wastewater treatment offers high removal efficiency with low input energy way. The efficiency of industrial wastewater treatment by microalgal cultures has proven by different studies as very promising. For example, Rai et al. (1981) reported that microalgae can remove heavy metals. Moreover, several toxic organic compounds can be cleaned up by microalgae (Redalje et al., 1989). It was also shown that microalgae can be successfully used for the treatment of olive oil mill wastewater and paper industry wastewater (Abeliovich and Weisman, 1978; Tarlan et al., 2002). Similarly, in another example, the rinse water from the olive oil extraction industry was used to cultivate and provide nutrient for microalgae (Hodaifa et al., 2008).

Besides nutrient removal ability, some scientist also investigated capacity of microalgae for removing dye from industrial wastewater. For example, El Sikaily et al. (2006) showed that methylene blue which is used in laboratory as an indicator can be removed from water by green algae.

2.6.1.3. Treatment of Animal Wastewater with Microalgae

It is known that microalgae can grow more abundantly in nutrient-rich (eutrophic) waters where the animal residue or fertilizer is allowed to run off surface water bodies like lakes and other slowing moving streams. This massive algal growth brings about generally algal blooms (Schenk et al., 2008). This rapid accumulation of microalgae make O_2 and food available conditions and the bacteria increase in number and use up the dissolved oxygen in the water and natural flora and fauna die off. Therefore, N and P are considered as key parameter of eutrophication of river, lake and seas (Lau et al., 1997).

In controlled algal system which is built next to dairy farms, algae can consume N and P components of the wastes (Olguin, 2003). For example, Shen et al. (2008) reported that algae can remove %88 of total nitrogen and 98% of total phosphorous from animal wastewater.

It was investigated that microalgae can be used for the treatment of dairy wastewater and BOD, TSS, NH_4-N, phosphorus, and *E. coli* removal were achieved by microalgae grown in different types of ponds (Park, Craggs et al., 2011).

In other instances, it was used swine effluent as a nutrient source for algae and was achieved 42-100% of the initial ammonium, and 58-100% of the total initial phosphorus removal (Kebede-Westhead, Pizarro et al. 2006; Mulbry, Kondrad et al., 2008).

2.6.2. Carbon Capture and Uptake

Microalgal biomass carbon content is nearly 50% of total by dry weight (Mirón et al., 2003). This carbon is taken from carbon dioxide. Since microalgae are the best known photosynthetic organisms, they can be used for CO_2 fixation. Sobczuk et al.(2000) stated that nearly 1.5 to 2.0 kg of CO_2 is needed to produce 1.0 kg of microalgae biomass. Also, Doucha et al. (2005) stated that 4.4 kg of CO_2 is required for 1 kg of *C. vulgaris* biomass.

Characteristically microalgae may be used the CO_2 derived from three different sources namely, atmospheric CO_2, CO_2 coming from emission of power plant and industry and dissolved CO_2 as carbonate (Wang et al., 2008). Since atmospheric CO_2 concentration is only 0.03–0.06% of total air, slow cell growth rate is observed due to mass transfer limitation (Chelf et al., 1993). On the other side, industrial emitted gases like flue gas contains nearly 15% CO_2 which is more efficient carbon source for microalgal bio fixation (Wang et al., 2008). The tolerance of microalgae to relatively high CO_2 content is critical in uptake of CO_2 and microalgal growth. Although many studies showed that high CO_2 content is harmful for microalgal photosynthesis (Silva and Pirt, 1984; Lee and Tay, 1991), a number of reports stated that microalgae can tolerate and grow under same CO_2 concentration present in the flue gases (Hanagata et al., 1992; Hirata et al., 1996; Maeda et al., 1995; Yun et al., 1997). Microalgal CO_2 tolerance is enhanced by several mechanisms such as preventing acidification of chloroplast parts and providing maintenance activity of Rubisco which is an enzyme work in CO_2 fixation (Solovchenko and Khozin-Goldberg, 2013).

Biomass measurements and growth rate evaluations are essential for interpreting the potential of a cultivation system for microalgae for direct CO_2 removal (Costa et al., 2004; Cheng et al., 2006). According to Chiu et al. (2009), the removal efficiency (%) can be obtained using this Equation 2-6:

$$\text{Efficiency } (\%) = \frac{[\text{Influent of } CO_2] - [\text{Effluent of } CO_2]}{[\text{Influent of } CO_2]} * 100 \qquad (2\text{-}6)$$

The CO_2 removal or fixation efficiency in a closed cultivation system depends on several parameters namely, species of microalgae, concentration of CO_2 flow, photobioreactor design and operating conditions (de Morais and Costa, 2007; Chiu et al., 2009).

Yun et al., (1997) stated that the CO_2 fixation rate could be obtained from the carbon content and specific growth rate of the microalgae. According to Yun et al. (1997), the fixation rate was determined by using following Equation 2-7:

$$R_{CO_2} = C_C * \mu * \frac{M_{CO_2}}{M_C} \qquad (2\text{-}7)$$

where R_{CO2} and μ are the fixation rate and the specific growth rate, respectively, while M_{CO2} and M_C are the molecular weights of CO_2 and elemental C, respectively. C_C is the average carbon content as measured by elemental analysis. The microalgal growth rate is determined in the linear growth regime, because most algal growth occurred during this phase.

2.6.3. Biosorption of Heavy Metals by Microalgal Cultures

Microalgae can also be used for heavy metals remediation from soil, water, and residues (Sekabira et al., 2011; Monteiro et al., 2012) and hence are accepted as promising biosorbents. Algae in heavy metal remediation have several advantages such as rapid metal uptake capacity, time and energy saving, ecologically and user friendly, reusable, cost effective, faster growth rate over higher plants, no toxic waste production, and applicability in both batch and continuous cultivation systems (Monteiro et al., 2012). It was reported that especially *Chlorella* and *Scenedesmus* are used for metal removal (de-Bashan and Bashan, 2010).

2.6.4. Organic Fertilizer

Microalgae biomass or extract residues have been used as organic soil fertilizer in coastal areas all over the world for many years (Skjånes et al., 2007). It was reported that these organisms are beneficial for plants via producing growth promoting factors, vitamins, proteins, antibacterial and antifungal compounds and plant growth improving polymers (de Mulé et al., 1999). Moreover, microalgal biomass increases not only water binding capacity but also mineral content of the soil (Riley, 2002). It was stated that 1 kg of microalgae could replace 60 kg of conventional fertilizer (De

La Noue and De Pauw, 1988). Dogan-Subasi and Demirer (2016) also reported the biofertilizer potential of *C. vulgaris*.

2.6.5. Human Nutrition

Microalgal biomass can be used as a human food source since its high nutritional content (Henrikson, 1989). *C. vulgaris* is one of the few microalgae that can be employed for human food supplement or additive in the form of tablets, powder, capsules or extracts (Liang et al., 2004; Yamaguchi, 1996). Besides of its nutritional richness in protein, carbohydrate and lipid, it also contains bioactive compounds. Therefore, algal biomass can be categorized as medicinally effective (Mayer and Gustafson, 2004). For example, it has not only immunological effects (Hayashi et al., 1998), but also antioxidant activity (Miranda et al., 1998) and promotion of intestinal bacteria (Pulz and Gross, 2004). Moreover, microalgae can be categorized as anti-bacterial, bronchodilator, polysynaptic blocker and analgesic (De La Noue and De Pauw, 1988).

2.6.6. Animal Feed

Becker (2007) estimated that approximately 30% of total microalgal production is sold for animal feed. Biomass of microalgae can play an important role in supplying not only human food but also animal feed. For example, *C. vulgaris* accumulates important amount of carotenoids under stress conditions and it can be used as feed for animals like fish and poultry. This substance shows pigmentation for fish flesh and chicken egg yolk. It also increases life expectancy of animals (Becker, 2007; Gouveia et al., 1996; Yamaguchi, 1996).

2.6.7. Biofuels

Algae and microalgae are categorized as third generation biofuel form. It can be seen as one of the alternatives to current biofuel crops namely, soybean, corn, sugarcane, and rapeseed since microalgae and algae do not compete with agriculture. Furthermore, microalgae and algae do not require arable land to grow. However, production cost of microalgae is still high and cannot compete with conventional fuel (Hannon et al., 2010). Microalgal biomass can be converted into biofuel with two methods which are classified as thermochemical (Bridgwater, 2003) and biochemical

methods (Yadvika et al., 2004). Thermo-chemical conversions include direct combustion providing electricity, gasification producing syngas, thermochemical liquefaction converting biomass into bio-oil and pyrolysis producing bio-oil, syngas and charcoal. Biochemical conversion methods include anaerobic digestion producing methane and hydrogen, alcoholic fermentation converting biomass to ethanol and photobiological hydrogen production (Brennan and Owende, 2010; Çalıcıoğlu and Demirer, 2015; Skjånes et al., 2007).

2.7. Cost Analysis of Wastewater Treatment and CO_2 Emission

Cost is a main parameter in the management and control of nutrient pollution. There are several cost associated impacts of the nutrient pollution such as water characteristics, geographic location, type of waterbody, type of nutrient source, level of pollution (EPA,2015). It can also be difficult to link between nutrient pollution and to estimate of external costs. In order to understand the economic feasibility of microalgae based wastewater treatment and CO_2 mitigation, several studies have performed techno-economic feasibility studies.

For example, Pruvost et al (2016) reported that an estimated yearly production of *C. vulgaris* in a photobioreactor is around 25–30 tons biomass per ha (i.e. average daily productivity of 7.68 g m^{-2} day^{-1}). It means that 40–50 tons of CO_2 fixed per year per ha by *C.vulgaris*.

Dogaris et al (2016) stated that estimated the capital cost of wall photobioreactor at full production to be $25,000 per hectare which includes cost of materials and manufacturing labor extrapolations. Moreover, it was estimated that open ponds ranges approximately from about $10,000 to almost $79,000 per hectare taking into account the costs of the liner and the paddlewheel.

It is known that raceway type open ponds are used in Israel, United States of America, China, and other countries. Although their low construction and operation costs, the average cost of these systems is $8-15 per Kg of dry biomass (Lee, 2001).

EPA (2015) reported that the 330 km^3 of municipal wastewater produced globally each year is enough to irrigate 40 million hectares which is equals to 15% of all currently irrigated land. Moreover, OECD 2009 report stated that global estimated wastewater treatment cost is $83.5 billion. Also, it was stated that due to improper wastewater treatment, CO_2 and CH_4 emissions are observed and this will reach 0.19 million tons of CO_2/day in 2025 (Rosso and Stenstrom, 2007).

As shown in Table 2-1, increasing amount of wastewater into aquatic systems such as canals, rivers, lakes and seas affects public health, environmental ecosystem and economic activities (UNEP, 2015). The values of these examples are classified as indirect costs.

Table 2-1. Examples of potential negative impacts of wastewater on public health, the environmental ecosystem and economic activities

IMPACTS ON	EXAMPLES OF IMPACTS
Health	• Increased burden of disease due to reduced drinking water quality • Increased burden of disease due to reduced bathing water quality • Increased burden of disease due to unsafe food (*contaminated fish, vegetables and other farm produce*) • Increased risk of diseases when working or playing in wastewater-irrigated area • Increased financial burden on health care
Environment	• Decreased biodiversity • Degraded ecosystems (*e.g. eutrophication and dead zones*) • Bad odours • Diminished recreational opportunities • Increased GHG emissions
Productive activities	• Reduced industrial productivity • Reduced agricultural productivity • Reduced market value of harvested crops, if unsafe wastewater irrigation • Reduced number of tourists, or reduced willingness to pay for recreational services • Reduced fish and shellfish catches, or reduced market value of fish and shellfish

CO_2 fixation is another popular concept since carbon credit can be traded on international market. In Turkey, total GHG emission was measured as 467.4 mt CO_2 in 2014 which mostly comes from energy based emission, industrial processes and agriculture (TUIK, 2014). This number showed that CO_2 emission increased by 125% compared to 1990 in Turkey. In 1990, it was reported that CO_2 emission was 3.77 ton per person; on the other hand, it was measured as 6.08 ton per person in 2014. According to Republic of Turkey Ministry of Energy and Natural Resources (General Directorate of Renewable Energy, 2012), the cost of CO_2 capture was between 25 and 60 €/ton CO_2, the cost of CO_2 transport was in the range of 1-4 €/ton CO_2, and the cost of CO_2 storage changed between 10 and 20 €/ton CO_2. As a result, it can be stated that the total cost of CO_2 capture, transport and storage changed between 36 and 84 €/ton CO_2.

Microalgae based domestic and industrial wastewater treatment CO_2 mitigation approach is a feasible alternative since it associates not only nutrient removal and CO_2 mitigation but also production of a wide range of commercial bioproducts and biomass. It can be calculated that approximately $83.5 billion from cost of global wastewater treatment and $889.8 billion from cost of global CO_2 capture and storage can be saved in a year by using microalgae. Furthermore, this produced biomass can be used in a wide range of commercial products and biofuel.

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental Sets and Procedures

3.1.1. Microalgae Cultivation

C. vulgaris culture was obtained from the Arac Creek (Karabük, Turkey). Prior to experiments, culture was enriched as presented in Section 3.1.2.

3.1.2. Microalgal Biomass Cultivation Medium and Cultivation Conditions

The collected microalgae was cultivated by using the Bold's Basal Medium with 3-Fold Nitrogen and Vitamins (3N-BBM+V) (Bilanovic et al., 2009) in a 3300 L cylindrical bubble column reactor with 3000 ml of working volume. The growth medium contained (concentration of the constituents are given in parentheses as g/L): $NaNO_3$ (0.75), $CaCl_2.2H_2O$ (0.025), $MgSO_4.7H_2O$ (0.075), $K_2HPO_4.3H_2O$ (0.075), KH_2PO_4 (0.175), NaCl (0.025), Na_2EDTA (0.0045), $FeCl_3.6H_2O$ ($5.84x10^{-4}$), $MnCl_2.4H_2O$ ($2.46x10^{-4}$), $ZnCl_2$ ($3x10^{-5}$), $CoCl_2.6H_2O$ ($1.2x10^{-5}$), $Na_2MoO_4.2H_2O$ ($2.4x10^{-5}$), Vitamin B1 (0.0012), Vitamin B12 (0.00001) (Bilanovic et al., 2009).

3.1.3. Characterization of Municipal Wastewater

The municipal (domestic) wastewater used in this study was collected from the exit point of the primary clarifier of Greater Municipality of Ankara Tatlar Domestic Wastewater Treatment Plant (Ankara, Turkey). Photobioreactor was fed with this wastewater which was collected at different dates and used as different batches (Table 3-1). Characterization of the wastewater indicated that each batch there is a variation in the characteristics of the wastewater. This is probably due to the

meteorological and seasonal variations (Tchobanoglous et al., 2003). Before analysis, each wastewater batch was filtered from a 0.3 mm pore size sieve and stored at 0°C and dark conditions. Characterization of municipal wastewater was made by standard measurement techniques (APHA, 2004). Characterization included the analyses of nutrients (NH_4^+ and PO_4^{3-}), pH and total COD levels. Domestic wastewater contains bacteria and other microorganisms. Therefore, microalgae culture was grown in autoclaved and non-autoclaved municipal wastewater to observe the impact of microorganisms on the growth of microalgae. Characterization of the municipal wastewater is given in Table 3-1.

Table 3-1. Municipal Wastewater Characterization

	Date of Collection			
Constituents	**17/12/2013**	**13/01/2014**	**11/02/2014**	**03/03/2014**
pH	7.3	7.2	7.7	7.4
NO_3-N (mg/L)	< 0.1	< 0.1	< 0.1	< 0.1
NO_2-N (mg/L)	< 0.01	< 0.01	< 0.01	< 0.01
PO_4^{3-}-P (mg/L)	1.5 ± 0.2	5.8 ± 1.5	3.9 ± 0.1	1.7 ± 0.1
TAN (mg/L)	61.5 ± 2.1	52.5 ± 1.9	53.2 ± 3.4	50.7 ± 0.1
N/P ratio	41.0	9.1	13.6	28.8
tCOD (mg/L)	274.4 ± 4.5	255.1 ± 3.9	230.3± 6.6	276.2 ± 3.5

3.1.4. Characterization of Industrial Wastewater

There are several processes in Karabük Kardemir Iron and Steel Factory. Different parts of the factory such as iron foundary, coking unit, electrofilter unit produce significant amounts of wastewater. After cleaning processes, final destination of purified industrial wastewater is Soganli Creek (Karabük, Turkey) which is near the factory. Wastewater originating from coking unit contains high level of TAN (Total ammonia nitrogen). Due to the requirement of high nitrogen concentrations for the

microalgal growth, coking process wastewater of Karabük Kardemir Iron and Steel Factory was used in the experiments. Analysis for water characterization revealed that unlike nitrogen concentration, phosphorous concentration was trace amount in wastewater. Yun et al (1997) used additional chemical agent containing phosphorous for algae growth in their research conducted by steel industry wastewater. Coke plant wastewater contains both metal and organic wastes therefore it can represent typical industrial wastewater. According to EPA (2002), iron and steel industry coking process wastewater includes benzene, naphthalene, anthracene, cyanide, ammonia, phenols, and cresols together with a range of more complex organic compounds known collectively as polycyclic aromatic hydrocarbons (PAH). Heavy metals which represent an important group of hazardous contaminants often found in industrial wastewater (Kratochvil and Volesky, 1998).

The optimum value of the N/P ratio for microalgae growth and productivity varies from 6.8 to 10 (Wang et al., 2010). In order to keep this ratio, additional phosphorus is added to wastewater. KH_2PO_4 was used as an external P source and N/P ratio was kept around 10 for the maximum growth of the microalgae. Due to high ammonia concentration, industrial wastewater was used after diluted 1/50 with distilled water. Characterization of the industrial wastewater is given in Table 3-2.

Table 3-2. Industrial Wastewater Characterization

Parameter	**Concentration**
TS (mg/L)	8471 ± 311
VS (mg/L)	136 ± 4
TS (%VS)	2
tCOD (mg/L)	11827 ± 150
sCOD (mg/L)	10225 ± 61
TN (mg/L)	3600 ± 90
TAN (mg/L)	3352 ± 78
NO_3-N (mg/L)	4 ± 0,2
NO_2-N (mg/L)	<0,01
Organic-N (mg/L)	244
O.PO_4-P (mg/L)	1 ± 0,1
Sulfate (mg/L) *	1509
Cyanide (mg/L) *	0,0125
Arsenic (µg/L) *	767,2
Mercury (µg/L) *	3,27
Iron (µg/L) *	9,26
Cadmium (µg/L) *	17
Chrome (µg/L) *	<1,8
Phenol (mg/L)*	950

* These parameters were measured by an accredited laboratory.

3.1.5. Collection and Characterization of Flue Gas

The flue gas was collected from Kardemir Iron and Steel Factory (Karabük, Turkey). Sample was collected during the manufacturing period. During sampling online gas composition measurements were made and reported in Table 3-3.

Table 3-3. The composition of the flue gas

Parameter	**Concentration**
CO_2	11.03 %
CO	379 ppm
NO	5 ppm
NO_2	1 ppm
NO_x	6 ppm
SO_2	0 ppm

The collected gas was stored in a pressurized 200 liter volume gas cylinder with 1.4-1.6 bar pressure (Figure 3-1). The gas cylinder had a regulator and a valve providing a connection to withdraw the flue gas during the experiments.

