INTEGRATED NUTRIENT REMOVAL AND CARBON DIOXIDE SEQUESTRATION BY USING MIXED MICROALGAE CULTURE

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

BY

ŞÜKRÜ BURAK ÇAKIRLAR

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

MAY 2017

Approval of the thesis:

INTEGRATED NUTRIENT REMOVAL AND CARBON DIOXIDE SEQUESTRATION BY USING MIXED MICROALGAE CULTURE

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ABSTRACT

INTEGRATED NUTRIENT REMOVAL AND CARBON DIOXIDE SEQUESTRATION BY USING MIXED MICROALGAE CULTURE

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May 2017, 154 pages

Microalgae can remove nitrogen (N) and phosphorus (P) in domestic and industrial wastewaters, which cause eutrophication in rivers, lakes and seas. Microalgae have also been recognized as a promising alternative for carbon dioxide (CO_2) sequestration from flue gas. However, it is necessary to design flexible and low-cost cultivation systems and, use suitable operating conditions to achieve enhanced biomass productivities and high CO_2 fixation efficiencies.

The aims of this study were: (i) to determine optimum hydraulic retention times (HRTs) for cultivation of microalgae in different types of wastewaters; (ii) to compare the microalgal productivities and nutrient removal rates at different N:P ratios (iii) to propose an integrated system for the utilization of wastewater and CO_2 in flue gas for the production of microalgae. Within this context, a mixed microalgae culture collected from Araç Creek in Karabük Province in Turkey was grown under batch and semi-continuous operation modes. Two types of culture mediums were used in the experiments: (i) primary treated domestic wastewater from Ankara Tatlar WWTP and (ii) KARDEMIR Coke Plant wastewater diluted with supernatant of sludge thickener tanks of Ankara Tatlar WWTP. While ambient air (0.03% CO_2) was supplied to the cultures grown with primary treated

domestic wastewater, CO_2 enriched air (4% CO_2) was sparged into the cultures grown with diluted industrial wastewater. Light and mixing (aeration) conditions were the same in all set-ups. The optimum inoculum volume was determined as 10% (v/v) conducting a batch study and was used in all experiments.

The optimum HRT was found to be 2 days for cultivation of microalgae in primary treated domestic wastewater. Mixed microalgae culture was able to remove 94.7% of Total Ammonia Nitrogen (TAN) and 93.8% of orthophosphate (PO_4^{3-}-P) from domestic wastewater at a HRT of 2 days. Although almost complete nutrient removal efficiencies were observed during steady conditions of the cultures with 4- and 8-day HRT, the steady-state conditions could not be maintained and cell washout was observed in the reactors due to nutrient limitation.

The TAN/PO_4^{3-}-P (g/g) ratio of 6 resulted in the maximum nutrient removal efficiency when the diluted coke plant wastewater was used in the batch-mode operation. Results of the semi-continuous study conducted with diluted coke plant wastewater revealed that HRT should be kept 8 days at minimum in order to achieve efficient TAN and PO_4^{3-}-P removal (>98%) and high steady-state biomass concentrations (>2.4 mg TS/L). The CO_2 removal rates were highest in the culture with 12 day-HRT and, it was obtained as 0.436 g CO_2/h.

The results demonstrated both effectiveness and potential application of the coupled system to remove nutrients from domestic and industrial wastewaters and simultaneous CO_2 removal from a point source.

Keywords: Microalgae, Nutrient Removal, Carbon Dioxide Sequestration.

ÖZ

KARIŞIK MİKROALG KÜLTÜRÜNÜN KULLANILMASI İLE ENTEGRE BESİYER MADDE GİDERİMİ VE KARBONDİOKSİT TUTULMASI

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Mikroalgler, ırmak, göl ve denizlerde ötrifikasyona neden olan, evsel ve endüstriyel atıksularda bulunan nitrojen (N) ve fosforu (P) giderebilirler. Mikroalgler, baca gazındaki karbondioksitin (CO_2) tutulması için de umut verici bir alternatif olarak kabul edilmektedir. Ancak, yüksek miktarda biyokütle üretimi ve CO_2 tutumu için esnek ve düşük maliyetli yetiştirme sistemlerinin geliştirilmesi ve uygun işletme koşullarının kullanılması gerekmektedir.