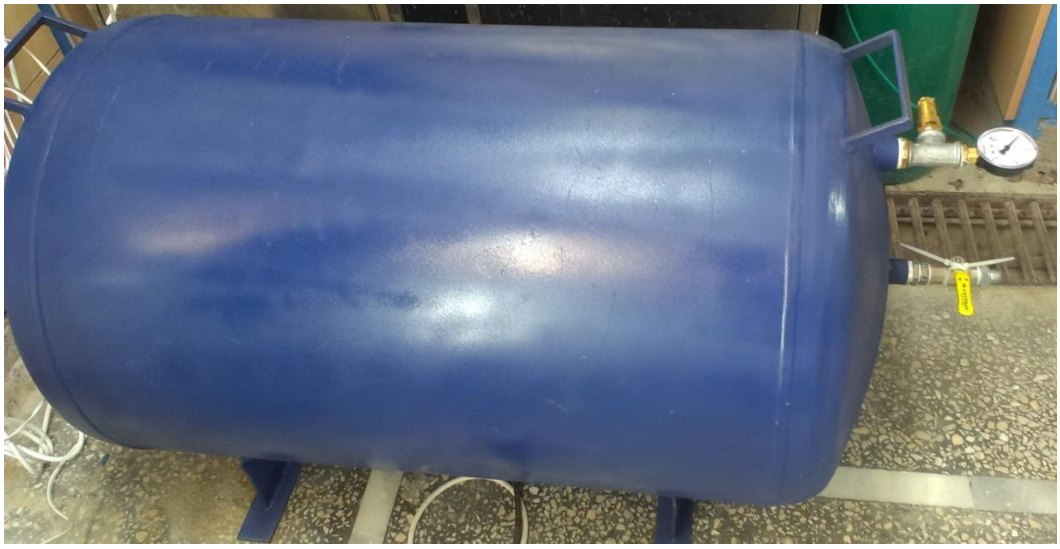

Figure 3-1. 200 liter volume gas cylinder

3.1.6. Reactors Used in Study

The reactors used in this study are summarized in Table 3-4 and described in detail in the Section 3.1.6.1, Section 3.1.6.2, Section 3.1.6.3 and Section 3.1.6.4.

Table 3-4. The reactors used in this study

Reactor Used	Purpose of Operation	Section
Semi-Continuous Cultivation Photobioreactor	To get enough microalgal biomass for Set-1, Set-2 and Set-3 experiments and analysis	3.1.6.1
Batch Cultivation Photobioreactor (Set-1)	To investigate the tolerance of high CO_2 levels by microalgae, removal of nutrients from domestic wastewater and the amount of artificial CO_2 biotransformed by microalgae	3.1.6.2
Batch Cultivation Photobioreactor (Set-2)	To investigate the performance of microalgae cultivation reactors fed by real flue gas, removal of nutrients from domestic wastewater and the amount of CO_2 biotransformed by microalgae	3.1.6.3
Fed-batch Cultivation Photobioreactor (Set-3)	To investigate the performance of microalgae cultivation reactors fed by real flue gas, removal of nutrients from industrial wastewater and the amount of CO_2 biotransformed by microalgae	3.1.6.4

3.1.6.1. Semi-continuous Cultivation Photobioreactor

High amount of microalgal biomass was needed for the Set-1, Set-2, and Set-3 experiments and for the determination of nutrient removal and CO_2 mitigation potentials of *C. vulgaris* cultures. Therefore, a semicontinuously operated photobioreactor simulating an open pond was designed and operated (Figure 3-2). The photobioreactor was inoculated by using microalgal cultures and the Bold-Basal Medium. After six days, photobioreactor was started with domestic wastewater as nutrient source instead of Bold-Basal Medium. Length, width and height values of photobioreactor were 32 cm, 25 cm and 50 cm, respectively.

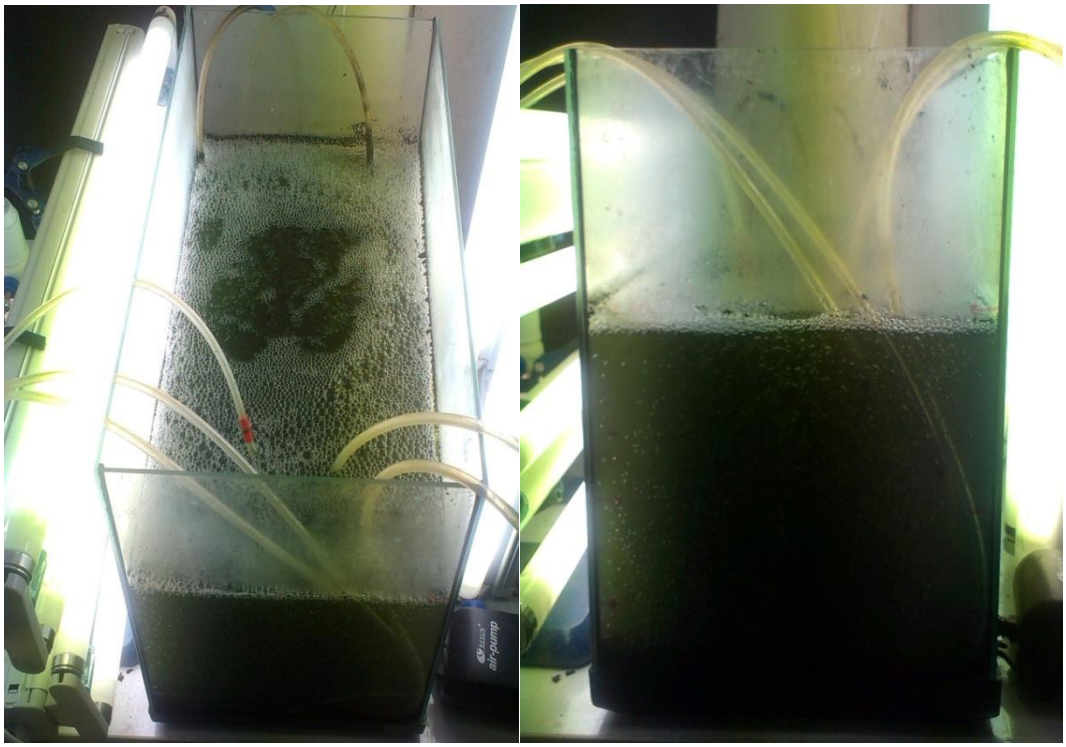

Figure 3-2. Semi-continuous Cultivation Photobioreactor

The photobioreactor was operated in semi-continuous mode. That is, when optical density started to increase in the reactor, wasting of wastewater was done. It was aerated with two air pumps (RESUN 9602, China). Each pump was connected to spargers. Dimensions of spargers are 25 cm x 2 cm x 2cm. Illumination of photobioreactor was provided with eight cool-white 18 W fluorescent lamps (OSRAM, L 18W/685, Korea). Lamps were placed to illuminate the four sides of the photobioreactors. Light intensity of the lamps measured as 120 μmol/m^2.s PAR at the surface of the photobioreactor and 80 μmol/m^2.s PAR in the center of the photobioreactor filled with wastewater. These light intensity values determined as the optimum range for growth of *C. vulgaris* (Filali et al., 2011). Lamps were 10 centimeters away from one side of the photobioreactor. Each lamp was 5 centimeters away from photobioreactor. In order to keep light intensity, photobioreactor was isolated by a black curtain. Photobioreactor was made of glass with total and operating volumes of 40 L and 24 L, respectively.

All plastic and glass parts of the photobioreactor and basal medium were autoclaved for 60 minutes at 121^0C and 5 psi pressure before cultivation. After, the photobioreactor cooled down 20 mL of culture was used to inoculate the photobioreactor under sterile conditions.

3.1.6.1.1. Operational Conditions

Photobioreactor was operated with 24 hours continuous illumination. In order to supply CO_2 for growth of microalgae, reactor aeration was done continuously with 0.5 volume air per volume broth per minute (vvm) air provided with air pump. Temperature was between 27-30 ^{0}C in the photobioreactor. First 6 days, pH was adjusted to around 7-8 via 0.1 M HCl. After the 6^{th} day, there was no need for adjustment of the pH since significant pH elevation was not observed.

Figure 3-3 summarizes the feeding protocol of photobioreactor. It was operated with varying feeding regimes. First 6 days, photobioreactor was filled with domestic wastewater gradually. After 6 days, wasting procedure started. Days between 6 and 12, 12 L wastewater and algae mixture taken from photobioreactor and 12 L domestic wastewater added into it daily. Optical density data showed that 12 L nutrient loading into the photobioreactor prevented microalgae growth as it was not seen any optical density increase during first 12 days. Therefore, it was decided that daily feeding regime was too short for growth of microalgae. In the days between 12 and 19, due to risk of wash out of remained microalgae, wasting procedure performed every second days. After the 12^{th} day, optical density started to increase until the 19^{th} day; however, after this point a decrease was detected in the optical density data again so it was concluded that every second day wasting time range was short for microalgae growth, too.

Days between 19 and 60, wasting was performed once every three days. Wasting was performed in successive days due to the risks of wash out of microalgae and overdose nutrient loading into the photobioreactor. As shown in Figure 3-3a, effective reactor volume of photobioreactor was kept constant at 24L. Daily feeding and wasting volumes of photobioreactor are shown in Figure 3-3b and c respectively.

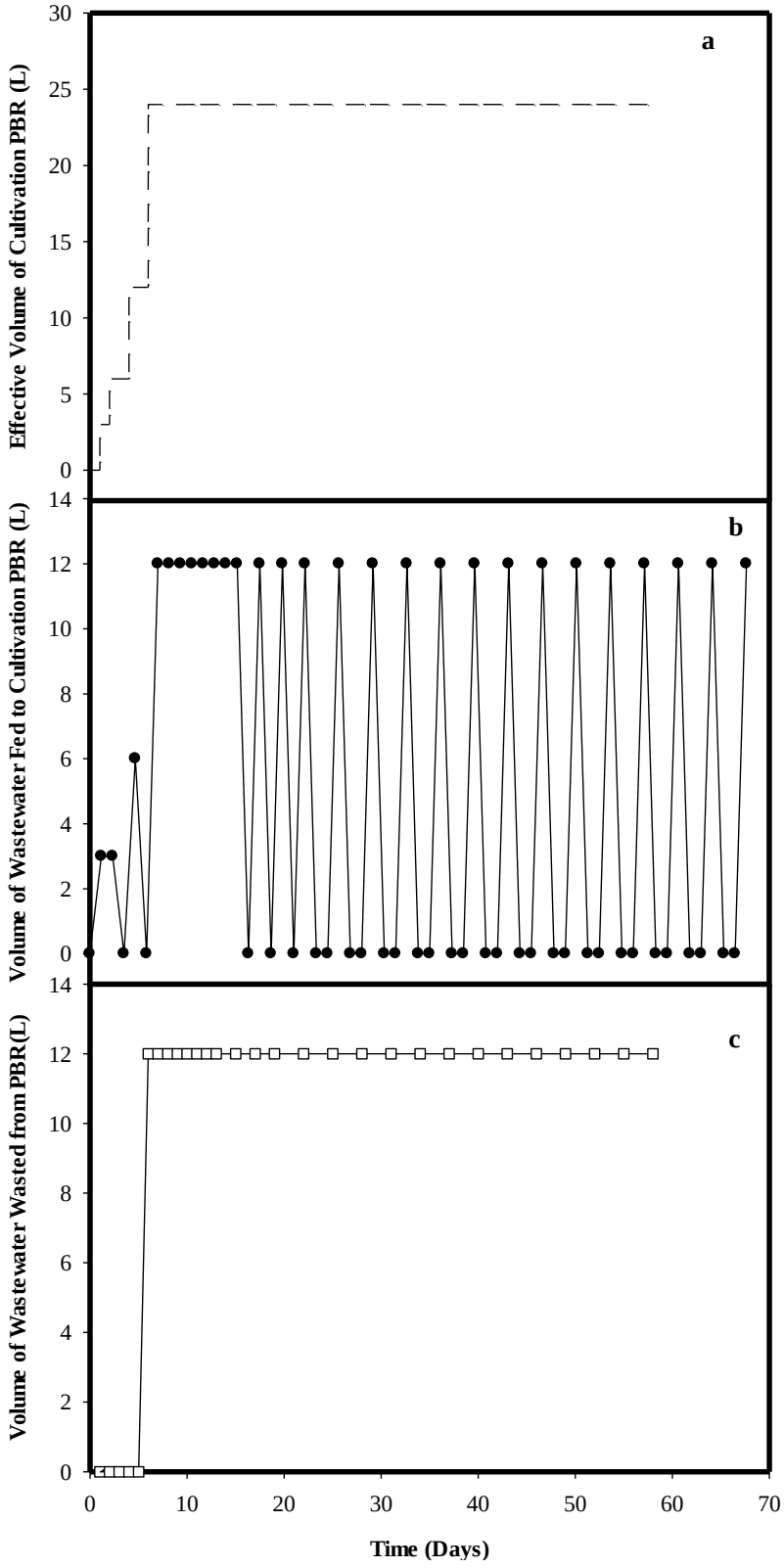

Figure 3-3. Summary of Feeding Protocol in Cultivation Photobioreactor

3.1.6.2. Batch Cultivation Photobioreactors (Set- 1)

In order to investigate the tolerance of high CO_2 levels by microalgae, removal of nutrients from domestic wastewater and the amount of artificial CO_2 biotransformed by microalgae, reactors were designed.

Serum bottles and caps were sterilized by autoclaving for 60 minutes at 121^0C and 5 psi pressure before used. Glass serum bottles with 500 mL total volume with tight caps were used for the experiments. 350 mL of microalgae and 50 mL of wastewater were added to each reactor. The reactors were illuminated with fluorescent lamps (OSRAM, L 18W/685, Korea) continuously receiving approximately 200 μmol/m^2.s light.

In order to achieve maximum contact time between microalgae and wastewater, the reactors were kept on a shaking table with rotational speed of 175 rpm. The number of reactors was 20 for this set of experiments.

Reactors were divided into 3 different categories based on their content. First type of reactors contained autoclaved domestic wastewater and microalgae. There were 8 replicas of these type reactors. Second type of reactors included raw domestic wastewater and microalgae. There were 8 replicas of these type reactors, as well. There were control group containing 4 reactors, as the third type of reactors. These control reactors contained autoclaved pure water, pure water, autoclaved domestic wastewater and raw wastewater, respectively. The batch cultivation reactors are illustrated in Figure 3-4. Characterization of the reactors is given in Table 3-5.

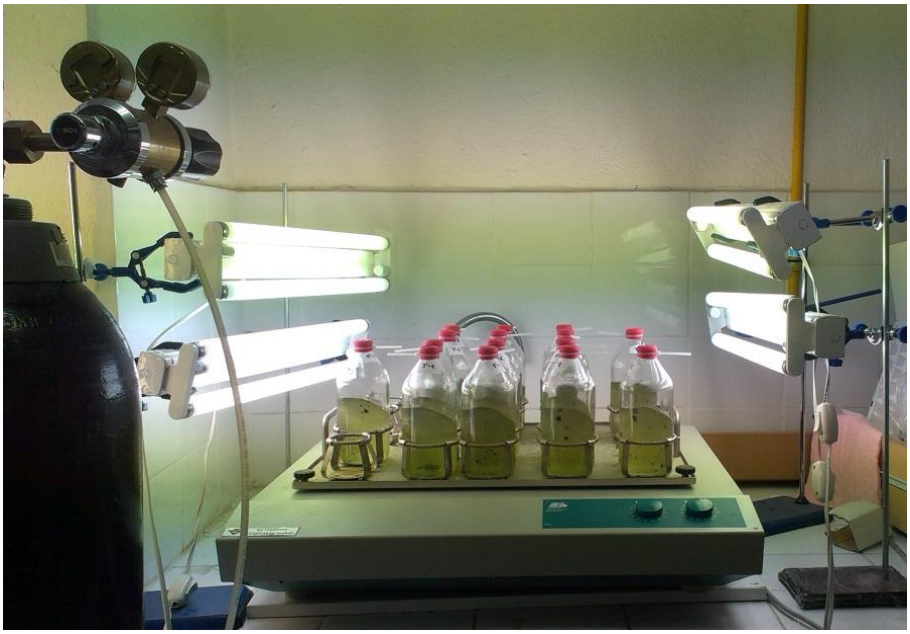

Figure 3-4. A photograph of Batch Cultivation Photobioreactors

Table 3-5. Set-1 Batch Cultivation Photobioreactors Characterization

Reactor Name	Reactor Number	Raw Domestic Wastewater	Autoclaved Domestic Wastewater	Distilled Water	Autoclaved Distilled Water	Algae	Total Volume
Control 1A	1	-	-	400 mL	-	-	400 mL
Control 1B	1	-	-	-	400 mL	-	400 mL
Control 2A	1	400 mL	-	-	-	-	400 mL
Control 2B	1	-	400 mL	-	-	-	400 mL
Reactor A	8	350 mL	-	-	-	50 mL	400 mL
Reactor B	8	-	350 mL	-	-	50 mL	400 mL

3.1.6.2.1. Operational Conditions

The headspace of the reactors was purged by pure CO_2 and air mixture to provide high levels of CO_2 (10-30%). The composition of headspace in terms of CO_2 was monitored every day to make sure CO_2 was not limiting (below 5%) for microalgae

growth in the reactors as well. This set of reactors which were operated with high levels of CO_2 (~20%), which may simulate typical flue gas composition of iron-steel and cement industries using the nutrients available in municipal wastewater.

When CO_2 level in the headspace decreased below 5 %, CO_2 addition was done. That is, reactors were operated in a fed-batch mode regarding CO_2. At each sampling time, that is every day during 7 days, one photobioreactor was terminated and the headspace CO_2 composition and aqueous phase TS, VS, TAN, and PO_4^{3-}, chlorophyll-a concentrations were measured. The operation of reactors was continued for 7 days, until the nutrients, especially PO_4, were exhausted.

In order to check gas leakage and CO_2 solubility in the distilled water, Control 1 reactors were used. Control 1A and Control 1B reactors contained 400 mL pure distilled water and autoclaved distilled water, respectively. To determine whether gas leakage and CO_2 solubility in the distilled water, these reactors were observed in terms of CO_2, O_2, and N_2 composition daily.

Second set of control reactors were inoculated with microalgae and domestic wastewater which was autoclaved at 121^0C for 30 min. In order to determine performance of heterotrophic bacteria originating from municipal wastewater, Control 2A and Control 2B reactors contained 400 mL raw domestic wastewater and autoclaved domestic wastewater, respectively, were operated.

Reactor A and Reactor B were experimental group reactors. One set of reactors which contained eight replicate raw domestic wastewater and microalgae mixture called Reactor A.

They contained 350 mL raw domestic wastewater and 50 mL microalgae. On the other hand, Reactor B groups which contained eight replicate autoclaved domestic wastewater and microalgae mixture. They contained 350 mL autoclaved domestic wastewater and 50 mL microalgae.

3.1.6.3. Batch Cultivation Photobioreactor (Set-2)

These reactors were run to investigate the performance of microalgae cultivation reactors fed by real flue gas, removal of nutrients from domestic wastewater and the amount of CO_2 biotransformed by microalgae.

Serum bottles and caps were autoclaved for 60 minutes at 121^0C and 5 psi pressure to be sterilized before used. Glass serum bottles with 500 mL total volume with tight caps were used for the experiments. 350 mL of microalgae and 50 mL of wastewater were added to each reactor. The reactors were illuminated with fluorescent lamps (OSRAM, L 18W/685, Korea) continuously which provided light intensity of 200 $\mu mol/m^2.s$.

In order to get maximum contact time between microalgae and wastewater, the reactors were kept on a shaking table with rotational speed of 175 rpm. The number of reactors was 18 for this set of experiments.

In this set of reactors operated to investigate CO_2 uptake from flue gas by microalgae growing in municipal wastewater. Flue gas with a 5-6% CO_2 was used to aerate this set of reactors. Characterization of the reactors is given in Table 3-6.

Table 3-6. Set-2 Batch Cultivation Reactors Characterization

Reactor Name	Reactor Number	Raw Domestic Wastewater	Autoclaved Domestic Wastewater	Algae
Control 2C	1	400 mL	-	-
Control 2D	1	-	400 mL	-
Reactor C	8	350 mL	-	50 mL
Reactor D	8	-	350 mL	50 mL

3.1.6.3.1. Operational Conditions

The headspace of the reactors was purged by flue gas. The composition of headspace in terms of CO_2 was monitored every day to make sure CO_2 was not limiting (below

5%) for microalgae growth in the reactors. As CO_2 level in the headspace decreased below 5%, CO_2 addition was done. That is, reactors were operated in a fed-batch mode regarding CO_2. At each sampling time, a reactor was terminated and the headspace CO_2 composition and aqueous phase TS, VS, TAN, PO_4 and chlorophyll-a concentrations were measured. The operations of reactors were continued for 7 days, until the nutrients, especially PO_4, were exhausted.

In order to determine performance of heterotrophic bacteria originating from municipal wastewater, Control 2C and Control 2D reactors contained 400 mL raw domestic wastewater and autoclaved domestic wastewater, respectively, were operated. Autoclaved domestic wastewater kept in 121^0C for 30 min.

Moreover, two different sets of reactors were designed to investigate performance of microalgae. One set of reactors which contained eight replicate raw domestic wastewater and microalgae mixture called Reactor C. Other set of reactors which contained eight replicate autoclaved domestic wastewater and microalgae mixture called Reactor D.

3.1.6.4. Fed-batch Cultivation Photobioreactor (Set-3)

The aim of this set of experiment is to investigate the coupled nutrient (nitrogen and phosphorus) and CO_2 removal by microalgae growth in photobioreactors operating fed-batch mode. Industrial wastewater from coking unit and flue gas from Kardemir Iron and Steel Factory were used for feeding of microalgae in reactors. Industrial wastewater was used as nutrient and flue gas was used as CO_2 sources in the test.

For the sterilization purposes, serum bottles and caps were autoclaved for 60 minutes at 121^0C and 5 psi pressure before used. Glass serum bottles with 120 mL total volume with tight caps were used for the experiments. 80 mL of effective volume and 40 mL of headspace were used for the each reactor. The reactors were illuminated continuously by fluorescent lamps (OSRAM, L 18W/685, Korea) receiving approximately 200 $\mu mol/m^2.s$ light.

In order to get maximum contact time between microalgae and industrial wastewater, the reactors were kept on a shaking table with rotational speed of 200 rpm. The number of reactors was 7 for this set of experiments. Characterization of the reactors is given in Table 3-7.

Table 3-7. Fed-batch Cultivation Reactor Characterization

Reactor Name	**Reactor Number**	**HRT**	**Raw Industrial Wastewater**	**Autoclaved Industrial Wastewater**	**Distilled Water**	**Algae**
Negative Control	1	8	-	-	70 mL	10 mL
Control 2E	1	8	80 mL	-	-	-
Control 2F	1	8	-	80 mL	-	-
Reactor E	2	4	40 mL	-	30 mL	10 mL
Reactor F	2	8	40 mL	-	30 mL	10 mL

3.1.6.4.1. Operational Conditions

In order to observe the performance of microalgae cultivation reactors fed by real flue gas and industrial wastewater, removal of nutrients from wastewater and the amount of CO_2 biotransformed by microalgae reactors were designed.

In order to investigate growth of microalgae, two different hydraulic residence times (HRTs) were used. Reactors were operated with 4 and 8 day hydraulic residence times (HRTs) and 40 mL of subsamples were taken and 40 mL of industrial wastewater were added at the same time. TS, VS, TAN, PO_4 and chlorophyll-a analyses were performed.

The headspace of the reactors was purged by flue gas. CO_2 concentration in the headspace will be kept at a level of 10-11% theoretically, but industrial CO_2 content is level of 5-6%. The composition of headspace in terms of CO_2 was monitored every day to make sure CO_2 was not limiting microalgae growth in the reactors. Once CO_2 decreased below 5%, CO_2 addition was done. The concentration of CO_2 in the

headspace was measured daily. The reactors were operated for 50 days with fed-batch feeding regimes. The pH of the industrial wastewater was between 10 and 11 and could be inhibitory on algal growth. Therefore, the pH value reduced to 7.0.

Industrial wastewater was taken from coking unit of Kardemir Iron and Steel Factory. Industrial wastewater was not used as nutrient for microalgae directly because of its excessive amount TAN and heavy metal concentration. High TAN and heavy metal content could be inhibitory on growth of microalgae (Abeliovich et al., 1976; Arunakumara et al., 2006; Gutierrez et al., 2016). In order to prevent microalgae from excessive TAN and heavy metal stress, industrial wastewater was used as 1/50 diluted form.

In order to get optimum growth of the microalgae, N/P ratio was kept as 10-11. Since phosphorous amount in industrial wastewater was limited for growth of the microalgae (see Table 3-2), KH_2PO_4 used as external P source.

In order to determine the effect of heterotrophic bacteria coming from industrial wastewater, Control 2E and Control 2F reactors were operated. Reactors named as Control 2E and Control 2F contained 80 mL raw industrial wastewater and 80 mL autoclaved industrial wastewater respectively. Autoclaved industrial wastewater kept in 121^0C for 30 min.

One set of reactors which contained two replicate of raw industrial wastewater and microalgae mixture which were called Reactor E, operated with 4 day hydraulic residence time. Other set of reactors which contained two replicate of raw industrial wastewater and microalgae mixture which were called Reactor F, operated with 8 day hydraulic residence time. The last reactor called as Negative Control included microalgae and distilled water which was operated to observe the effect of industrial wastewater on microalgae.

3.2 Analytical Methods

Several measurements were done during this study. pH, temperature, light intensity, Optical Density, Total Solids (TS), Volatile Solids (VS), Total Suspended Solids

(TSS), Volatile Suspended Solids (VSS), Total Chemical Oxygen Demand (tCOD), Soluble Chemical Oxygen Demand (sCOD), Total Kjeldahl Nitrogen (TKN),Total Nitrogen (TN), Total Ammonifiable Nitrogen (TAN), Total Nitrate (NO^{3-} - N), and Orto phosphate (PO_4^{3-} -P) were measured. Besides these chemical measurements gas analyses such as O_2, N_2, and CO_2 of reactors were done as well.

3.2.1. pH Measurements

pH was measured for samples from all the batch and fed-batch photobioreactors using a pH meter (HI 8314, Hanna Instruments) and pH probe (HI 1230, Hanna Instruments).

3.2.2. Temperature

In order to monitor the temperature of the culture, temperature was measured using a submerged thermometer (Sensorex, p350, Garden Grove, CA, USA).

3.2.3. Light Intensity

In order to evaluate the amount of light intensity striking the microalgal cells, the light intensity measurements were done using a light meter (Li-Cor, 250 A, Lincoln, Nebraska, USA). It was done with the help of the underwater quantum sensor apparatus.

3.2.4. Optical Density

Optical density was measured using spectrophotometer (HACH, DR 2800, Berlin, Germany) at 685 nm wavelength with 1cm light path. Macro-cuvettes were used. There were different wavelength values for microalgal measurement. In order to determine the optimum value, optical density values at different wavelengths within the range of 450 to 800 nm were measured. 685 nm was accepted as optimum value owing to the highest absorbance (Ras et. al., 2011). In order to get maximum absorbance, it must be within the range of 0.1 to 1. Ideally absorbance values must be above 0.1 and below 1 and sample dilution was made if necessary.

3.2.5. Total Solids, Volatile Solids, Total Suspended Solids and Volatile Suspended Solids

TS, VS, TSS and VSS values were determined according to Standard Methods 2540 (APHA, 2004). In order to get suspended portion of any sample glass fiber filters were used (Millipore, AP40, Billerica, MA, USA). Vacuum filtration unit (Millipore, WP8 11 2250, MA, USA) was performed for filtration processes.

3.2.6. Total Chemical Oxygen Demand

Total COD values were determined by using Standard Methods 5220 were used (APHA, 2004).

3.2.7. Soluble Chemical Oxygen Demand

The samples were filtered through 0.45 μm cellulose- acetate filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250, Billerica, MA, USA).

Medium-range (0 – 1500 mg/L COD) and low-range (0-150 mg/L COD) test kit vials (Lovibond, Aqualytic, Dortmund, Germany) were used for determination of sCOD values. At first, vials were heated up to 150 ^{0}C, digested for 120 minutes and then cooled to ambient temperature prior to COD value detection using a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany). After cooling process, vials were put in photometer and values were recorded.

3.2.8. Total Kjeldahl Nitrogen

TKN values were determined by using Standard Methods 4500-N_{org} Macro Kjeldahl Method was used (APHA, 2004).

3.2.9. Total Soluble Nitrogen

The samples were filtered through 0.45 μm cellulose- acetate filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250, Billerica, MA, USA). The filtrate was then used to measure TN values of the samples.

TN values were determined using test kit vials (Lovibond, Vario 535560, Aqualytic, Dortmund, Germany). After samples preparation, a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany) was used to measure TN concentration.

3.2.10. Total Ammonifiable Nitrogen

The samples were filtered through 0.45 µm cellulose- acetate filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250, Billerica, MA, USA). The filtrate was then used to measure TAN values of the samples. TAN (NH_4^+-N + NH_3-N) values were detected using test kit vials (Lovibond Vario 535600, Aqualytic, Dortmund, Germany). After preparation of samples, TAN concentration was determined using a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany). This process took 20 minutes.

3.2.11. Nitrate-N

The samples were filtered through 0.45 µm cellulose- acetate filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250, Billerica, MA, USA). The filtrate was then used to measure NO_3^- -N values of the samples. NO_3^- -N values were detected using test kit vials (Lovibond Vario NitraX 535580, Aqualytic, Dortmund, Germany). After addition of samples, NO_3^- -N values were determined using a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany) within ten minutes.

3.2.12. Ortho-Phosphate

The samples were filtered through 0.45 µm cellulose- acetate filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250, Billerica, MA, USA). The filtrate was then used to measure PO_4^{3-}- P values of the samples. PO_4^{3-}- P values were detected using Lovibond phosphorus tablet pack (Lovibond, Vario 515810, Aqualytic, Dortmund, Germany). After preparation of samples, PO_4^{3-}- P values were determined using a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany).

3.2.13. Gas Analysis

A 10 mL gas-tight syringe (Sanitex-Italy) was used to obtain gas samples from the headspace of the photobioreactors and analyses was made by using a gas chromatograph (GC) (Thermo Electron Co., Thailand) equipped with a thermal conductivity detector (TCD). In the GC measurements, helium (He) was the carrier gas supplied at a constant pressure of 100kPa. Temperature of inlet and detector were programmed to 50 ^{0}C and 80 ^{0}C respectively. The calibration of GC for hydrogen (H_2), carbon dioxide (CO_2), oxygen (O_2), nitrogen (N_2) was made by pure standards of the gases.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Results of Semi-continuous Cultivation Photobioreactor

4.1.1. Cultivation Photobioreactor Biomass Production and Operation

High amount of microalgal biomass was needed for the Set-1, Set-2, and Set-3 experiments and analysis to determine the nutrient removal and CO_2 mitigation potentials of *C. vulgaris* cultures. In order to provide that, a semi-continuously operated photobioreactor simulating an open pond was designed and operated for 58 days.

The culture used in this study was analyzed by Ayşe Özgül Çalıcıoğlu in Environmental Engineering Department of Middle East Technical University, Ankara, Turkey (Çalıcıoğlu, 2013). Algae were characterized under microscope and it was found that the dominant algal species in the culture was *C. vulgaris*.

In this study, microalgae were fed with domestic wastewater with varying feeding regimes. Growth of microalgae was monitored with optical density, TAN and PO_4 removal rates, and pH data.

Optical density measurements were known as a good indicator for microalgae biomass production (Toennies and Gallant, 1949; Shuler and Kargi, 2005). In order to measure optical density of *C. vulgaris*, 685 nm wavelength was used throughout the experiment (Ras et. al., 2011).

As shown in Figure 4-1, first 19 days were observed as the acclimation period for microalgae. It can also be called as a lag or an induction phase. First 19 days the algae were adapting to the environment and the conditions and were not reproducing yet. After 19th day, microalgae entered exponential growth phase. Algal cells reproduced well and had an optical density of approximately 1.00 Abs after 25th day. Moreover, it was shown a gradual decline in microalgae growth rate after 25th day. This decline was thought to be a result of self-shading of microalgae in cultivation photobiorector. Self-shading is a phenomena resulting from the limitation in light penetration due to high density of microalgal culture and small light path (Cheah et al, 2014). As Dickinson et al. (2015) stated, the growth of microalgae was light limited due to the high density. When optical density value of microalgae reached up to 1.00 Abs, it pointed out poor light diffusion inside the reactor. After 31st day, microalgae reached stationary phase and the number of new cells was limited due to the self-shading effect. The highest optical density was recorded as 1.38 Abs in 58th day.

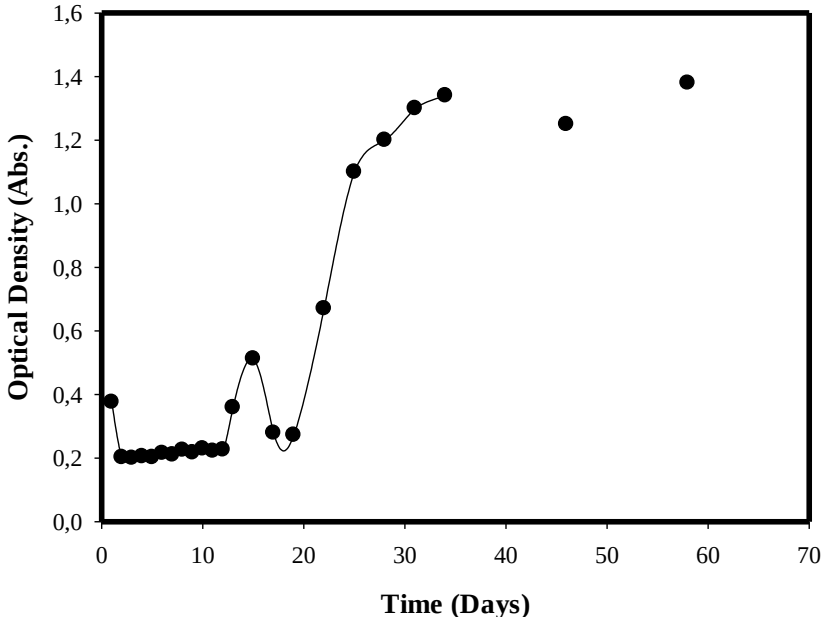

Figure 4-1. Optical density graph of Cultivation Photobioreactor against time

Besides optical density, pH values of the cultivation photobiorector were also monitored in daily basis. pH is another important parameter for growth and metabolic activities of microalgae (Ho et al., 2011). As seen in Figure 4-2, no significant change in pH data was detected after 6th day. In contrast, pH fluctuated and increased up to 10 during the first 6 days.

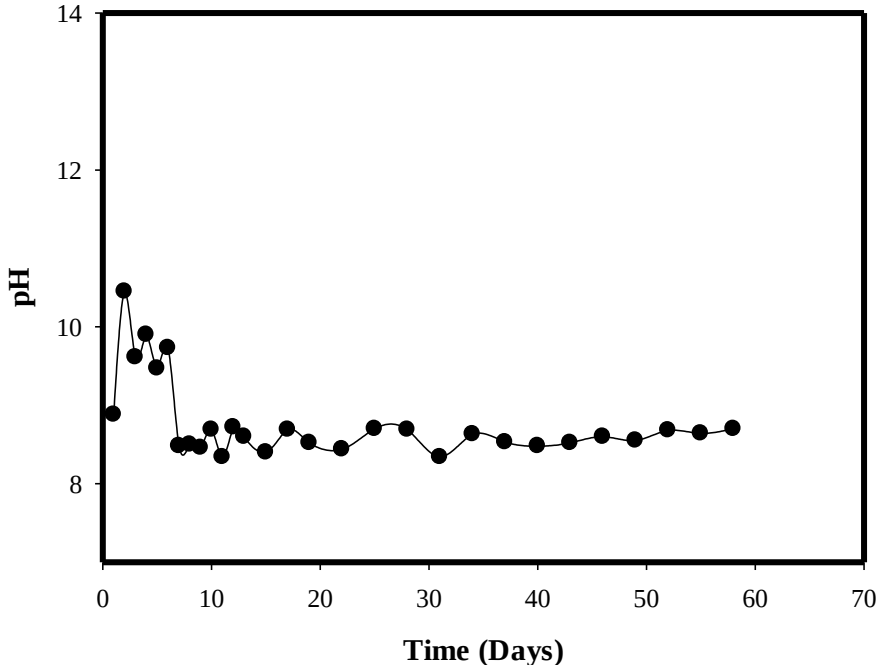

Figure 4-2. pH data of Cultivation Photobioreactor

Microalgae adapted and grew the pH ranges between 8.2 and 8.7 which is the optimum range for growth of many microalgae species (Bitog et al., 2011). During the first 6 days, pH values measured as around 9-10 level and they were adjusted to pH ranges 8.0 and 8.5 with 0.1 N HCl after feeding/wasting operation. This arrangement was found necessary to do to prevent ammonia volatilization due to elevated pH induced by microalgae and to keep pH in optimum ranges for microalgae growth (Hammouda et al., 1995). During the first 6 days, instability of pH and adjustment necessity is probably due to accelerated metabolic reactions and growth activity during this acclimation period.

In order to prevent significant pH changes, mixing is generally used since it regulates pH by promoting gas exchange between air and water as reported by Oswald (1988). Unfortunately, no mixing sources could be used in this set of the experiment due to size of the aquarium. For this reason, deficiency of the continuous mixing could be another reason for instability of pH during first 6 days.

In addition, in order to fill reactor with wastewater completely, daily nutrient loading during first 6 days was done and after these 6 days, two different feeding regimes were applied namely every second days and every third days. This nutrient loading fluctuation was another cause for instability of pH.

After the first 6 days of reactor operation, cultivation photobioreactor reached 24 L effective volume and there was no need to do adjustment since pH values were measured around 8-8.5 and did not change significantly. pH stability within the optimal range indicates that balanced growth and stabilized metabolic activities of microalgae.

Cultivation photobioreactor was fed with domestic wastewater which was taken from the exit point of primary clarification operating in the municipal wastewater treatment plant at four different times. Domestic wastewater samples which were taken in different months was analyzed and used as nutrient source for microalgae as shown in Table 3-1 (See Section 3.1.3). Inorganic N/P ratio was a significant indicator for algal growth (Wang et al., 2010). The ratio stated by Redfield (1934) indicates that N/P ratio of algal mass equals to 7.2 g N/g P on average. Similarly Roels (1938) found a stoichiometric equation for *C. vulgaris*. According to this equation, N/P ratio of algal mass equals to 7 g N/g P. Proportions between the quantity of nitrogen and phosphorous of all wastewater in this study were higher than the ratio stated by Redfield (1934) and Roels (1938), significantly. These higher ratios which were namely 41.0, 9.1, 13.6, and 28.8 indicated that there was a phosphorous limitation in domestic wastewater. Additional phosphorous sources were not preferred and feeding regime did not change due to phosphorous deficiency. Moreover, nitrogen concentration was also analyzed since nitrogen limitation can change metabolic activities of microalgae (Ras et al., 2011). For example, in order to

take precaution against nitrogen starvation, lipid accumulation starts in the microalgae cells. Brennan and Owende (2010) stated that lipid accumulation means that increased concentration of lipids within the microalgae cells without consideration of the overall biomass production, and is just opposite to the specific productivity. In other words, nitrogen deficiency causes lower specific productivity.

4.2. Results of Batch Cultivation Photobioreactors (Set-1)

The aim of this part of the study was the removal of nutrients (nitrogen and phosphorus) from domestic wastewater by microalgae growth using CO_2 enriched air, simulating flue gas.

4.2.1. Nutrient Removal from Wastewater

In this part of the study, nutrient removal potential of *C. vulgaris* cultivated in domestic wastewater was investigated. The nitrogen and phosphorus removal were monitored.

At the end of experiment, TAN and PO_4 removal efficiencies of Reactor A and Reactor B were observed as 99.95 - 98.75% and 90.57 - 94.11% respectively. In order to see if there is a significant difference between TAN and PO_4 removal efficiencies of Reactor A and Reactor B, the Student's t test was applied. It was found that the TAN and PO_4 removal efficiencies were not significantly different ($p=0.09$ and $p=0.12$, respectively).

The results proved that *C. vulgaris* is one of the dominant algae strain and shows high performance in wastewater treatment like other studies. For example, it was reported that 95.3% and 96% of nitrogen and phosphorus removal efficiencies were obtained, respectively, in swine industry effluents by Kim et al., (2013). Another study was conducted by Li et al.,(2013) indicated that 90.9- 93.6% of nitrogen and 89.9- 91.8% of phosphorus removal efficiencies, in municipal wastewater.

It can be seen from Figure 4-3b and 4-4b, that the general trend of TAN and PO_4 concentrations in Reactor A and B were gradually decreasing by time and reached to values near zero.