Bu çalışmanın amaçları: (i) farklı tür atıksularda yetiştirilen mikroalg için en uygun hidrolik bekletme sürelerinin (HBS) bulunması; (ii) farklı N:P oranları için mikroalg üretim verimlerinin ve besiyer madde giderim hızlarının karşılaştırılması; (iii) mikroalg üretiminde atıksuyun ve baca gazındaki CO_2'in kullanıldığı entegre bir sistem önermektir. Bu bağlamda, Türkiye'nin Karabük ilinde bulunan Araç Çayı'ndan alınan karışık alg kültürü, kesikli ve yarı kesikli işletme modlarında yetiştirilmiştir. Deneylerde iki farklı kültür ortamı kullanılmıştır; (i) Ankara Tatlar Atıksu Arıtma Tesisi'nden alınan ve ön arıtmaya tabi tutulmuş evsel atıksu ve (ii) Ankara Tatlar Atıksu Arıtma Tesisi'ne ait çamur yoğunlaştırma tanklarının süzüntü sularıyla seyreltilmiş KARDEMİR Kok Fabrikası atıksuyu. Birincil olarak arıtılmış evsel atıksu ile yetiştirilen kültürlere ortam havası

(%0.03 CO_2) beslenirken, endüstriyel atıksu kullanılarak yetiştirilen kültürlere CO_2 yönünden zenginleştirilmiş hava (%4 CO_2) sağlanmıştır. Aydınlatma ve karıştırma (havalandırma) koşulları tüm düzenekler için aynı tutulmuştur. En uygun inokulum hacmi %10 olarak belirlenmiş ve tüm çalışmalarda bu oran kullanılmıştır.

Birincil olarak arıtılmış evsel atıksular için en uygun HBS'nin 2 gün olduğu tespit edilmiştir. Karışık alg kültürü, 2 günlük HBS süresinde evsel atıksudan 94.7% Toplam Amonyak Azotu (TAN) ve 93.8% ortofosfat (PO_4^{3-}-P) giderimi sağlayabilmiştir. Denge koşullarında, 4 ve 8 günlük bekletme süresine sahip kültürlerde besiyer madde giderimi tama yakın olduğu gözlemlenmiş olsa da, denge durumunun korunamadığı ve besiyer madde limitasyonu sebebi ile alg kültürlerinin çöktüğü gözlemlenmiştir.

Seyreltilmiş endüstriyel atıksu ile kesikli modda yapılan çalışmada, TAN/PO_4^{3-}-P (g/g) = 6 oranı maksimum besiyer madde giderimi ile sonuçlanmıştır. Seyreltilmiş endüstriyel atıksu ile gerçekleştirilen yarı-kesikli çalışmanın sonuçları ise yüksek (>98%) TAN ve PO_4^{3-}-P giderimlerine ve yüksek (>2.4 mg TS/L) biyokütle konsantrasyonlarına ulaşmak için hidrolik bekletme süresinin en az 8 gün olarak seçilmesi gerektiğini göstermiştir. 12 günlük bekletme süresine sahip kültürde CO_2 giderim oranının en yüksek olduğu belirlenmiş ve 0.436 g CO_2/saat olarak kaydedilmiştir.

Deneysel sonuçlar, evsel ve endüstriyel atıksularda bulunan besiyer maddelerin giderimi ve noktasal kaynaklı CO_2 giderimi sağlayan birleştirilmiş sistemin etkinliğini ve potansiyel uygulamasını göstermiştir.

Anahtar Kelimeler: Mikroalg, Besiyer Madde Giderimi, Karbondioksit Tutulması.

To my family with endless love

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere gratitude to my supervisor Prof. Dr. Göksel N. Demirer for his guidance, patience and understanding, through my master's studies. I also thank the members of my thesis defense jury, Prof. Dr. İpek İmamoğlu, Prof. Dr. Ayşegül Aksoy, Assoc. Prof. Dr. Emre Alp and Assoc. Prof. Dr. Selim L. Sanin for their valuable contributions to this thesis.

I also gratefully acknowledge the financial support provided by the Scientific and Technological Research Council of Turkey (TUBİTAK) for the project (Project No: 111Y205) which this thesis has emerged from.

My dear project members, Özgül Çalıcıoğlu and Gece Cansu Öğüt; I thank you for always being there for me, whether it was work or personal. Güneş Tunçay, Engin Koç, Mehmet Gazaloğlu, İlke Çelik, Eray Gür, Begüm Aytaç, Elif Sena Uzunpınar and Nilüfer Ülgüdür and, all those who have contributed to the completion of this thesis one way or the other, I thank you for your friendship and support. I would like to specially thank Emin Calbay, Yiğit Özşen, Melih Can Akman, Emrehan Berkay Çelebi and Eda Bayar for their endless laughs. Zeynep Melis Çin, I will be forever grateful for your love and faith in me.

Last but not the least, I am forever indebted to my family for supporting me during all the ups and downs of this thesis. Any number of words I would use here are not sufficient to thank my family. I can never thank them enough for their unwavering love and support. I love you guys.

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μ	:	Specific Growth Rate, (d^{-1})
ATP	:	Adenosine triphosphate
BM	:	Basal Medium
BNR	:	Biological Nutrient Removal
BOD	:	Biochemical Oxygen Demand
C	:	Carbon
CAPEX	:	Capital Expenditure
CO	:	Carbon Monoxide
CO_2	:	Carbon Dioxide
COD	:	Chemical Oxygen Demand
DO	:	Dissolved Oxygen
EBPR	:	Enhanced Biological Phosphorus Removal
EU	:	European Union
g	:	Grams
GHG	:	Greenhouse Gas
H_2O	:	Water
ha	:	Hectares
HRP	:	High Rate Algal Pond
HRT	:	Hydraulic Retention Time
L	:	Litres
mg	:	Miligrams
N	:	Nitrogen
NH_3	:	Free Ammonia
NH_4^+	:	Ammonium Ion
NH_4^+-N	:	Ammonium - Nitrogen
NO_3^-	:	Nitrate Ion
NO_3^--N	:	Nitrate - Nitrogen

NO_x	:	Nitrogen Oxide
O_2	:	Oxygen
OD	:	Optical Density
OPEX	:	Operating Expenditure
PO_4^{3-}-P	:	Orthophosphate
P	:	Phosphorus
P	:	Productivity
PAR	:	Photosynthetically Active Radiation, (nm)
PAO	:	Polyphosphate Accumulating Organisms
PBR	:	Photobioreactor
PM	:	Particulate Matter
sCOD	:	Soluble Chemical Oxygen Demand
SO_x	:	Sulfur Oxide
TAN	:	Total Ammonia Nitrogen
tCOD	:	Total Chemical Oxygen Demand
TKN	:	Total Kjeldahl Nitrogen
TN	:	Total Nitrogen
TP	:	Total Phosphorus
TS	:	Total Solids
TSS	:	Total Suspended Solids
VS	:	Volatile Solids
VSS	:	Volatile Suspended Solids
vvm	:	Volume Gas per Volume of Broth per Minute

CHAPTER 1

INTRODUCTION

Global warming is the term used to describe the gradual increase in the average temperatures of lower atmosphere that causes climate change. It is believed that the main reason of global warming is the large amount of anthropogenic greenhouse gases emitted during energy production, industrial activities including waste management, cultivation of crops and livestock, and transportation (Mata et al., 2010).

CO_2 is considered as the most abundant greenhouse gas contributing the global warming. Atmospheric concentration of CO_2 has increased significantly from the level of 278 ppm in preindustrial period to current level of 400 ppm in the last 150 years-period and, it is predicted to reach 500 ppm by the year of 2100 (Johnston et al., 2003). Combustion of fossil fuels is the source of about 75% of the total anthropogenic emissions (López et al., 2009) and extensive use of fossil fuels for energy production is considered to be the main reason of accumulation of CO_2 in the atmosphere. However, it is predicted that the use of fossil fuels for energy production will continue in the foreseeable future.

In the recent years, various strategies have been investigated and many technologies have been developed for CO_2 mitigation (Filali et al., 2011). Increasing the use of renewable sources for energy production, developing energy efficient technologies and improvements in combustion processes can help reducing CO_2 emissions (Fernández et al., 2012).

Current technologies available for the removal or capture of CO_2 in the flue gases can be divided into two categories, namely physicochemical and biological methods.