In A Reactors TAN and PO_4 uptake started almost immediately without any lag phase. 3.2 mg/L PO_4 and 49.87 mg/L TAN were uptaken in Reactor A at the end of 6 days of operation. On the other hand, in B Reactors TAN and PO_4 uptake started after 1 - 1.5 day acclimation period. It was calculated that 0.68 mg/L PO_4 and 14.43 mg/L TAN were uptaken in Reactor B at the end of 4 days of operation. (See Figure 4-3a and Figure 4-4a). It is noteworthy to note that that more than 95 % of the TAN and PO_4 were removed by microalgae in reactors approximately in 5 days.

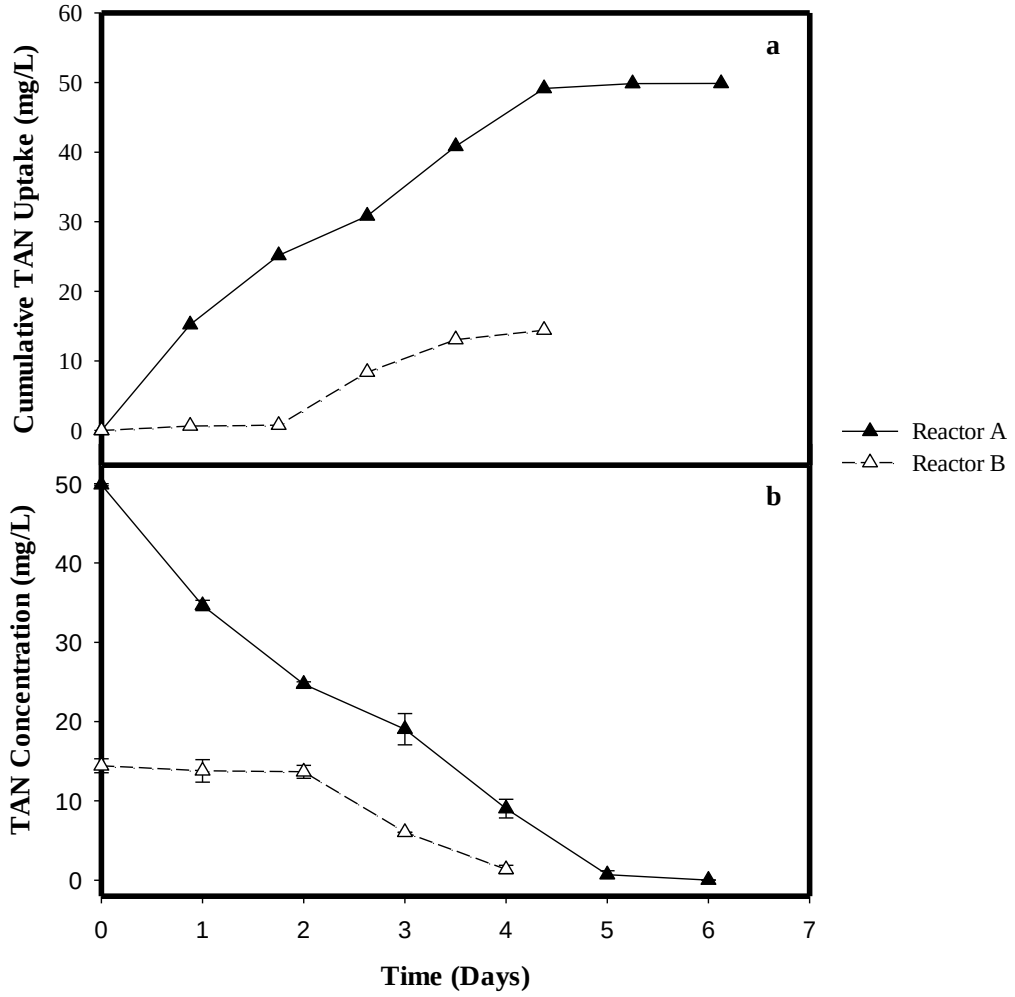

Figure 4-3. a) Cumulative TAN uptake; b) TAN concentration of Reactor A and Reactor B

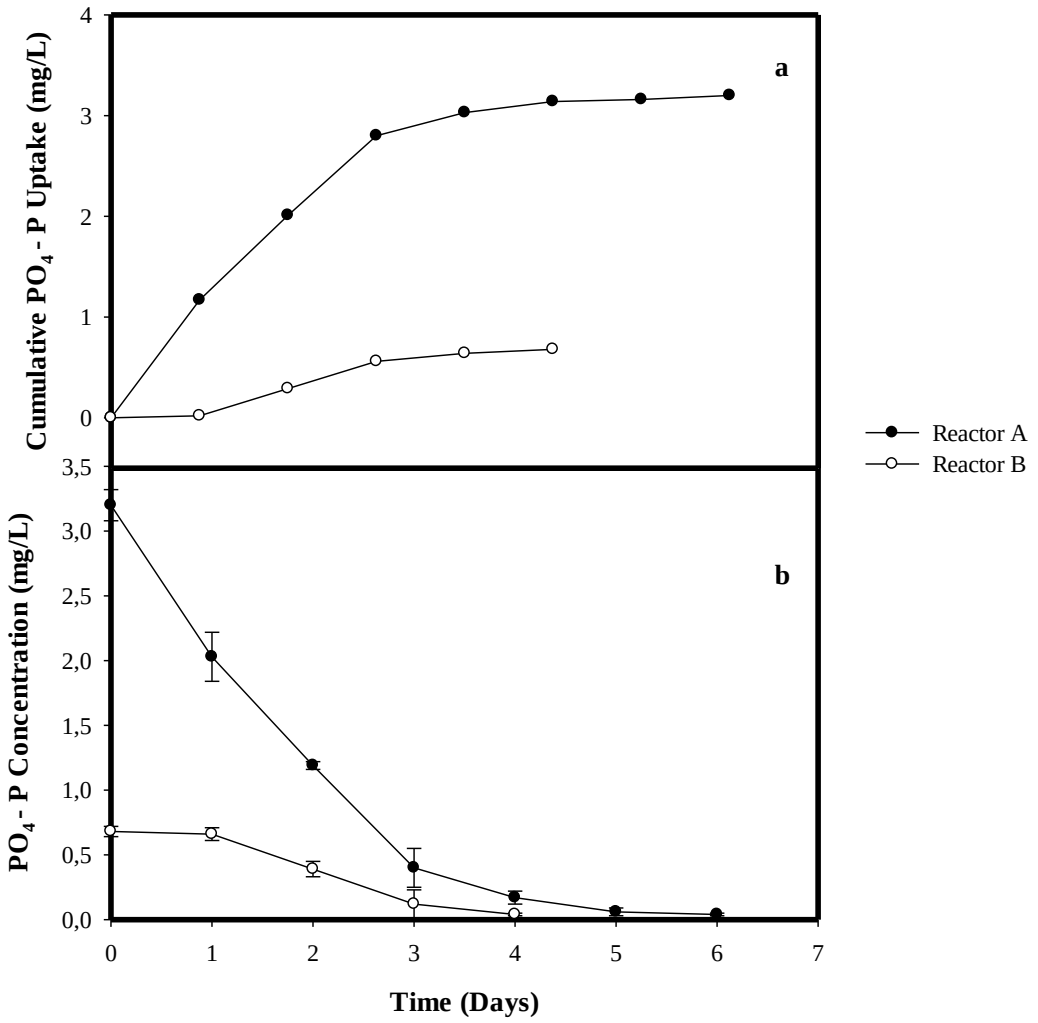

Figure 4-4. a) Cumulative PO_4-P uptake; b) PO_4-P concentration of Reactor A and Reactor B

The N and P removal rates for Reactor A were 7.19 mg/L.day and 0.81 mg/L.day, respectively. For Reactor B these rates were 3.34 mg/L.day and 0.15 mg/L.day, respectively. These results are similar to other studies. For example, Cabanelas et al., (2013) demonstrated *C. vulgaris* in wastewater showed nitrogen removal rate about 3.4 mg/L.day and phosphorous removal rates varied from 0.5 to 0.7 mg/L.day. Similarly Su et al., (2012) showed that the N and P removal rates for *C. vulgaris* were 4.39 mg/L.day and 0.76 mg/L.day respectively.

As seen in Table 4-1, the results of this study were in agreement with the related literature. Li et al., (2013) performed semi-continuous and continuous cultivation

reactors which operated with *C. vulgaris* in municipal wastewater treatment plant secondary effluent. Total nitrogen and total phosphorus removal efficiencies of these semi-continuous and continuous cultivation reactors were 90.3 - 93.6% and 89.9-91.8%, respectively. In another study conducted by Kim et al. (1998) showed 95.3% and 96% removal efficiencies of nitrogen and phosphorus, respectively, by *C. vulgaris* in 25% secondarily treated swine wastewater after four days of incubation. Yun et al., (1997) reported *C. vulgaris* was able to remove 100% of ammonia, by addition of external phosphate salts. After ammonia was depleted, 50% nitrate removal was also achieved. Lau et al., (1995) performed laboratory-scale experiments, more than 90% of nitrogen and 80% of phosphorus were removed from primary treated sewage by *C. vulgaris*. Similarly, Woertz et al., (2009) studied with *C. vulgaris* cultivated in municipal wastewater. Over 99% of ammonium and orthophosphate removal was achieved for CO_2 sparged treatments with both 3 and 4 days HRT.

Table 4-1. Comparison of this study with related literature in terms of phosphorous and nitrogen removal efficiencies (%)

Reference	**Microalgal Species**	**Nitrogen Removal Efficiency (%)**	**Phosphorous Removal Efficiency (%)**
Li et al. (2013)	*C. vulgaris*	90.3 - 93.6	89.9 - 91.8
Kim et al., (1998)	*C. vulgaris*	95.3	96.0
Lau et al., (1995)	*C. vulgaris*	90.0	80.0
Yun et al., (1997)	*C. vulgaris*	100.0	50.0
Woertz et al., (2009)	*C. vulgaris*	Over 99	Over 99
Tam and Wong (1996)	*C. vulgaris*	95 - 100	N.D
Reactor A (In this study)	*C. vulgaris*	99.9	98.7
Reactor B (In this study)	*C. vulgaris*	90.5	94.1

Table 4-2 indicates that TAN and PO_4 removal rate was higher in Reactor A than Reactor B. As previously mentioned, Reactor A was operated with raw domestic

wastewater and Reactor B was operated with autoclaved domestic wastewater as nutrient source for microalgae. TAN and PO_4 removal rate difference is due to the presence of bacteria and bacterial assimilation of nutrients associated with microalgae growth in wastewater of Reactor A.

Table 4-2. Comparison of Reactor A and Reactor B in terms of TAN and PO_4 removal rates

Reactor	**TAN Removal Rate (mg/L.day)**	**PO_4 Removal Rate (mg/L.day)**
Reactor A	7.19	0.81
Reactor B	3.34	0.15

When microalgae are cultivated with wastewater rich in nutrients and organic matter, symbiotic relationship with bacteria can be observed (Nurdogan, 1980). As shown in Figure 4-5, some bacteria and algae can live together having a mutually beneficial relationship. Bacteria can help degrade some compounds to ammonium, nitrogen, phosphate and carbon dioxide so that microalgae can use them easily (Zhang et al., 2012). According to the literature, interaction between microalgae and bacteria has been tested in ponds and photobioreactors which could achieve cost effective high rate nitrogen, phosphorus removal (Boelee et al., 2014; He et al., 2013; de-Bashan et al., 2002; de Godos et al., 2009).

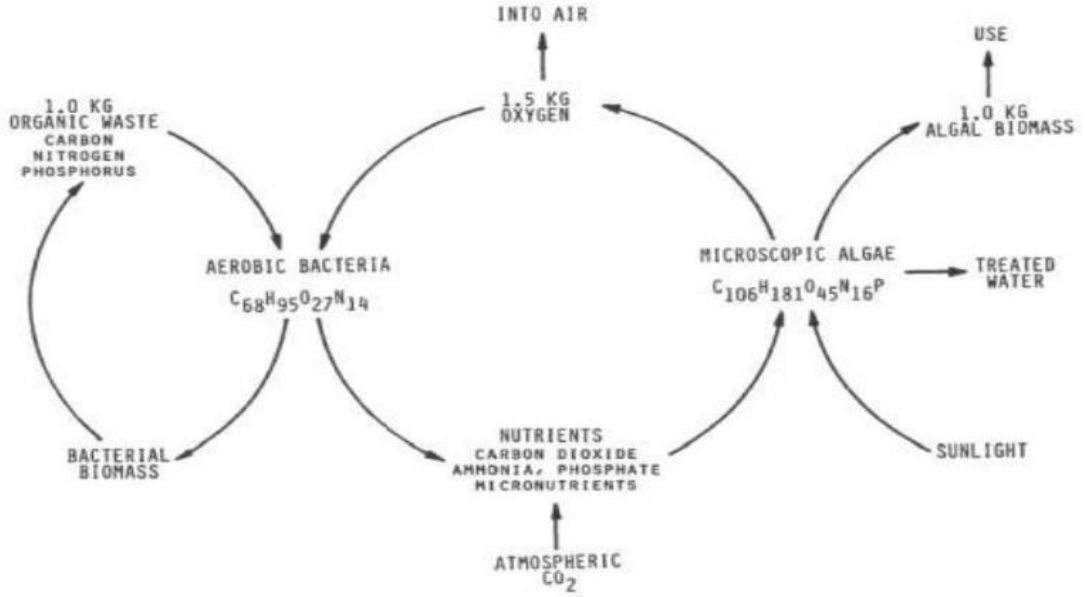

Figure 4-5. Symbiotic relationship between bacteria and microalgae in a wastewater-fed system (Nurdogan, 1980).

At the beginning of this part of the experiment, initial TAN and PO_4 amount of Reactor A was almost four times greater than Reactor B (Table 4-3).

Table 4-3. Characterization of Reactor A and Reactor B

Reactor	**TAN (mg/L)**	**PO_4 (mg/L)**
Reactor A	49.87	3.2
Reactor B	14.43	0.68

The reason behind this difference was the sterility of wastewater in the Reactor A and B. During the autoclave process, wastewater present in Reactor B waited for 60 minutes at 121^0C and 5 psi pressure. After the autoclave process Reactor B was cooled down to room temperature and pH was measured as 10.3, which can be accepted as a high rate. The reason for having high pH might be from the result of increased temperature and pressure. Because of the abrupt rise of pH, ammonia stripping and phosphate precipitation were observed (Hoffman, 1998). Removal of

nitrogen and phosphate from wastewater due to the autoclave effect caused a significant difference between initial TAN and PO_4 amount of Reactor A and Reactor B. It can be concluded that, TAN and PO_4 removal rate difference between Reactor A and Reactor B might be attributed to initial TAN and PO_4 amount of these reactors.

4.2.2. CO_2 Sequestration and Dissolution

In this part of the study, CO_2 sequestration potential of *C. vulgaris* grown in raw and autoclaved domestic wastewater was investigated. In order to understand CO_2 tolerance of microalgae and the amount of CO_2 biotransformed by microalgae, CO_2 concentration was monitored regularly.

Pure distilled water and autoclaved distilled water containing control reactors (Control 1A and Control 1B) were prepared to understand the dissolution of CO_2 in distilled water, to check if there can be any leak from the reactors, and any sort of possible contaminations. Due to the CO_2 and H_2CO_3 equilibrium, CO_2 amount decrease in the headspace of these reactors. Raw domestic wastewater and autoclaved domestic wastewater included reactors (Control 2A and Control 2B) were used to test the dissolution amount of CO_2 in wastewater and leak check of the reactors.

Figure 4-6 showed that CO_2 dissolution in both domestic wastewater and distilled water ceased at the end of first 24 hours. After first 24 hours any significant gas content change was not observed. It indicated that there was not any gas leakage from reactor and there was not any risk of bacterial contamination. CO_2 solubility of Control 1A, Control 1B, Control 2A and Control 2B reactors calculated as 57.1%; 60.6%; 25.3%; 40.8%, respectively. It was observed that CO_2 was more soluble in distilled water than domestic wastewater. Moreover, CO_2 was the most soluble in autoclaved distilled water when compared to all of the control reactors which may be caused from homogeneity came from autoclave process. Similarly, the reactor Control 2B (containing autoclaved domestic wastewater) had more CO_2 gas solubility than Control 2A (containing raw domestic wastewater). All these results

demonstrated that homogeneity due to autoclave is an important factor for CO_2 dissolution.

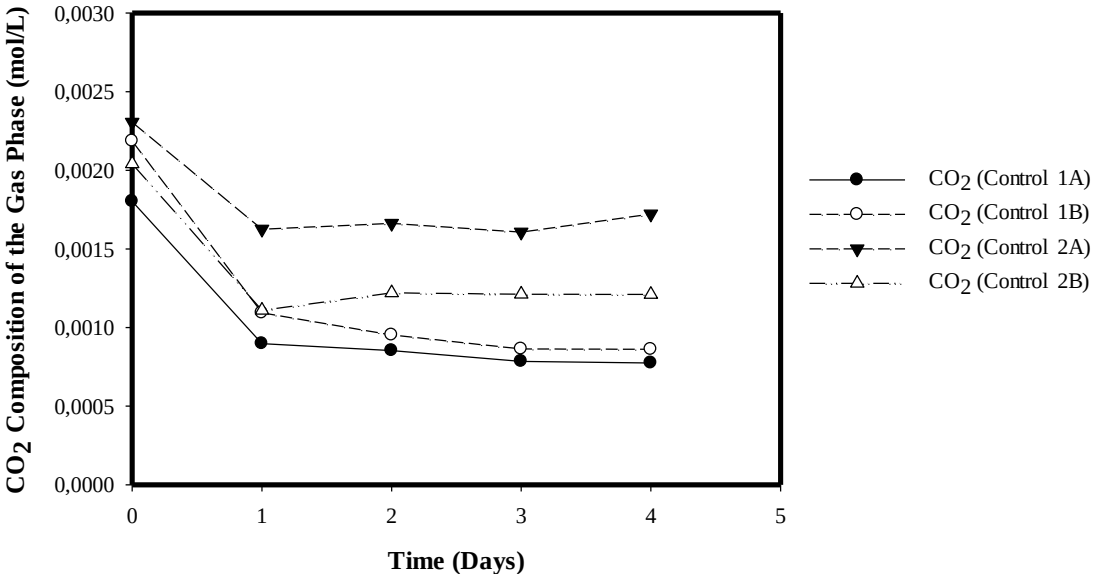

Figure 4-6. CO_2 composition of the gas phase data of Control Reactors (Control 1A-1B-2A-2B Reactors)

In order to observe the CO_2 sequestration potential of *C. vulgaris*, two different types of reactor were operated. The first one was Reactor A which contained raw wastewater and microalgae and second was Reactor B which contained autoclaved wastewater and microalgae. In Reactor A, there was a sharp decrease in CO_2 concentration during the first 6 days (Fig4-7a). CO_2 was added if the CO_2 concentration in the headspace was below 5% by mole. The addition of CO_2 was indicated by arrows in Figure 4-7a. At the end of seven days, 303.73 mg/L CO_2 was used by algae and CO_2 consumption rate was calculated as 42.09 mg/L.day for this reactor. On the other hand, in Reactor B, a decreasing trend in CO_2 (%) in the headspace was observed in first 4 days. Following the dissolution of CO_2 in aqueous solution, CO_2 was uptaken, in other words, biotransformed by microalgae. At the end of four days, 100.75 mg/L CO_2 is used by algae. CO_2 consumption rate was

calculated as 21.11 mg/L.day for this reactor. Since nearly all nitrogen and phosphorous sources were consumed in Reactor B, extra CO_2 addition was not needed.