Physicochemical methods include absorption and injection into deep oceans and geological formations. However, these methods are far away from being sustainable due to their high costs and high energy consumption. On the other hand, biological CO_2 mitigation refers to CO_2 fixation with terrestrial plants and microalgae via photosynthesis. Microalgae are considered as one of the most effective approaches to fix CO_2 since their photosynthetic efficiency and CO_2 fixation capability are notably higher than terrestrial plants (Douskova et al., 2009). The carbon fixed by microalgae is incorporated into cellular components such as carbohydrates, lipids and proteins (Costa et al., 2011). Therefore, biofuels, fine chemicals, or foods can be produced from the harvested algal biomass (Bhakta et al., 2015). When coupled with biofuel production and wastewater treatment, algae-based CO_2 sequestration can be a more economically feasible and environmentally sustainable process.

Nutrient enrichment (mainly nitrogen and phosphorus) in surface water due to agricultural activities and inadequately treated urban and industrial wastewater discharge lead to deterioration of water quality. Algal bloom which cause decreased water transparency, oxygen depletion, odor and fish kills is also a serious environmental problem caused by nutrient rich conditions in lakes, rivers and coastal waters (Nyenje et al., 2010). In addition to being an environmental problem of great importance, algal bloom has significant economic and socio-cultural consequences when public health costs of illnesses and impacts on fisheries and tourism are considered (Cai et al., 2013). In order to reduce the impacts of nutrients on surface waters, it is essential to treat wastewaters prior to discharge. Many activated sludge treatment plants have been upgraded to remove nutrients however upgrades are not cost-effective, especially for nitrogen, due to high energy demand (U.S. EPA, 2008). Nutrients in domestic and industrial wastewaters can also be removed by microalgae in the engineered systems namely, open ponds and photobioreactors. Algal biomass produced in these controlled systems can then be transformed to high value chemicals and biofuels such as biomethane, bioethanol or biohydrogen.

The costs for microalgae cultivation and harvesting can be reduced by integration of CO_2 sequestration, wastewater treatment and production of useful industrial products. In this context, the objective of this study to investigate nutrient removal from domestic and industrial wastewaters coupled with CO_2 mitigation using mixed algal cultures.

The tasks undertaken are:

- Determination of optimum inoculum culture and volume for the cultivation of mixed microalgae culture in primary treated domestic wastewater;
- Evaluation of the effect of hydraulic retention time (HRT) on the steady-state biomass concentrations and nutrient removal from primary treated domestic wastewater using semi-continuous cultures;
- Evaluation of the kinetics of microalgae growth and nutrient removal from primary treated domestic wastewater;
- Investigation of the effect of the N:P ratio on the microalgal growth and nutrient removal from the diluted coke plant wastewater;
- Investigation of simultaneous CO_2 sequestration and nutrient removal potential of semi-continuous cultures grown in diluted coke plant wastewater under continuous CO_2-enriched air supply

CHAPTER 2

LITERATURE REVIEW

2.1. Nutrient Pollution

Nutrient pollution in the surface and groundwater has received much attention in recent years world-wide and it is ranked as one of the most significant causes of degradation of water quality (Liu et al., 2005; Subramanian, 2012). Nutrient pollution can be caused by point and non-point sources. Anthropogenic nutrient inputs such as municipal and industrial effluents which are point sources; and agricultural runoff which is a non-point source constitute serious threats for both surface and groundwater. Excess nitrogen and phosphorus from these inputs lead to eutrophication in coastal waters, rivers and lakes which causes reduction of biodiversity and replacement of dominant species, increased water toxicity, and increased turbidity of the water and decreased lifespan of the lakes (Cai et al., 2013). These changes in water quality directly affect the economic activities and human health.

Nutrients such as nitrates and phosphates in the groundwater are particularly an important issue where communities use groundwater as daily water supply for their domestic and agricultural activities (Jayasingha et al., 2012). Contamination of groundwater with nutrients occurs due to fertilizer application, sewage leakage and animal manures. Nitrate is one of the most common groundwater contaminants in rural areas and excessive concentrations of nitrate in drinking water can lead to a serious disease in infants known as methemoglobinemia, or blue baby disease (Ota et al., 2013; WHO, 2011).