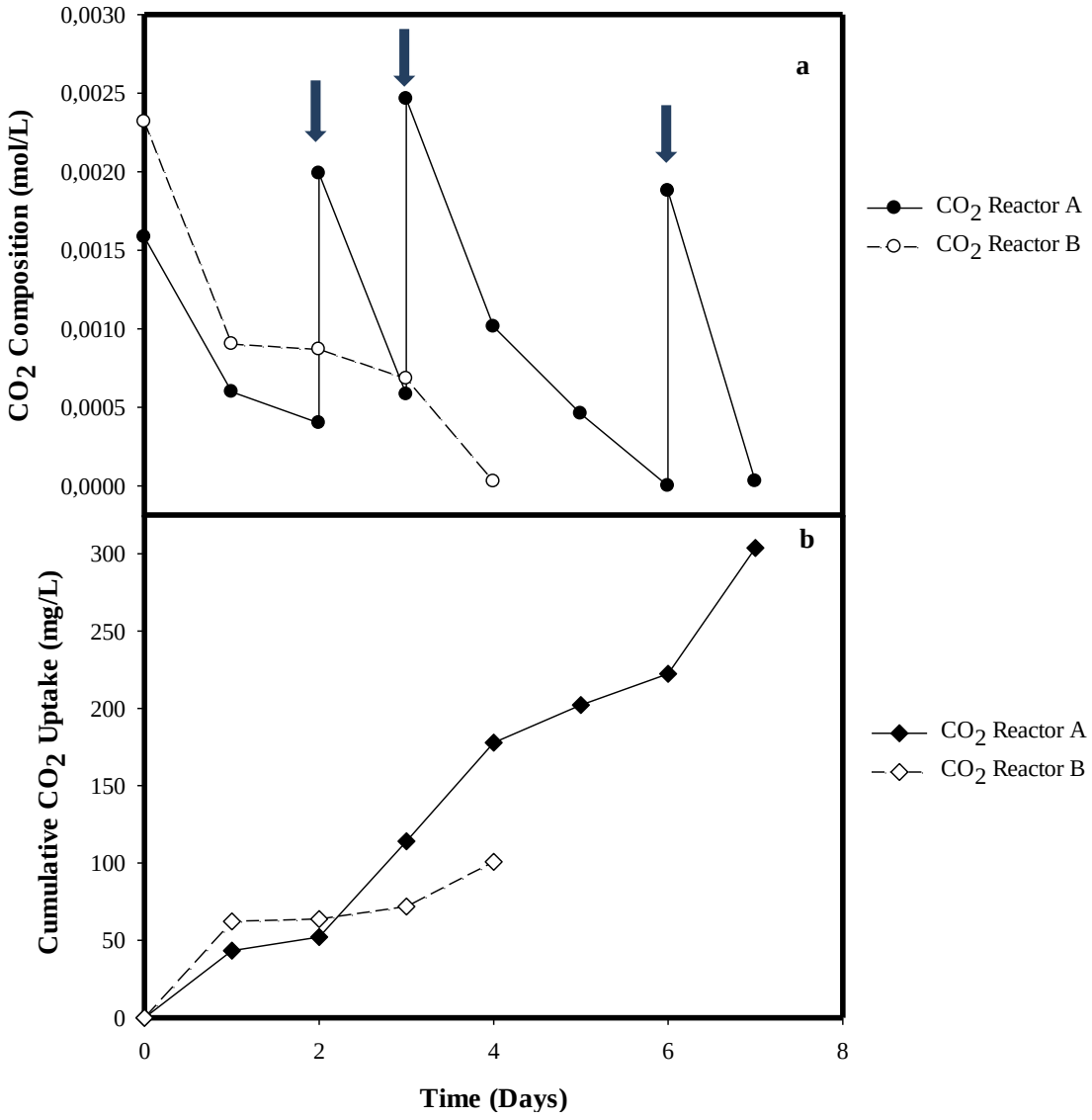

Figure 4-7. a) Daily CO_2 composition change and b) Cumulative CO_2 uptake data of Reactor A and Reactor B

Figure 4-7b represents the CO_2 uptake differences between Reactor A and B. Presence of higher TAN and PO_4-P concentrations in the raw domestic wastewater was the main reason for this difference. Due to the autoclave process, the initial composition of the wastewater changed significantly in terms of TAN from 49.87 to 14.43 mg TAN/L, while PO_4-P concentration dropped from 3.2 to 0.68 mg PO_4-P/L. The CO_2 uptake by microalgae could be observed until the depletion of nutrients in the reactors. Cumulative CO_2 uptake was parallel to cumulative TAN and PO_4 uptakes of Reactor A and B. Another significant reason for the difference in CO_2 uptake of microalgae grown in autoclaved and raw domestic wastewater is bacterial activity in the raw domestic wastewater. Since there was raw domestic wastewater in the Reactor A, symbiotic relationship with bacteria and microalgae could be observed (Nurdogan, 1980). Owing to this mutually beneficial relationship, more microalgal activity and more CO_2 uptake could be measured. For example, Vasseur et al. (2012) reported that the maximum carbon conversion efficiency increased up to 6.3% due to the bacteria in the algae–bacteria system.

The comparison of this study with the related literature is presented in Table 4-4 based on CO_2 consumption rate of *C. vulgaris*. In this study, 42.09 and 21.11 mg/L.day CO_2 fixation rates were obtained in Reactor A and Reactor B, respectively. These values are relatively lower than the value studied by Sydey et al., (2010); Adamczyk M (2016) where CO_2 fixation rate of *C. vulgaris* in different types of culture medium fed with different CO_2 concentration varies between 75 and 550 mg/L.day for different studies. The reason of much lower CO_2 fixation rate in this study might be attributed to phosphorus limitation due to the domestic wastewater. In high CO_2 fixation performance studies, different types of culture media were used as nutrient source. However, microalgae were fed with domestic wastewater which was phosphorous deficient, as a nutrient source in this study. Key parameters for microalgae growth, productivity and CO_2 fixation are CO_2, nitrogen, and phosphorus. Previous research has repeatedly stated that excessive supply of phosphorus is recommended to avoid nutrient limitation (Larsdotter, 2006; Mostert and Grobbelaar 1987). As stated by Adamczyk M (2016), a high concentration of biomass means that both a large quantity of biomass with the same volume of culture and an increase of the CO_2 biofixation. That is, it can be stated that low algae

biomass productivity leads to a low carbon mitigation effect. In the light of this information, lower CO_2 fixation rate in this study was expected when compared to all of the other studies in Table 4-4.

Table 4-4. Comparison of literature in terms of CO_2 consumption rate

Microalgal Species	**Reference**	**CO_2 (%)**	**Cultivation**	**CO_2 Consumption Rate (mg /L. day)**
C. vulgaris	Scragg et al., (2002)	Air	Watanabe medium	75
C. vulgaris	Sydney et al., (2010)	5	Modified Bristol Medium and Zarouk Medium	252
C. vulgaris	Adamczyk et al., (2016)	8	BBM medium	550
C. vulgaris	This study (Reactor A)	20-25	Raw Domestic Wastewater	42.09
C. vulgaris	This study (Reactor B)	20-25	Autoclaved Domestic Wastewater	21.11

4.2.3. Growth and Development of Microalgae

In this part of the study, growth, development and biomass accumulation potential of *C. vulgaris* cultivated in domestic wastewater were investigated. To this purpose, total solid, volatile solid and chlorophyll-a concentration were monitored.

In all batch reactors (wastewater and algae mixtures), the average A664b/A665a ratio can be used an indicator for the physiological condition of the microalgae. A664b/A665a ratio of 1.5 - 1.7 indicates pure chlorophyll-a and a good physiological condition of microalgae (Van Den Hende et al., 2011). It was determined as 1.48 - 1.59 for Reactor B and 1.30 - 1.62 for Reactor A after second day of operation. These results showed that the microalgae populations in these reactors were adapted the growth conditions of domestic wastewater.

Total solid (TS) and volatile solid (VS) contents of the microalgae used in this experimental setup are given in Figure 4-8. As seen in Figure 4-8, microalgal biomass increased in both Rector A and B. It was noticed that total solid content increased from 0.84 g/L to 1.19 g/L in Reactor A. In addition, volatile solid content of Reactor A increased from 0.12 g/L to 0.42 g/L. Similarly, total solid content increased from 0.90 g/L to 1.11 g/L and volatile solid content increased from 0.11 g/L to 0.34 g/L in Reactor B.

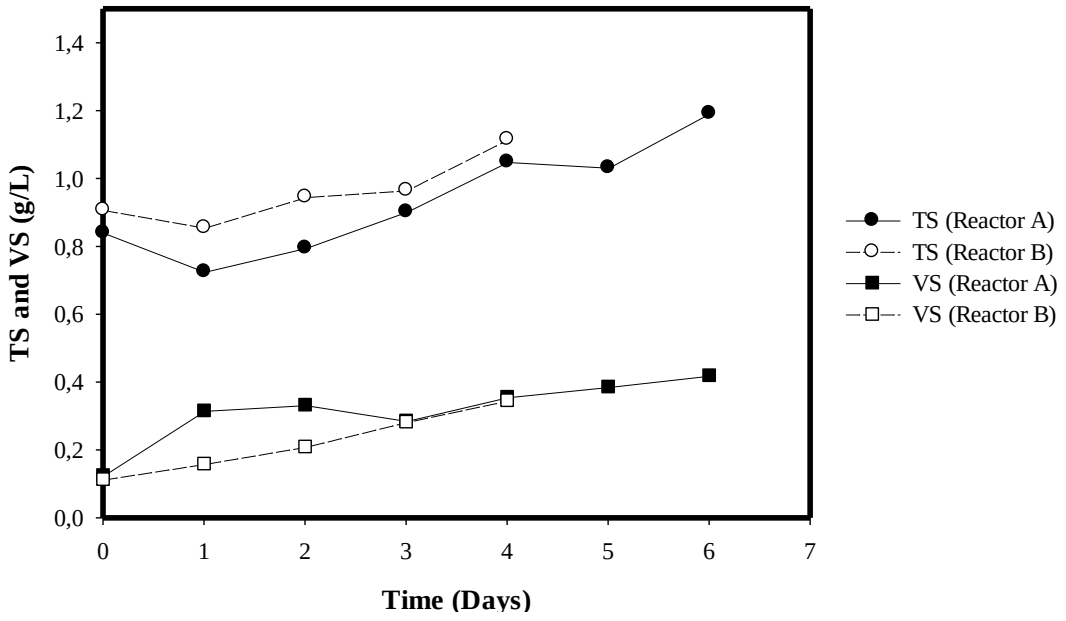

Figure 4-8. TS and VS contents of Reactor A and Reactor B

The maximum net specific growth rate was observed 0.20 and 0.28 d^{-1} in Reactor A and Reactor B, respectively. The results of this study were also supported by the related literature. De Morais and Costa (2007) studied with *C. vulgaris* in basal medium fed with air and CO_2 enriched air and found maximum specific growth rate between 0.12 and 0.31 d^{-1}. In another study, Li et al., (2013) cultivated *C. vulgaris* in different modes and reported maximum net specific growth rate between 0.2 and 0.37 d^{-1}. The results of the study conducted by Chinnasamy et al. (2010) also support this study. Maximum net specific growth rate of *C. vulgaris* was founded as 0.22 d^{-1}.

However, other studies were conducted by Martinez and Orus (1991) and Filali et al., (2011) stated specific growth rates of 1.92 d^{-1} and 1.95 d^{-1} respectively. In those studies, *C. vulgaris* was cultivated in Rodriguez-Lopez medium with glucose, and Bristol 3N medium, respectively. Since domestic wastewater was used instead of medium as a nutrient source in present study, lower specific growth rate data might have been obtained.

In this study, 49 mg/L.d and 58 mg/L.d productivity were obtained in Reactor A and Reactor B, respectively during exponential phase of *C. vulgaris* cultivation, which is supported value reported by Scragg et al., (2002) and Çalıcıoglu Ö. (2013). In another study, Mata et al., (2010) recorded biomass productivity of *C. vulgaris* between 0.02 and 0.25 g/L.d. However, higher biomass productivity data was recorded in the Yoo et al., (2010)'s study which was noted as 104.76 mg/L.day. The higher biomass productivity obtained in Yoo et al. (2010)'s study was attributed to the use of higher CO_2 concentrations.

4.3. Results of Batch Cultivation Photobioreactors (Set-2)

The aim of this part of the study was to investigate the performance of microalgae cultivation reactors fed by flue gas, removal of nutrients from domestic wastewater and the amount of CO_2 biotransformed by microalgae. Set-1 reactors were fed with CO_2 enriched air, simulating flue gas but Set-2 reactors used real flue gas in this set.

In this part of the study, batch photobioreactors were purged by real flue gas collected from iron-steel industry and its level of CO_2 was measured as 5-6% by volume. The reactors were operated until the nutrients were completely used up by microalgae and/or bacteria which lasted 7 days. In order to control number of heterotrophic bacteria coming from domestic wastewater, Control 2C and Control 2D reactors were operated. These control reactors contained 400 mL raw domestic wastewater and autoclaved domestic wastewater respectively.

4.3.1 Nutrient Removal from Wastewater

In order to observe nutrient removal potential of *C. vulgaris,* it was cultivated with industrial flue gas and domestic wastewater. For that purpose, nitrogen and phosphorus removal monitored daily.

At the end of experiment, TAN and PO_4 removal efficiencies of Reactor C and Reactor D were observed as 89.59 - 98.4% and 99.75 - 82.25% respectively. The Student's t test was applied and it was found that TAN and PO_4 removal efficiencies of Reactor C and Reactor D were not significantly different ($p=0.11$ and $p=0.26$, respectively).

After 7 days of operation, nitrogen and phosphorous sources in the reactors were probably almost depleted. Considering literature review, it is again proven that *C. vulgaris* is a dominant algae strain and showed high performance in wastewater treatment (Kim et al., 2013; Li et al., 2013).

It can be seen from Figure 4-9b and 4-10b that the general trend of TAN and PO_4 concentration in Reactors C and D were decreasing by time and reached to values near zero. The total amount of TAN and PO_4 were consumed in the C and D Reactors are shown in Figure 4-9a and 4-10a.

Initial TAN and PO_4 in the Reactor C were 40.38 mg/L and 13.33 mg/L respectively and decreased to 4.20 mg/L and 0.20 mg/L at the end of Day 7 (See Figure 4-9b and 4-10b). It is noteworthy that 89.59 % of the TAN and 98.4% of PO_4 were removed by microalgae in Reactor C in 7 days. On the other hand, in D Reactor initial TAN and PO_4 were 16.07 and 10.76 mg/L respectively and decreased to 0.04 and 1.91 mg/L at the end of Day 7 (See Figure 4-9b and 4-10b). TAN and PO_4 removal efficiencies of Reactor D were 99.75% and 82.25% respectively, which was in agreement with other studies (Li et al., 2013; Kim et., 1998; Yun et al., 1997; Lau et al., 1995).

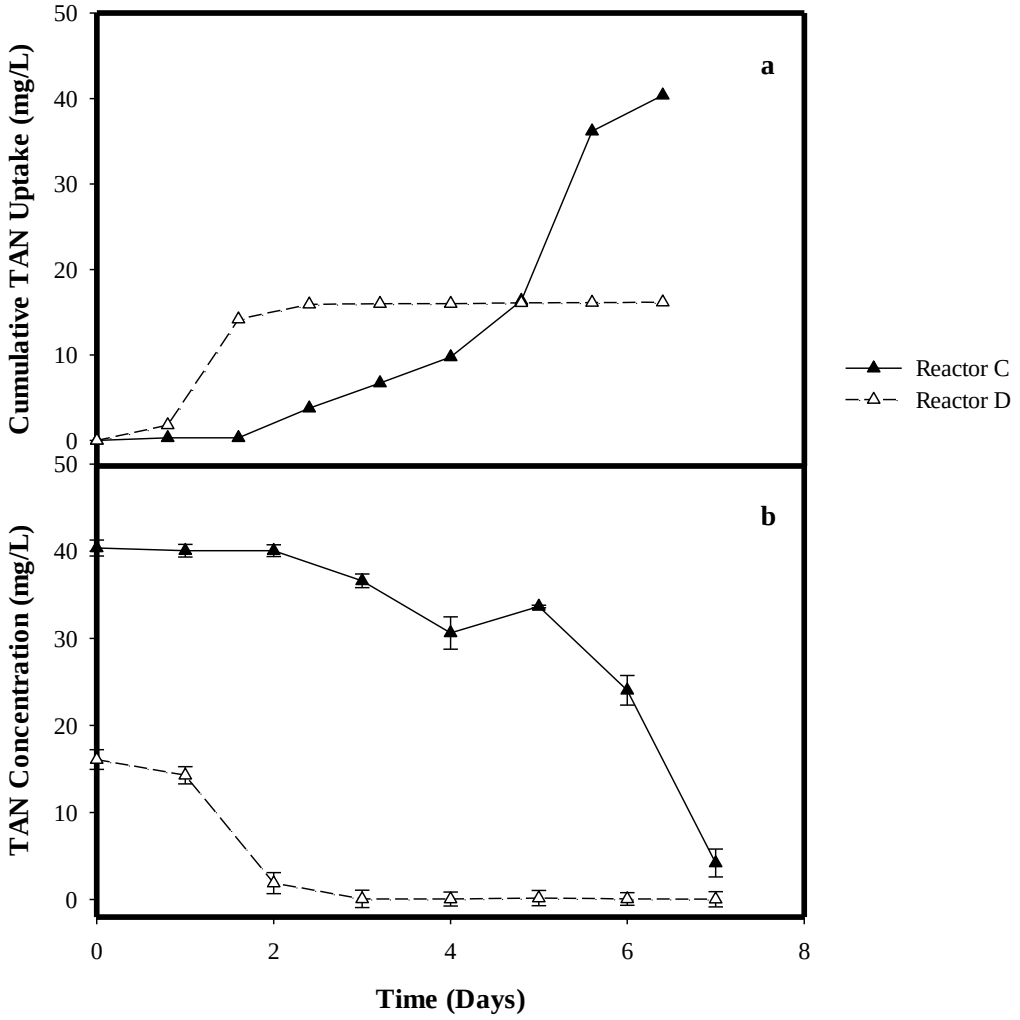

Figure 4-9. a) Cumulative TAN uptake, b) TAN concentration of Reactor C and Reactor D

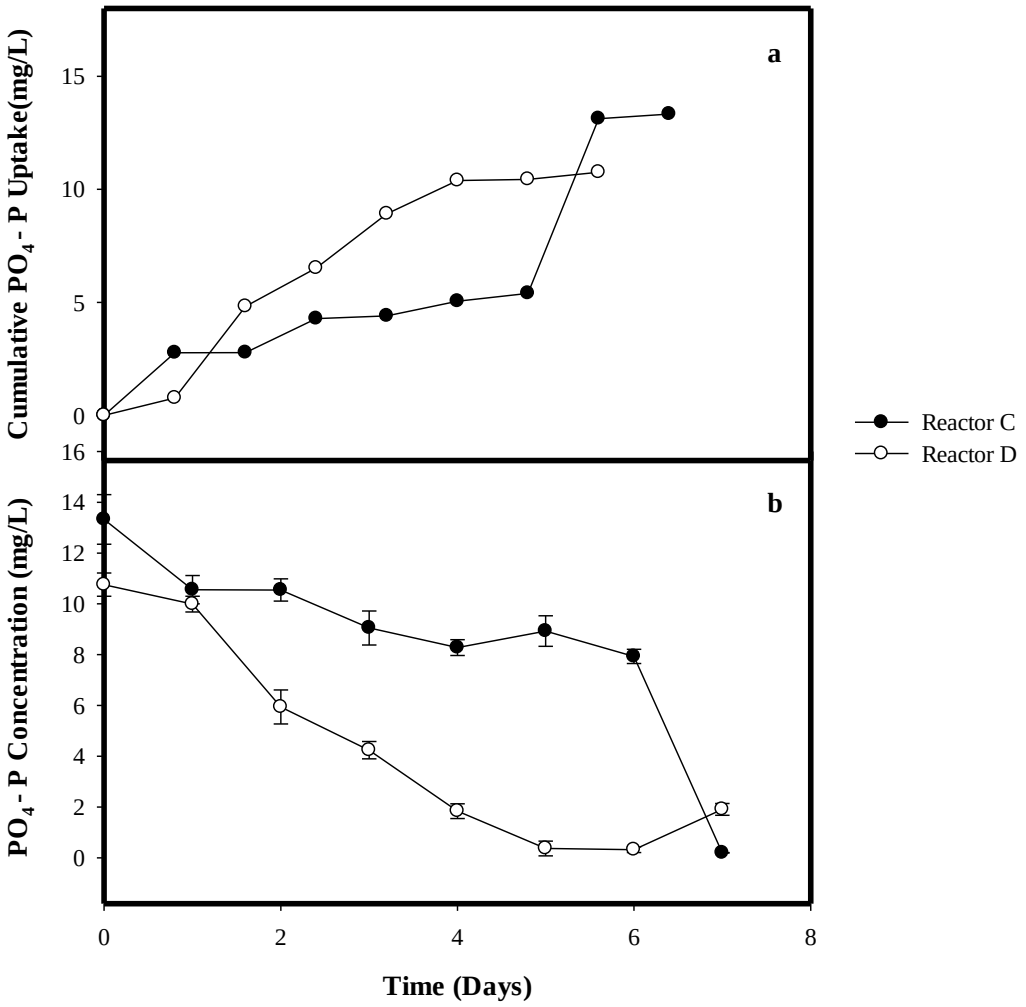

Figure 4-10. a) Cumulative PO_4-P uptake, b) PO_4-P concentration of Reactor C and Reactor D

The comparison of this set (Set-2) with Set-1 is presented in Table 4-5 based on grams of nutrients which were uptaken per gram of microalgae. Total TAN and PO_4 uptake normalized with respect to biomass at the end of the experiments were calculated as 1.06 g TAN/g TS and 0.14 g PO_4/g TS in the Reactor A which contained raw domestic wastewater. The amounts of TAN and PO_4 uptaken by microalgae grown in Reactor B containing autoclaved domestic wastewater were lower and equal to 0.31 g TAN/g TS and 0.07 g PO_4/g TS. On the other hand, second set nutrient uptake values were lower than that obtained in first study. The amounts of TAN and PO_4 uptaken by microalgae grown in Reactor C containing wastewater were 0.10 g TAN/g TS and 0.04 g PO_4/g TS and lower uptake values observed as

0.05 g TAN/g TS and 0.03 g PO_4/g TS in Reactor D containing autoclaved wastewater.