2.1.1. Eutrophication

European Commission Directive 98/15/EC on urban wastewater treatment defines the eutrophication as the enrichment of water by nutrients, especially compounds of nitrogen and/or phosphorus, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned. Eutrophication poses a significant threat to long-term health and functioning of coastal and enclosed water bodies in several regions of the world (Ruiz et al., 2013). As a result of eutrophication, low dissolved oxygen level, fish kills and depletion of desirable flora and fauna are observed (Sathasivan, 2009).

There are natural and anthropogenic causes of eutrophication. Natural run-off of nutrients from the soil and the weathering of rocks are natural eutrophication processes. Untreated sewage discharge and run-off of inorganic fertilizers and manure from farms containing nitrates, ammonia and phosphate are the significant sources of human-caused eutrophication. Beside direct causes, there are also indirect causes. For instance, deforestation following human activities leads to eutrophication because it results in increased nutrient accumulation rate in water bodies due to increased surface runoff. The main effects of eutrophication on water bodies are algal blooms and increased vegetation, development of anoxic conditions, increase in turbidity and decrease in species diversity. Consequently, eutrophic water bodies become non-potable, and unsuitable for drinking, agricultural and industrial purposes (Nyenje et al., 2010).

Between 1961 and 2013, total phosphorus consumption has increased fivefold and reached 31 Tg (Chen and Graedel, 2016). Human activities have caused a dramatic increase in the annual accumulation rate of the phosphorus in ecosystems during this period (Calicioglu, 2013). Phosphorus mostly enters the aquatic ecosystems from non-point sources such as agricultural operations and phosphate rock mining. Point sources such as municipal and industrial effluents are also a significant source of total phosphorus loading to water bodies. Since phosphorus is the key-growth limiting nutrient for algae in fresh-water systems, excessive loading of phosphorus into freshwater systems causes eutrophication (Usher et al., 2014). Therefore, it is important to remove phosphorus in the

effluents before being discharged to surface water to prevent eutrophication (Cordell et al., 2011).

Eutrophication is one of the most significant global environmental problems. According to a survey conducted by International Lake Environment Committee, 54% of the lakes or reservoirs in Asia are impaired by eutrophication. In Europe, the percentage is 53%, in North America it is 48%, in South America it is 41%, and in Africa it is 28% (Cai et al., 2013). The survey results show that both developing and developed countries are suffering from this problem. Research, monitoring and evaluation activities are very critical for determining impacts and sources of eutrophication in water bodies in order to formulate appropriate policy responses.

2.2. Nutrient Removal Technologies

Conventional biological processes designed to meet secondary treatment effluent standards typically do not remove total nitrogen (TN) and total phosphorus (TP) to the extent needed to protect water quality. Discharges from conventional wastewater treatment plants still contain high concentrations of nitrogen and phosphorus, which causes eutrophication in receiving water bodies. To meet the discharge limits for both N and P, conventional plants are now required to add tertiary treatment systems (Selvaratnam et al., 2014).

Biological Nutrient Removal (BNR) processes have been developed to protect receiving water bodies from eutrophication. In BNR processes, nitrogen in wastewater is converted to inert nitrogen gas and phosphorus is trapped in the solids, which are removed from effluent (EPA, 2004). These processes include different combinations of anaerobic, aerobic, and anoxic zones with internal recirculation such as Bardenpho, A_2O, UCT, and their modifications to remove ammonium, nitrate and phosphate in wastewater (Mennaa et al., 2015). Conventional suspended growth biological treatment plants can also be modified to BNR systems.

The main disadvantages of these biological wastewater treatment technologies have been given as high costs, complex operation and great volume of waste sludge production (Von et al., 2002). Moreover, external carbon sources, such as methanol and sodium acetate, are required to improve the process efficiency since the carbon (COD) is limiting for both nitrogen and phosphorus removal for the treatment of wastewater with low C:N ratio (Yuan et al., 2016).

2.2.1. Nitrogen Removal Technologies

There are physical-chemical and biological methods for nitrogen removal from wastewaters (Sathasivan, 2009). Physical-chemical methods include ammonia stripping, selective ion exchange and breakpoint chlorination. These techniques can be applied for direct removal of nitrogen in ammonia form and thus the cost of converting ammonia to nitrate in biological treatment processes is eliminated. Furthermore, physical-chemical nitrogen removal methods are unaffected by toxic compounds, which can have adverse effects on the performance of biological treatment methods. Ammonia stripping process consists of raising to pH to values up to 11.5, formation and reformation of water droplets in stripping tower and agitation by air through the tower to provide air-water contact. However, air pollution problems may arise due to the atmospheric reactions (Behera et al., 2013) and also, dry and wet deposition of stripped ammonia may negatively affect the quality of sensitive water bodies (Yadav et al., 2016).

In the selective ion exchange process, natural zeolites, synthetic zeolites and other synthetic ion exchange resin can be used for the removal of ammonia from wastewaters. The process includes a regeneration step. In this step, the absorbed ions are removed and then replaced with less selective binding ions. Therefore, it is a costly step due to the requirement of concentrated salt or alkaline solutions (Rahmani et al., 2006).

Breakpoint chlorination process is used for converting ammonia in wastewater to chloramines and then nitrogen gas by the addition of chlorine to the breakpoint. It has been reported that 95 to 99 percent ammonia in domestic wastewater could be converted

to nitrogen gas. However, additional alkalinity is required to neutralize the acidity produced by oxidation of ammonia to nitrogen gas (U.S. EPA, 2008).

Stripping, ion exchange and breakpoint processes for ammonia removal are not feasible because of technical, regulatory, and economical concerns and therefore, they are not preferred by municipalities in most countries, including the USA (U.S. EPA, 2008). The traditional method is the biological methods, which is carried out by three main steps called ammonification, nitrification and denitrification. In the first step, organic nitrogen is converted to ammonia by hydrolysis and microbial activity. In nitrification step, ammonia is oxidized to form nitrate and nitrite by *Nitrosamanas* and *Nitrobacter*. Then, nitrate is converted to nitrogen gas under anoxic conditions in the last step commonly by *Pseudomonas sp.* (Tchobanoglous et al., 2003). However, denitrification efficiency is dependent on C:N ratio and, sufficient carbon must be available in the wastewater to completely denitrify nitrite to nitrate (Shahrom et al., 2012).

2.2.2. Phosphorus Removal Technologies

Removal of phosphorus present in the wastewater can be achieved by chemical and biological methods. Combination of these methods might also be employed in some cases to attain desired effluent concentrations (U.S. EPA, 2004).

In chemical phosphorus removal process, a trivalent metal cation such as ferric ion or aluminum ion is used for the precipitation of orthophosphate. Metal ion addition is performed in primary clarifiers or in the secondary processes. Alkalinity is required for the chemical reaction to be completed and generally, lime is used in order to supply sufficient alkalinity level. Chemical phosphorus removal process produce additional sludge which is the main disadvantage of the process and, there is a risk of phosphorus release if sludge is in an anaerobic environment.

Biological process is also utilized for phosphorus removal from wastewaters. Activated sludge systems can be designed and operated to treat phosphorus are called as Enhanced Biological Phosphorus Removal (EBPR). Biological phosphorus removal is achieved by enhancing the accumulation of phosphorus in microorganisms called as Polyphosphate

Accumulating Organisms (PAO) in the form of polyphosphates under cyclic anaerobic and aerobic operation conditions (Seviour et al., 2003). Phosphorus is removed from system as a fixed biological material in the waste sludge after sedimentation. Microorganisms also remove phosphate in conventional activated sludge process during biological oxygen demand (BOD) utilization however, removal levels are 2.5 to 4 times higher in EBPRs (WEF and ASCE, 1998). It is important to note that ratio of C:P is an important factor to achieve high phosphate removal efficiencies. In a previous work, it has been observed that as the C:P ratio was higher, P removal was also enhanced (Zhao et al., 2008).

2.2.3. Nutrient Removal Using Microalgae

Biological wastewater treatment with microalgae is very attractive since microalgae can convert solar energy into useful biomass via photosynthesis and also remove nutrients such as nitrogen and phosphorus from wastewater (de la Noue et al., 1988). In addition to nutrients, microalgae can also remove coliform bacteria, chemical and biological oxygen demand and heavy metals in both domestic and industrial wastewater (Hammouda et al., 1995; Abdel-Raouf et al., 2012).

Microalgae have direct and indirect effects on nutrient removal. For example, nutrient uptake into algal biomass is direct removal and, ammonia stripping and orthophosphate precipitation due to high pH resulted from photosynthetic activity is indirect removal (Garcia et al., 2000).