Table 4- 5. Comparison of Set-2 Reactors with Set-1 Reactors

Reactor	**Operation Mode**	**gram TAN/gram Algae**	**gram PO_4/ gram Algae**
Reactor A	Batch/CO_2 enriched air	1.06	0.14
Reactor B	Batch/CO_2 enriched air	0.31	0.07
Reactor C	Batch/Flue gas	0.10	0.04
Reactor D	Batch/Flue gas	0.05	0.03

It was observed that, lower TAN and PO_4 removal of Reactor C and D in this set when compared with the results of Set-1 reactors (Reactor A and B) in terms of grams of nutrients which were uptaken per gram of microalgae. There may be several reasons for observing low microalgae activity in these reactors: 1) The low solubility of CO_2 in flue gas in water, 2) inhibition of bacterial activities by the other gases in flue gas, 3) granulation of microalgae with bacteria in the presence of organics, 4) low initial microalgae density and inoculation time, 5) limitation of phosphorus in the domestic wastewater.

(1) Low water solubility of CO_2 in flue gas may affect activity and growth of microalgae. It was stated that improved CO_2 solubility enhances more CO_2 fixation and biomass production (G. Kim et al., 2013).

(2) Inhibition of microalgal and bacterial activities due to contaminant gases in flue gas can be other cause for low microalgal activity in the reactors. It is known that impurities of flue gas such as CO_2, NO_x, SO_x can inhibit microalgal growth (Yen et al., 2015).

(3) Other limitation for microalgal activity might be the granulation of microalgae and bacteria. Microalgae and bacteria aggregate compact flocs and these large and

compact flocs settle down easily due to its high gravity. Besides positive effects of these symbiotic interactions, there are also negative impacts on bacterial and microalgal growth such as pH increase or shading (Valigore, 2011).

(4) Initial microalgae inoculum density and inoculation time are significant effect on the activity and productivity of microalgae (López-Elías et al., 2011). It was proved that removal efficiencies of nitrogen, phosphorus and COD in the wastewater by *C. vulgaris* are superior in the superconcentrated culture (Lau et al., 1995).

(5) According to Larsdotter (2006), high ratio between nitrogen and phosphorus such as 30:1 suggests P-limitation and these ratios mostly found in wastewater. Phosphorus limitation in domestic wastewater can be another main cause for reducing algal growth and microalgal activity.

4.3.2. CO_2 Sequestration and Dissolution

This part of the study included the CO_2 sequestration potential determination of *C. vulgaris* fed by flue gas and grown in domestic wastewater. In order to evaluate the CO_2 tolerance of microalgae and the amount of CO_2 biotransformed by microalgae, CO_2 concentration was monitored regularly. Raw domestic wastewater contained reactor called as Control 2C and autoclaved domestic wastewater contained reactor called as Control 2D were used to test the dissolution amount of CO_2 in wastewater and check the leak of the reactors. CO_2 composition change in the headspaces of the Control 2C and 2D Reactors were shown in Figure 4-11.

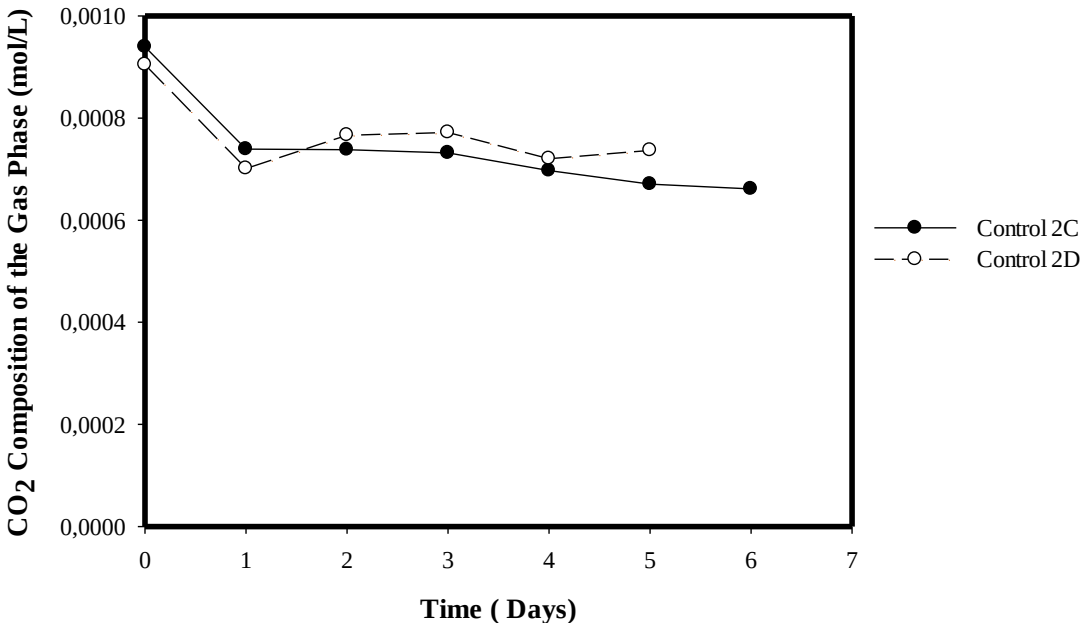

Figure 4-11. CO_2 composition of the gas phase data of Reactor 2C and 2D.

CO_2 solubility of Control 2C and Control 2D reactors were calculated as 29.3% and 18.5%, respectively. In addition, CO_2 solubility was lower in Reactor 2C and 2D compared to Reactor 1A, 1B, 2A, 2B in previous set up. This was attributed to lower solubility of flue gas. Flue gas contained several other components and gases such as CO, NO, NO_2, NO_x so these components may affect solubility of CO_2 in flue gas in water. Since the CO_2 concentration in flue gas is quite low (approximately 5-6%), this effects its partial pressure and solubility (Brunetti et al., 2010).

CO_2 composition change in the headspaces of the Reactor C and Reactor D was shown in Figure 4-12a. The addition of CO_2 was made whenever the CO_2 amount in the headspace was below 5%. Arrows were shown in the Figure 4-12a indicates the addition of CO_2. CO_2 addition was performed 2 and 4 times into the headspaces of Reactor C and D, respectively.

The CO_2 uptake by microalgae could be observed until the depletion of nutrients in the reactors. Cumulative CO_2 uptake was parallel to cumulative TAN and PO_4 uptakes of Reactor C and D.

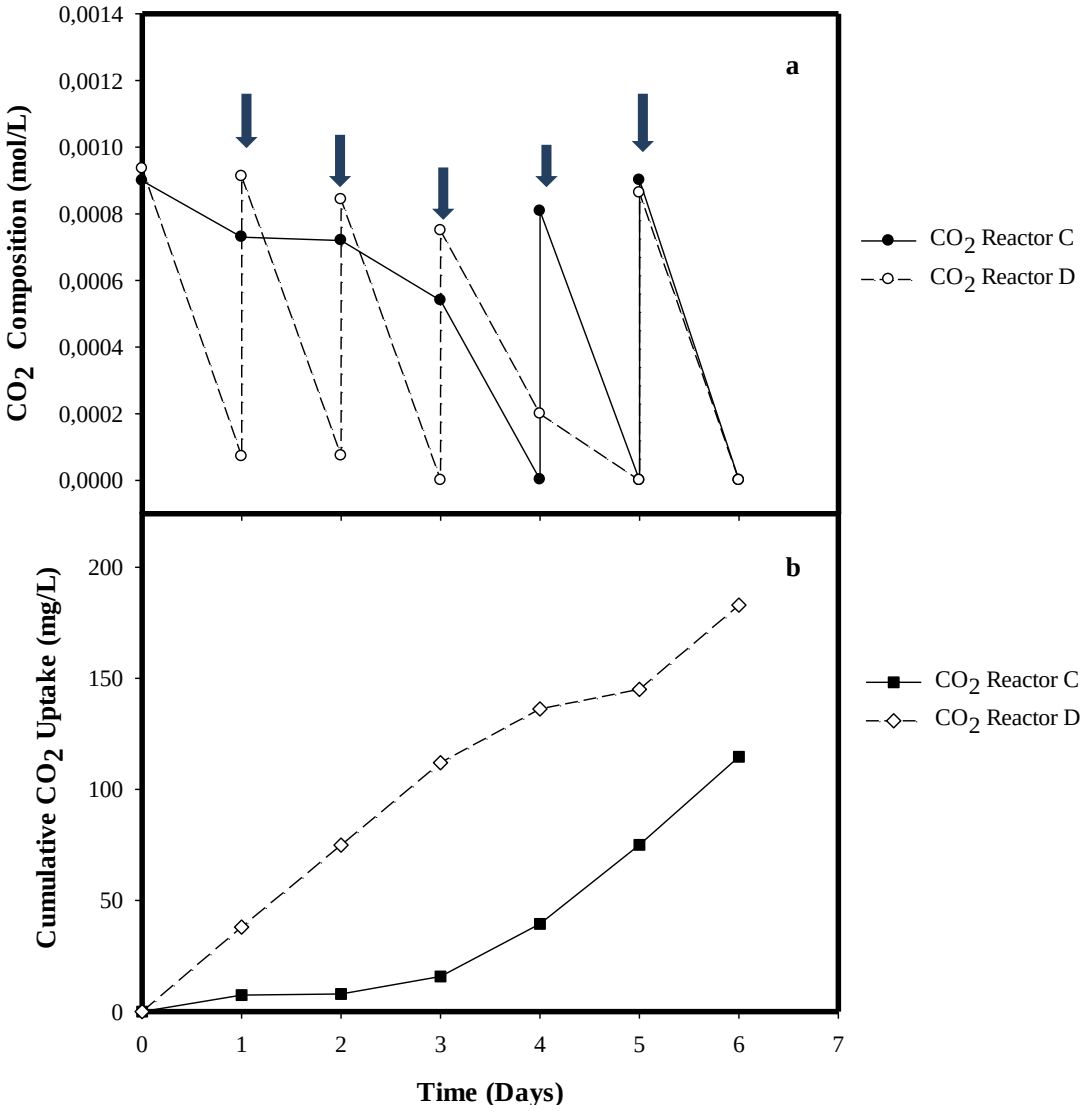

Figure 4-12. a) Daily CO_2 composition change and b) Cumulative CO_2 uptake data of Reactor C and Reactor D

Figure 4-12b shows the cumulative CO_2 uptake differences between Reactor C and D briefly. The rates of CO_2 uptake were calculated as 18.2 mg/L.day and 29.4 mg/L.day in the Reactor C and D, respectively. These values are lower than the

values reported by Douskova et al. (2009). The reported CO_2 uptake rate for *C.vulgaris* grown by real flue gas in a growth medium solution is 4.4 g/L.day. The data from different studies were in the range from 0.65 to 4.0 g CO_2/L.day (Kurano et al., 1995; Yoshihara et al., 1996; Murakami and Ikenouchi, 1997). The rates are much higher than the rates observed in this study, which may be due to the nutrient concentrations in the growth medium optimized for maximum growth. Moreover, it was noted that photobioreactor design optimization with a very thin microalgal suspension layer and intensive illumination can be another cause for high carbon dioxide fixation rate (Douskova et al., 2009).

It can be seen from Figure 4-13 that granulation of microalgae which significantly reduced surface and contact area for gas exchange. Therefore, CO_2 uptake rate of the microalgae decreased significantly in these reactors. Granulation may be caused from flue gas content or bacteria and gas content relationship. Moreover, during growth phase of microalgae CO_2 consumption was observed and this situation causes pH level elevation. Increased pH cause a process known as autoflocculation (Nurdogan, 1988).

Figure 4-13. Granulation of microalgae in reactors

Finally, the low solubility of CO_2 in flue gas in water, inhibition of symbiotic bacterial activities by the other gases in flue gas, granulation of microalgae with bacteria in the presence of organics, low initial microalgae density and limitation of phosphorus in the domestic wastewater may result in lower microalgae activities hence lower CO_2 uptake when raw wastewater was used to grow microalgae. (See Section 4.3.1).

4.3.3. Growth and Development of Microalgae

The aim of this part was to observe the growth, development and biomass accumulation potential of *C. vulgaris* fed with flue gas and cultivated in domestic wastewater.

As seen in Figure 4-14, it was noticed that total solid content increased from 0.94 g/L to 1.47 g/L in Reactor C. In addition, volatile solid content of Reactor C increased from 0.28 g/L to 0.78 g/L. Similarly, total solid content increased from 0.95 g/L to 1.76 g/L and volatile solid content increased from 0.33 g/L to 0.79 g/L in Reactor D. Maximum biomass concentration in the batch reactors used for *Chlorella sp.* wild type growth using flue gas (25% CO_2) in the study of Chiu et al., (2011) was reported as 1500 mg/L, which is similar to the results obtained from Reactor C and D.

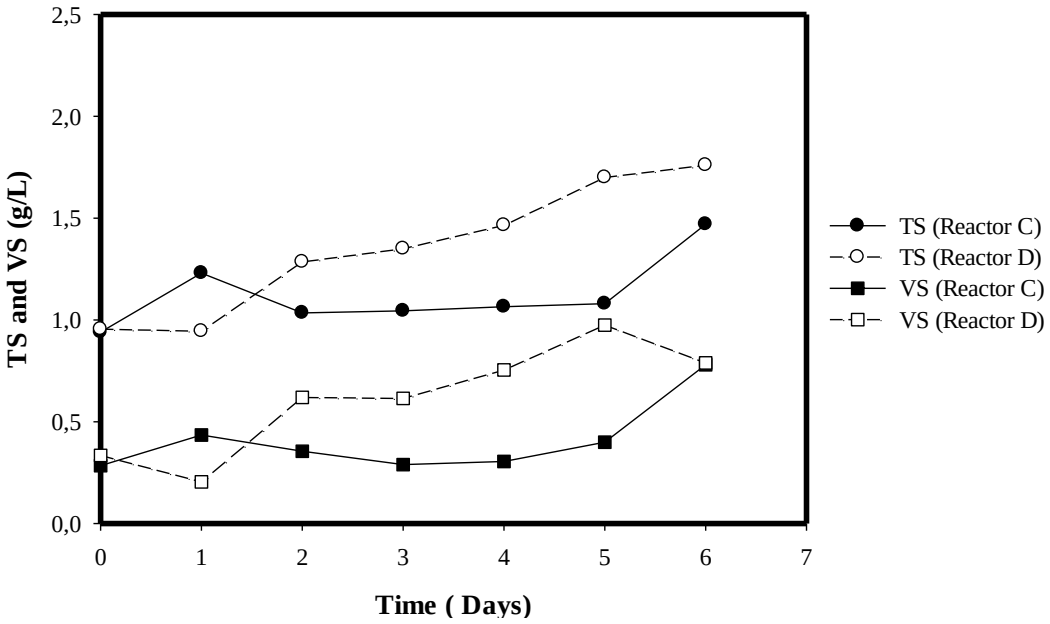

Figure 4-14. TS and VS data of Reactor C and Reactor D

In this study, 82 mg/L.d and 75 mg/L.d productivity was obtained in Reactor C and Reactor D, respectively during exponential phase of *C. vulgaris* cultivation. In another study, an isolated thermal and CO_2 tolerant strain of *Chlorella sp*. MTF-7 has been inoculated in batch reactors aerated by flue gas from iron-steel industry with 25% CO_2 content (Chiu et al., 2011) and the average biomass accumulation rate has been reported as 0.37 g/L.day. The reported microalgae cultures are grown in a growth medium prepared in the laboratory to optimize the growth for maximum

while wastewater was the growth medium in this study. Therefore, the nutrient concentrations may be controlling the biomass accumulation in the Reactor C and D.

As it was previously mentioned that A664b/A665a ratio of 1.5 - 1.7 shows good physiological condition of microalgae (Van Den Hende et al., 2011). It was also checked for Reactor C and D which were 3.4 and 1.2 respectively at the end of cultivation time. This result proved that microalgae in this set were not as good physiological condition as Set-1's microalgae. CO_2 and nutrient uptake amounts also supported this idea.

4.4. Results of Fed-batch Cultivation Photobioreactors (Set-3)

Fed-batch cultivation reactor set was conducted to investigate the activity, growth, development and biomass accumulation potential of *C. vulgaris* fed with flue gas and cultivated in industrial wastewater. Industrial wastewater from coking unit and flue gas from Kardemir Iron and Steel Factory were used for feeding of microalgae in photobioreactors. Set-3 photobioreactors were operated fed-batch mode with two different hydraulic residence times which were HRT 4 and 8 day.

After first four days, photobioreactors were operated in fed-batch mode with 4 day hydraulic residence times kept without any nutrient loading in order to prevent the system wash out. Since four days was too short as hydraulic residence time for microalgae nutrient uptake, nutrient loading rate exceeded the microalgal nutrient consumption and the culture started to wash out. That means there is not enough time for the utilization of substrate and therefore, wash-out of microbial cultures is a strong possibility (Speece, 1996). The same approach was also valid for microalgae in this study. In order to overcome this problem, photobioreactors were operated at a 4 day HRT kept without any nutrient feeding and wasting. However, gas exchange and gas analysis lasted for 50 days.

In order to analyze the activity and growth of microalgae, the CO_2 composition of the headspace in the reactors was monitored daily. Furthermore, microalgal activity was measured by monitoring TAN, PO_4 during the operation of the reactors.

In order to control the number of heterotrophic bacteria coming from industrial wastewater, Control 2E and Control 2F reactors which contained raw industrial wastewater and autoclaved industrial wastewater respectively were operated. In order to observe the effect of industrial wastewater on microalgae, Negative Control was used. It had only microalgae and distilled water in it.

In order to investigate the performance of microalgae cultivation reactors fed by flue gas and industrial wastewater, two different sets of photobioreactors were operated. One set of photobioreactors which contained two replicate of raw industrial wastewater and microalgae mixture which were called Reactor E, operated with 4 day hydraulic residence time. Other set of photobioreactors which contained two replicate of raw industrial wastewater and microalgae mixture which were called Reactor F, operated with 8 day hydraulic residence time.

4.4.1. Nutrient Removal from Wastewater

C. vulgaris was cultivated with industrial flue gas and industrial wastewater as carbon and nutrient source for the growth of microalgae, respectively. Nitrogen and phosphorus removal rates were monitored daily to control microalgae nutrient uptake rate.

Control 2E and Control 2F were operated with HRT 8. Although microalgae were not put into these control reactors, these reactors were contaminated by microalgae and turned green color. For this reason, it could not be taken any results from Control 2E and Control 2F.

As shown in Figure 4-15b and Figure 4-16b, TAN and PO_4 uptake started almost immediately without any lag phase in E reactors. It was calculated that 321.03 mg/L TAN and 88.07 mg/L PO_4 were uptaken in Reactor E at the end of 50 days of operation. Similarly, any lag phase was not observed in F reactors and 800.32 mg/L TAN and 171.94 mg/L PO_4 were uptaken at the end of 50 days of operation.

In other words, the N and P removal rates for Reactor E were 8.85 mg/L.day and 1.75 mg/L.day respectively. For Reactor F these rates were 18.28 mg/L.day and 4.03

mg/L.day, respectively. Moreover, total TAN and PO_4 uptake normalized with respect to biomass at the end of the experiments was calculated as 1.26 g TAN/g TS and 0.48 g PO_4/g TS in the Reactor E. The amount of TAN and PO_4 were uptaken by microalgae grown in Reactor F were 2.23 g TAN/g TS and 0.25 g PO_4/g TS. When it was compared with Set-1 and Set-2's nutrient uptake results, it was obvious that nutrient uptake capability of this set is superior. It may be caused from optimum nutrient content ratio of industrial wastewater. As it was mentioned in Section 3.1.3, external phosphorus source was used and N/P ratio was arranged around 10-11 in order to obtain maximum growth and productivity of microalgae. Moreover, industrial wastewater was used after diluted 1/50 with distilled water to prevent high ammonia inhibition and KH_2PO_4 was used as an external P source for the maximum growth of the microalgae.

Microalgal nutrient removal, CO_2 uptake, and biomass increase stopped at the same time in Reactor E. As seen in Figure 4-15a and Figure 4-16a, there was no significant nutrient uptake (2.80 mg/L.day of TAN and 0.57 mg/L.day of PO_4) in Reactor E after 8^{th} day. This situation might be caused from presence of some inhibitor factors against microalgal growth and performance in Reactor E. Wash out rate of microalgae was higher than growth rate of it, therefore, system came to a standstill. After first the 8 days, it was decided to stop continuous nutrient exchange of Reactor E to prevent the system wash out. It can be concluded that high concentration of industrial wastewater component may inhibit microalgal growth therefore hydraulic residence time for Reactor E was seen as too short to overcome this inhibition.

According to EPA (2002), wastewater from iron and steel industry contains organic and inorganic pollutants such as cyanides, sulfur compounds, phenol, dust, heavy metals, ashes, slags, and ore particles. Due to these pollutants in industrial wastewater, short hydraulic residence time could be not suitable for microalgal growth and nutrient uptake. Frequent loading of these components into the system created stress on microalgae (Pinto et al., 2003; Miazek et al., 2015). For all these reasons, after the 8^{th} day of the operation of Reactor E, operation mode was switched to semi-batch mode.

On the other hand, Reactor F operated with longer hydraulic residence time of 8 day. Two replicates of Reactor F were monitored daily and they operated fed-batch mode without any break. Sample taken from Reactor F showed that there was not any problem related with nutrient removal.

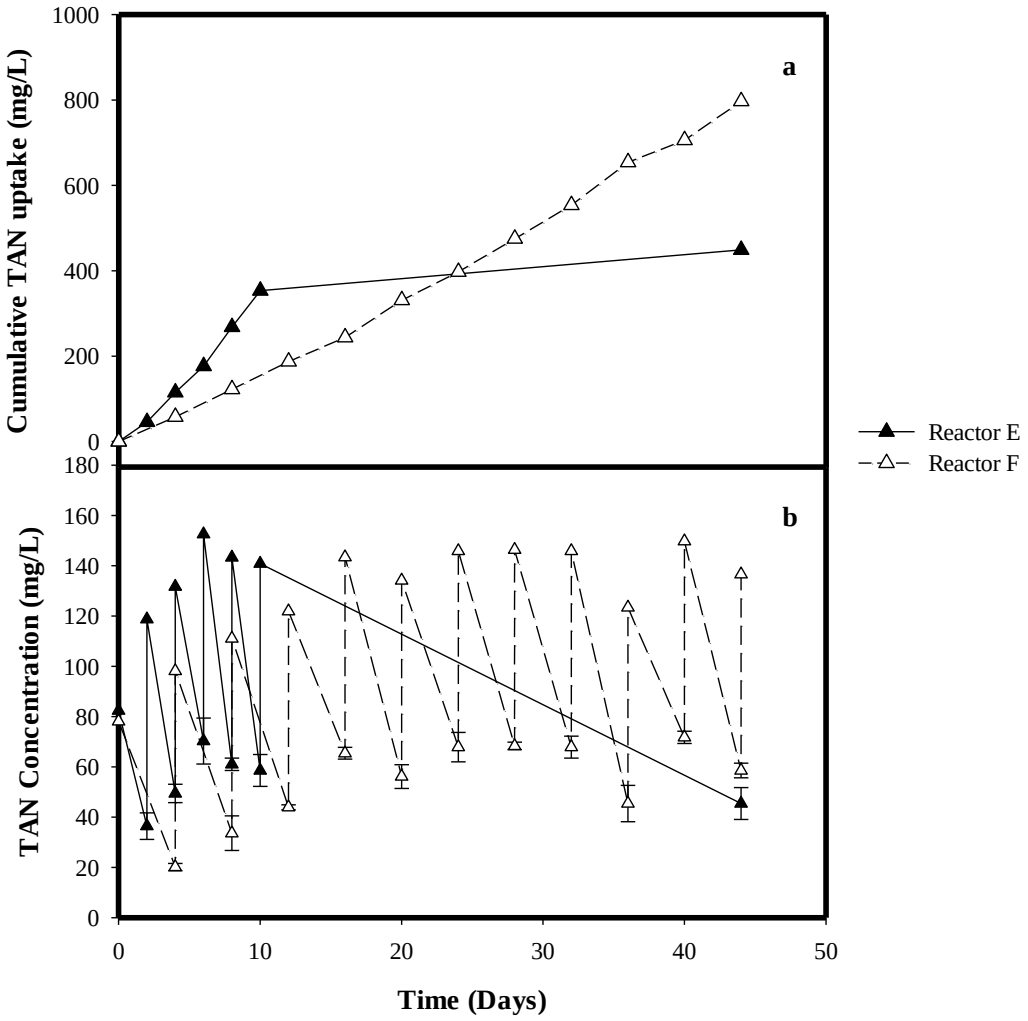

Figure 4-15. a) Cumulative TAN uptake, b) TAN concentration of Reactor E and Reactor F

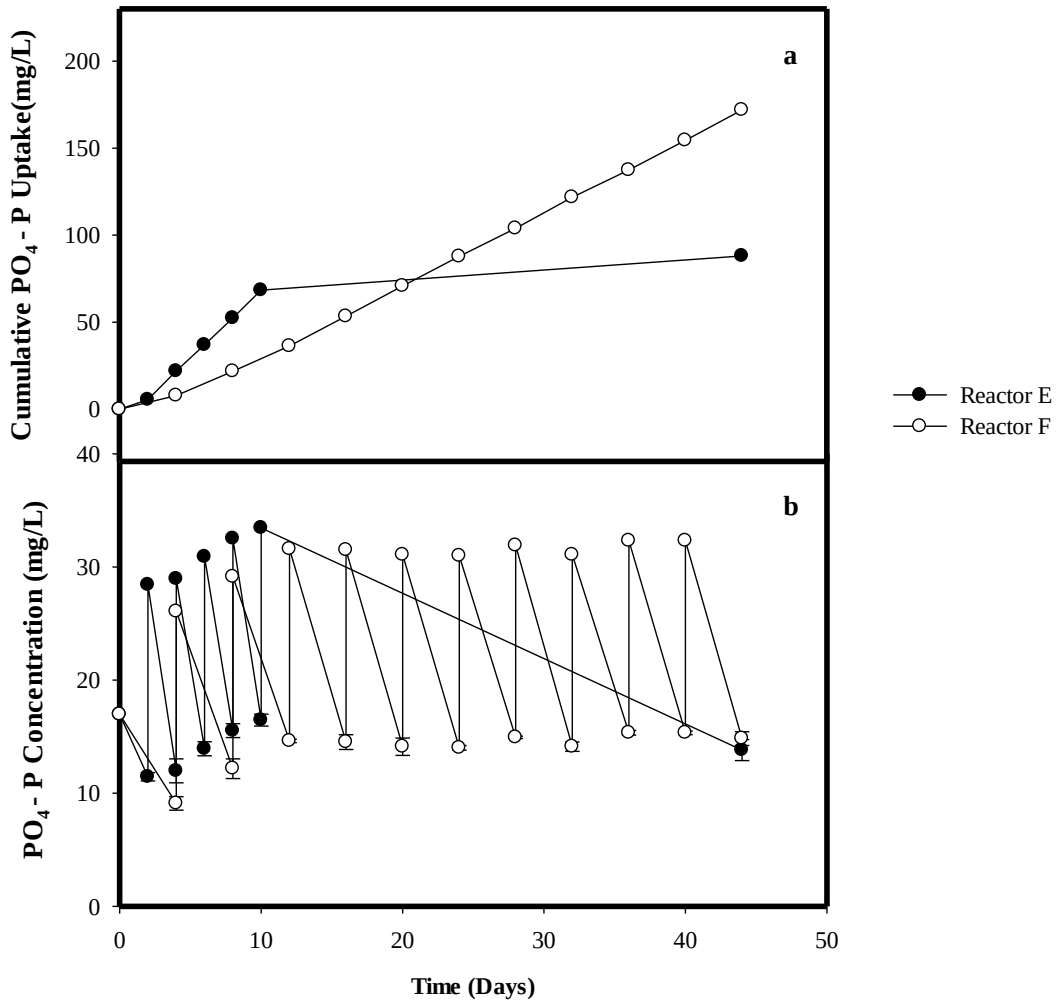

Figure 4-16. a) Cumulative PO_4-P uptake; b) PO_4-P concentration of Reactor E and Reactor F

Negative Control Reactor was used to check the reliability of the analysis which was not containing any wastewater as a nutrient source. Along with that, there was not any phosphorous and nitrogen source for the reactor apart from the unpreventable amount which comes with microalgae while it was taken from the stock, microalgae had to survive in this condition. It was calculated that 11.69 mg/L TAN and 3.89 mg/L PO_4 were uptaken in Negative Reactor at the end of 50 days of operation (Figure 4-17).

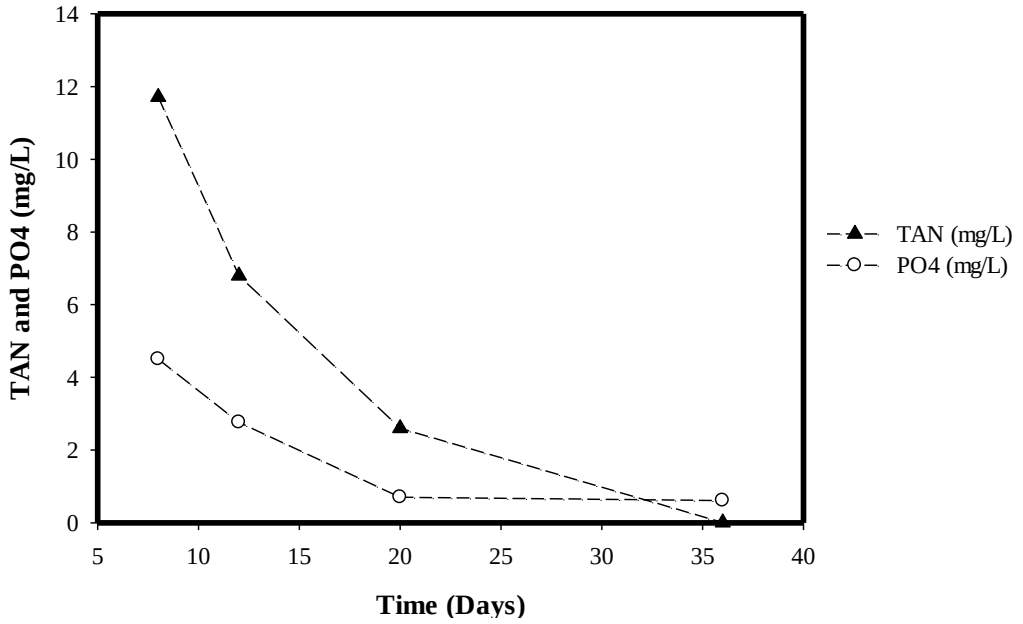

Figure 4-17. TAN and PO_4 values of Negative Control

4.4.2. CO_2 Sequestration and Dissolution

This experiment was designed to observe CO_2 sequestration (uptake) potential of microalgae fed with industrial gas and industrial wastewater. Gas composition of photobioreactors which were operated fed-batch mode with two different hydraulic residence times and control reactors were monitored regularly.

Daily CO_2 composition change and cumulative CO_2 uptake rates of Reactor E are shown in Figure 4-18a and b. As it can be seen in Figure 4-18a and b, microalgae in Reactor E stopped CO_2 mitigation shortly after the start of reactor operation. In order to enhance microalgae activity and prevent any microalgal inhibition in this reactor, the operation mode of Reactor E was changed from fed-batch to batch mode at the 8^{th} day of the performance.

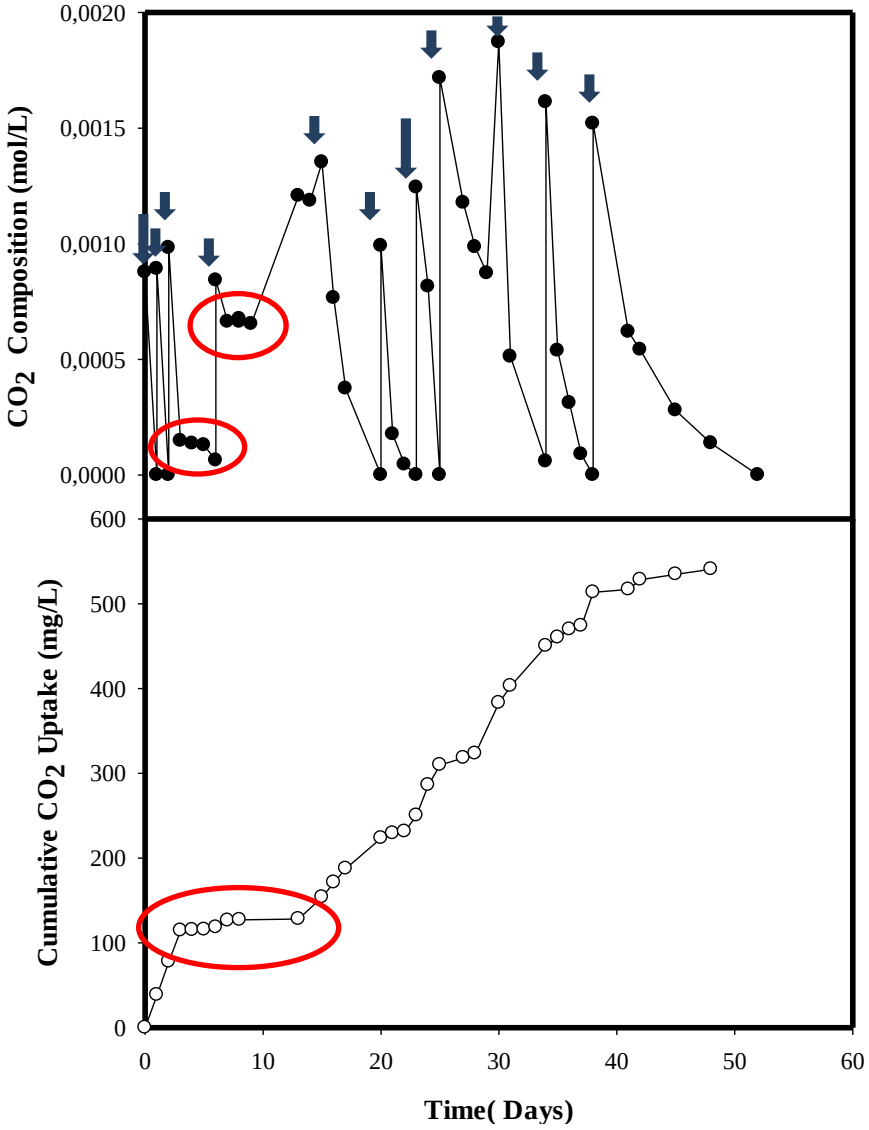

Figure 4-18. a) Daily CO_2 composition change and b) Cumulative CO_2 uptake data of Reactor E

In the Reactor E, total CO_2 uptake rate was calculated as 8.58 mg/L.day during first 13 days. This uptake rate significantly increased and reached almost to 13.52 g/L.day after the 13^{th} day. During the first 13 and last 37 days of operation of Reactor E, there were two different CO_2 uptake rates. It can be stated that this difference could be resulted from microalgal acclimation period. Since growth period of microalgae

was longer in industrial wastewater and flue gas culture condition, acclimation period was necessary for microalgae in this culture.

On the other hand, Reactor F replicates were operated in a fed-batch mode at HRT 8 day and CO_2 uptake of them were analyzed regularly. During 50 days of operation of Reactor F, microalgae CO_2 uptake rate of 11.45 g/L.day was recorded. It was obviously shown that, the growth rate of microalgae reached steady state easily and CO_2 removal went on without any disruption (Figure 4-19a and b). Since the contact time between microalgae and industrial wastewater was long enough to avoid from wash-out of microalgae, microalgae in Reactor F could maintain activities without any acclimation period.

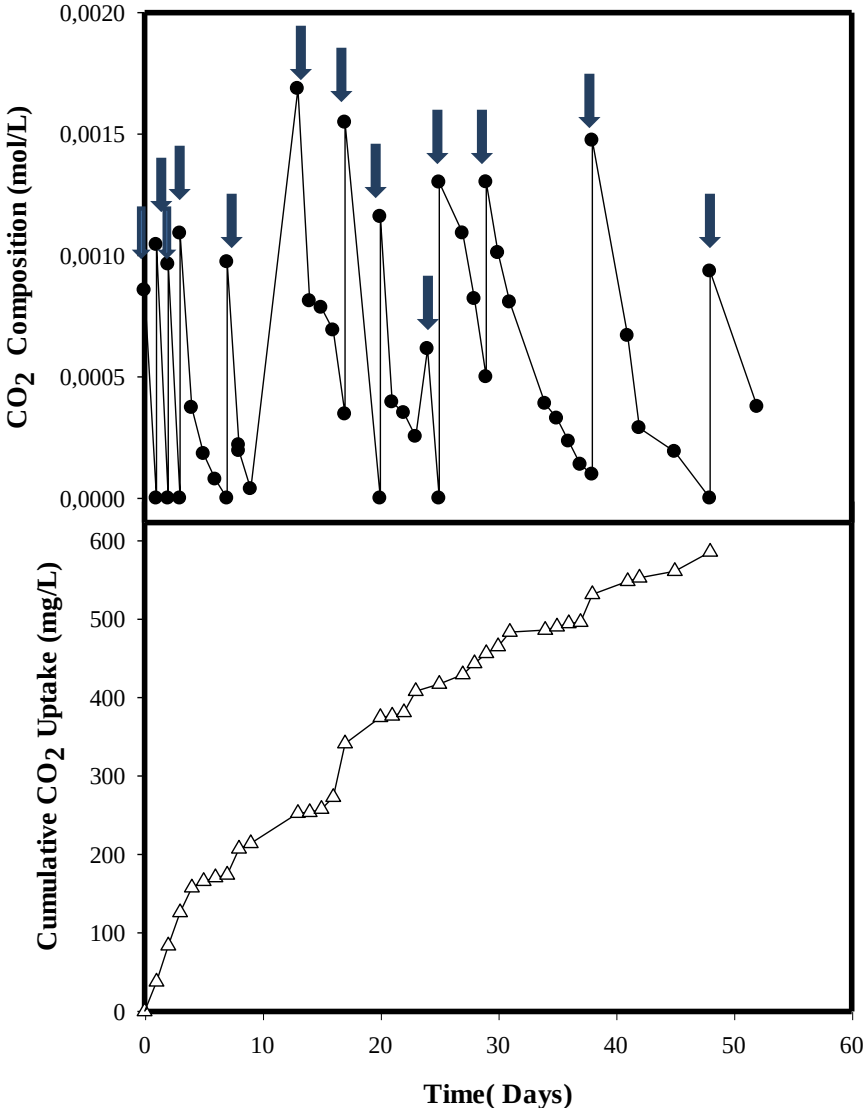

Figure 4-19. a) Daily CO_2 composition change and b) Cumulative CO_2 uptake data of Reactor F

Daily change of CO_2 composition and cumulative CO_2 uptake values of Negative Control are shown in Figure 4-20a and b. It was calculated that totally 869.11 mg/L CO_2 was uptaken with the rate of 21.27 mg/L.day in the Negative Control. Since Negative Control Reactor contained distilled water instead of industrial wastewater, microalgae could grow more easily and consume more CO_2 than other reactors such as Reactor E and F (Figure 4-20).

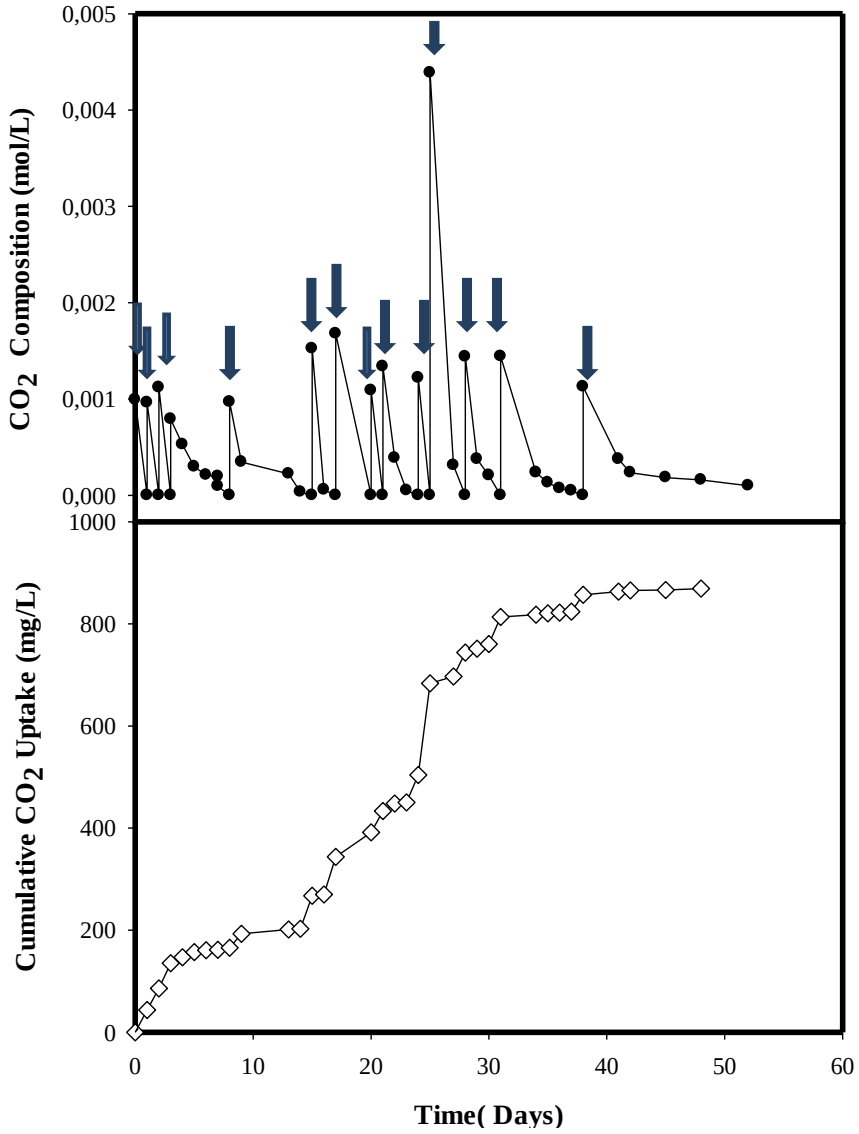

Figure 4-20. a) Daily CO_2 composition change and b) Cumulative CO_2 uptake data of Negative Reactor

Maximum CO_2 uptake was achieved in Negative Control in which initial TAN and PO_4 concentration were 11.69 mg/L and 3.89 mg/L respectively. In this reactor, wasting process was done with distilled wastewater instead of industrial wastewater, so microalgae could grow easily without any stress and inhibition until the end of the entire nutrient supply in reactor. It can be concluded that contents of industrial

wastewater had a negative effect on CO_2 uptake of microalgae in Reactor E and F (Figure 4-21).

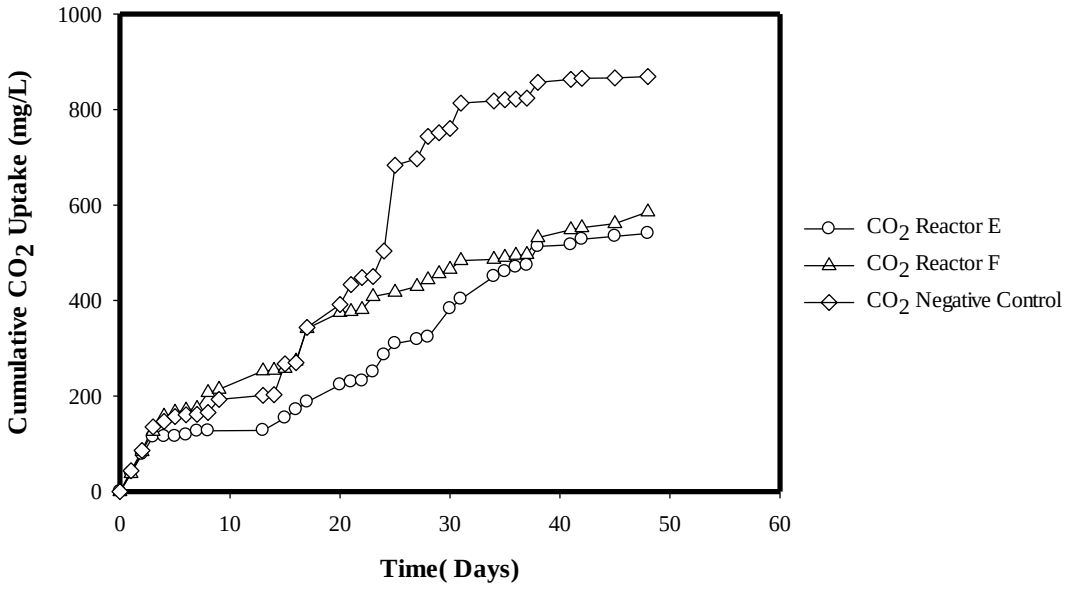

Figure 4-21. Cumulative CO_2 uptake data of Fed-batch Reactors

4.4.3. Growth and Development of Microalgae

Specific to this set of experiments, since the sample taken for here is very small compared to the rest of the experiments, therefore, the TS and VS measurements could not be conducted thus presented.

4.5. Comparison of Three Sets of Reactors

4.5.1. CO_2 Uptake Performance

Present study indicates that potential CO_2 capture of microalgae ranges from 0.32 to 6.44 grams per grams of microalgae as shown in Table 4-6. Compared to literature, this range is higher than some of the other studies conducted by Lardon et al., (2009); Herzog and Golomb, (2004); Doucha et al., (2005).

Table 4-6. Comparison of three sets of reactors in terms of CO_2 capture data

Reactor	**Operation Mode**	**Total CO_2 Uptake Amount (mg/L)**	**CO_2 Uptake Rate (mg/L.day)**	**Gram CO_2/Gram TS Algae**
Reactor A	Batch/Artificial gas	303.73	42.09	6.44
Reactor B	Batch/Artificial gas	100.75	21.11	2.13
Reactor C	Batch/Flue gas	114.65	18.2	0.32
Reactor D	Batch/Flue gas	182.96	29.4	0.51
Reactor E	Fed-batch/Flue gas	540.74	11.49	1.51
Reactor F	Fed-batch /Flue gas	585.81	11.45	1.64
Control 1A	Batch/Artificial gas	45.88	-	-
Control 1B	Batch/Artificial gas	58.31	-	-
Control 2A	Batch/Flue gas	25.63	-	-
Control 2B	Batch/Flue gas	36.52	-	-
Control 2C	Batch/Flue gas	12.23	-	-
Control 2D	Batch/Flue gas	7.37	-	-
Control 2E	Fed-batch /Flue gas	Contamination	-	-
Control 2F	Fed-batch Flue gas	Contamination	-	-
Negative Control	Fed-batch /Flue gas	869.11	21.27	2.43

As shown in Table 4-6, total CO_2 uptake normalized with respect to biomass at the end of the experiments was calculated as 6.44 g CO_2/g TS (1.75 g C/g TS) in the Reactor A. The amount of CO_2 fixed by microalgae grown in Reactor B was lower and equal to 2.13 g CO_2/g TS (0.58 g C/g TS).

Furthermore, total CO_2 uptake of Reactor C and D were normalized with respect to biomass at the end of the experiments as 0.32 g CO_2/g TS (0.08 g C/g TS) and 0.51 g CO_2/g TS (0.14 g C/g TS), respectively. Furthermore, same analysis was done for Negative Control and 2.43 g CO_2/g TS (0.66 g C/g TS) was measured.

Normalized total CO_2 uptake with respect to biomass at the end of the experiments were measured in Reactor E and F were 1.51 g CO_2/g TS (0.41 g C/g TS) and 1.64 g CO_2/g TS (0.45 g C/g TS), respectively.

When it was compared, the best CO_2 uptake performance was achieved in the first set of the experiments. Set-1 experiments were conducted with artificial gas instead of flue gas. It can be claimed that iron-steel industry flue gas affects CO_2 mitigation performance and metabolic activities of microalgae. Also, Set-3 experiments showed second maximum CO_2 fixation performance which may be caused from operation mode of reactors and higher nutrient content of industrial wastewater. Both second and third sets of reactors were operated with flue gas, but industrial wastewater was used as nutrient source only in Set-3 reactors. Furthermore, Set-3 reactors were operated with fed-batch mode. It was calculated the lowest amount of CO_2 fixed by every gram of microalgae in Set-2 reactors. There may be several causes for obtaining low microalgae CO_2 fixation performance in these reactors such as solubility problem of flue gas, granulation process, low inoculation density, phosphorus limitation etc. (See Section 4.3.1).

Compared to literature, CO_2 uptake performance of per gram of microalgae in this study is in the range of 0.32 and 6.44 which is higher than some of the other studies. For example, Lardon et al., (2009) reported that 1.8 gram of CO_2 consumed by per gram of microalgae in an open raceway pond. In another study, it was reported that between 1.6 and 2 grams of CO_2 is captured for every gram of algal biomass produced (Herzog and Golomb 2004). Moreover, it was estimated that 4.4 kg of CO_2 is needed for production of 1 kg (dry weight) algal biomass in an outdoor open thin-layer photobioreactor (Doucha et al., 2005).

Furthermore, the one-way analysis of variance (ANOVA) was used to determine whether there are any statistically significant differences between the means of these three sets. The CO_2 uptake rates and the amount of captured CO_2 by per gram of microalgae values of these three sets were not significantly different ($p=0.26$ and $p= 0.23$, respectively).

4.5.2. Nutrient (TAN and PO_4) Uptake Performance

This study indicates that TAN and PO4 uptake rates of microalgae can be dependent on initial concentration of these nutrients in culture. As shown in Table 4-7, since initial nutrient concentrations coming from industrial wastewater were maximum in Reactor E and Reactor F, TAN and PO_4 uptake rates of microalgae were achieved the maximum level as well. Furthermore, Reactor A and C showed second maximum nutrient uptake performances which may be caused from high initial nutrient concentrations of raw domestic wastewater. As it was mentioned in Section 4.2.1, autoclave process caused a decrease in amount of initial TAN and PO_4 concentration. Therefore TAN and PO_4 uptake rates of Reactor A which contained raw domestic wastewater as nutrient source are higher than Reactor B contained autoclaved domestic wastewater. Similarly, TAN uptake rate of Reactor C is higher than Reactor D. However, PO_4 uptake rate of Reactor D is the only one that does not fit into this generalization. That is, PO_4 uptake rate of Reactor D should be lower than the values of Reactor C due to autoclave effect. However, PO_4 uptake rate of Reactor D which is 1.69 mg/L.d was higher than Reactor C which is 1.50 mg/L.d. This may come from experimental error.

Besides PO_4 and TAN uptake rates, PO_4 and TAN removal efficiencies of the reactors were analyzed. The results of this study were in agreement with the related literature. For example, Kim et al. (1998) showed 95.3% and 96% removal efficiencies of nitrogen and phosphorus, respectively, by *C. vulgaris* in 25% secondarily treated swine wastewater after four days of incubation. Yun et al., (1997) reported *C. vulgaris* was able to remove 100% of ammonia, by addition of external phosphate salts. Lau et al., (1995) indicated that more than 90% of nitrogen and 80% of phosphorus were removed from primary treated sewage by *C. vulgaris*. Furthermore, the one-way analysis of variance (ANOVA) was used to determine whether there are any statistically significant differences between the means of these three sets in terms of TAN and PO_4 uptake rates. TAN and PO_4 uptake rates of these three sets were not significantly different ($p= 0.19$ and $p= 0.21$, respectively).

As shown in Table 4-7, nutrient removal efficiencies in batch operated reactors reached up to 99% however removal efficiencies of around 50 - 60% was observed in fed-batch operated reactors. Also one way ANOVA analysis was done and p values of nitrogen and phosphorous removal efficiencies are 0.007 and 0.02, respectively. It shows that phosphorous removal efficiencies of these three sets are significantly different. This may come from inhibitory effect of industrial wastewater on microalgae grown in Reactor E and F (Set-3) (Abeliovich et al., 1976; Arunakumara et al., 2006; Gutierrez et al., 2016) (See Section 4.4.1). Li et al., (2013) performed semi-continuous and continuous cultivation reactors which operated with *C. vulgaris* in municipal wastewater treatment plant secondary effluent. Total nitrogen and total phosphorus removal efficiencies of these semi-continuous and continuous cultivation reactors were 90.3 - 93.6% and 89.9 - 91.8%, respectively. Similarly, Woertz et al., (2009) studied with *C. vulgaris* cultivated in municipal wastewater with both 3 and 4 days HRT. Over 99% of ammonium and orthophosphate removal was achieved. It can be stated that the reason for the lower nutrient removal efficiencies of Reactor E and F is industrial wastewater, not operation mode.

Table 4-7. Comparison of three sets of reactors in terms of nutrient removal rate and removal efficiencies data

Reactor	**Operation Mode**	**TAN Uptake Rate (mg/L.day)**	**PO_4 Uptake Rate (mg/L.day)**	**Nitrogen Removal Efficiency (%)**	**Phosphorous Removal Efficiency (%)**
Reactor A	Batch/Artificial gas + air mixture	7.19	0.81	99.95	98.75
Reactor B	Batch/Artificial gas + air mixture	3.34	0.68	90.57	94.11
Reactor C	Batch/Flue gas	5.12	1.50	89.59	98.40
Reactor D	Batch/Flue gas	1.86	1.69	99.75	82.25
Reactor E	Fed-batch /Flue gas	8.85	1.75	50.01	57.99
Reactor F	Fed-batch /Flue gas	18.28	4.03	52.60	57.75

CHAPTER 5

CONCLUSION

Carbon mitigation through CO_2 bioconversion is natural mechanisms for microalgae culture. Microalgae have ability to absorb atmospheric CO_2 and CO_2 from flue gas, causing reduction of greenhouse gas concentration. Microalgae based CO_2 mitigation strategies will reduce greenhouse effect and provide ecosystem preservation. Also, microalgal culture can be utilized in biological wastewater treatment as an alternative method. Microalgae do not only remove nutrients from wastewater but also produce biomass which can be used as energy crops. In this regard, coupled nutrient removal and CO_2 mitigation using microalgae culture is a sustainable, cost effective and environmentally friendly method.

In the first set batch reactors, the use of CO_2 enriched air (18-20% CO_2) for the growth of freshwater microalgae *C. vulgaris* culture brought about the CO_2 uptake rate of 0.02-0.04 g/L.day. Moreover, the total CO_2 uptake was found to be 0.1 and 0.3 g/L when autoclaved and raw domestic wastewater used as growth media, respectively. Microalgae growth was slightly higher in raw domestic wastewater because of higher nutrient concentrations than autoclaved domestic wastewater and production of additional CO_2 as a result of bacterial activities. The use pure CO_2 resulted in higher amounts of CO_2 fixed by microalgae which was measured indirectly by dry weight (TS) as 2.13 and 6.44 g CO_2 /TS for autoclaved and raw domestic wastewater growth media, respectively.

In the second set batch reactors, *C. vulgaris* culture carried out photosynthetic reactions in domestic wastewater fed by flue gas collected from iron-steel industry.

The microalgae strains in culture could tolerate CO_2 in the flue gas (5%), which resulted in the CO_2 uptake rate of 0.02-0.03 g/L.day. Moreover, the total CO_2 uptake was found to be 0.18 and 0.11 g/L when autoclaved and raw domestic wastewater used as growth media, respectively. Also, total CO_2 uptake of these microalgae which were cultivated in autoclaved and raw domestic wastewater, were normalized with respect to biomass at the end of the experiments as 0.51 g CO_2/TS and 0.32 g CO_2/TS, respectively. It could be seen that total CO_2 uptake and microalgae growth were halted in the reactors operated with raw wastewater. There may be several reasons for observing low microalgae activity in these reactors: 1) low solubility of CO_2 in the flue gas, 2) bacterial inhibition in the presence of flue gas, which would stimulate microalgae growth otherwise by producing CO_2, 3) formation of microalgae-bacteria flocs in the presence of organics, which may reduce the growth of microalgae, 4) low initial microalgae density and inoculation time, 5) limitation of phosphorus in the domestic wastewater.

In the third set fed-batch reactors, microalgae were cultivated with industrial wastewater and flue gas collected from iron-steel industry. Total CO_2 uptake was found to be 0.54 and 0.58 g/L when reactors were operated 4-day HRT and 8-day HRT, respectively. Moreover, normalized total CO_2 uptake with respect to biomass at the end of the experiments were measured in reactors operated 4-day HRT and 8-day HRT as 1.51 g CO_2/TS and 1.64 g CO_2/TS, respectively. The rate of CO_2 uptake was around 0.01 g/L.day in both reactors. Also, totally 869.11 mg/L CO_2 was uptaken with the rate of 21.27 mg/L.day in the Negative Control. Since Negative Control contained distilled water instead of industrial wastewater, microalgae could grow more easily and consume more CO_2 than other fed-batch reactors. Moreover, the amount of CO_2 fixed by microalgae which was measured by dry weight (TS) was 2.43 g CO_2 /TS for Negative Control.

Nitrogen and phosphorus removal efficiencies of these three sets were 82-99%. This study proved that *C. vulgaris* can be used for both domestic and industrial wastewater treatment and also microalgal-bacterial symbiotic cultures can also help nutrient removal from wastewater.

This study is important for showing CO_2 fixation using fast-growing microalgal species can be a very promising alternative for mitigation of CO_2 which is the most prominent greenhouse gas. Moreover, this study showed that coupled wastewater treatment and CO_2 mitigation by *C. vulgaris* culture can be an economically feasible and environmentally sustainable approach. Therefore, this approach can be used in pilot scale studies after lab-scale studies has completed and progressed.

Also, this study was proved that microalgal biomass can be produced by using domestic and industrial wastewater as nutrient source. This approach can be a feasible alternative to get high amount of microalgal biomass which could have several applications such as cosmetics, antioxidants, biofertilizer, nutrient supplements, pharmaceuticals, biofuels (Spolaore et al. 2006).

The results obtained point out that there is a need to increase microalgae growth rate when flue gas is used as the source for CO_2 supply. Also, it was observed that CO_2 uptake rates were lower than the values reported in the other studies. This might be caused from low nutrient concentrations in domestic and industrial wastewater compared to growth media used in other studies. For further experiments, micronutrients, phosphorous, trace elements, vitamins and silicon additions should be considered at proper concentrations.

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