Microalgae based wastewater treatment systems are considered as a promising alternative to biological nutrient removal (BNR) processes. Unlike previously described BNR processes, nutrient removal using microalgae-based wastewater treatment systems do not require the addition of chemicals, construction of numerous tanks for operations and internal recirculation of partially or fully processed wastewater (Mennaa et al., 2015). Microalgae biomass has a high potential for commercial application of large scale production of bio-compounds that are easily converted into biofuels (Costa et al., 2011).

Besides, ability of microalgae to capture CO_2 can reduce a treatment facility's environmental footprint (Packer, 2009).

Microalgae are utilized in two types of wastewater treatment systems, namely, facultative waste stabilization ponds and high rate algal ponds (HRPs). Intentionally use of microalgae for wastewater treatment has been developed by Oswald in the beginning of 1950's with the introduction of stabilization ponds (Oswald et al., 1957). Waste Stabilization Ponds are large and shallow basins used for treatment of wastewater by biological processes involving both algae and bacteria (Hammouda et al., 1995; Abdel-Raouf et al., 2012) . Microalgae produce oxygen necessary for bacteria to remove the organics and contribute to nutrient assimilation in facultative waste stabilization ponds (Woertz et al., 2009). These ponds do not have any artificial energy input and, mixing is provided by wind and hydraulic dilution. Facultative ponds are cost-effective, reliable and easily-operated method for wastewater treatment nevertheless they have lower biomass productivity than HRPs. Nitrogen cycle in waste stabilization ponds is shown in Figure 1.

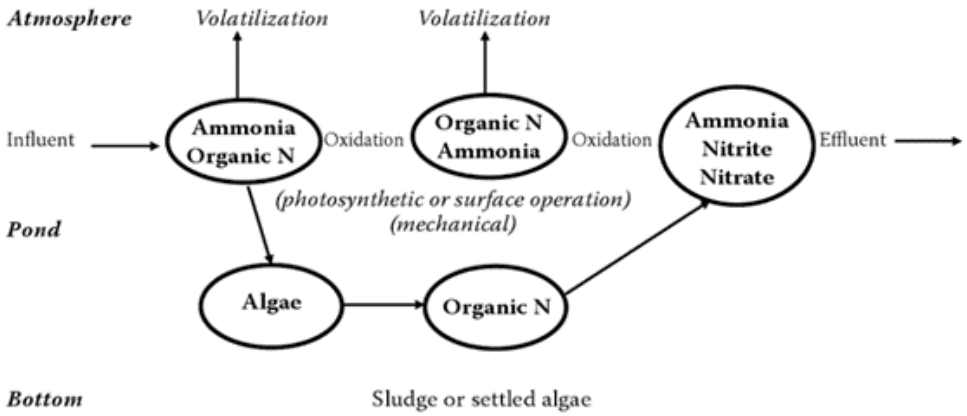


Figure 1 Nitrogen Cycle in Waste Stabilization Ponds (Spellman and Drinan, 2014)

HRPs are modified versions of facultative algae ponds and they have been developed to achieve higher Biological Oxygen Demand (BOD) and Total Suspended Solids (TSS) removal. HRPs are shallow and raceway shaped ponds and, mixing and circulation are

provided by paddle wheels. HRPs have shorter hydraulic retention times and less area requirement than conventional ponds and, they have high nutrient removal capability. Green et al. (1995) have measured ammonia and phosphorus removal efficiencies of 89 and 49 percent, respectively, in the treatment of municipal wastewater with facultative-HRP sequences. In a recent study, ammonia removal efficiencies up to 85% and phosphate removal efficiencies between 51% and 57% have been achieved at HRPs with different operation conditions treating anaerobically digested wastewater (de Godos et al., 2016).

Chlorella, *Scenedesmu*s and *Spirulina* are widely used microalgae cultures for nutrient removal (Xin et al., 2010). Currently, most studied strains are *Chlorella vulgaris* and *Scenedesmus acutus* in the studies utilizing real wastewater (Ale et al., 2014) Although there are many studies investigating nutrient removal using microalgae, most of the research was on cultivation of monocultures in synthetic wastewater with batch mode operations (Aslan and Kapdan, 2006; Hsueh et al., 2007; Hu et al., 2012; Li et al., 2011b; Ruiz-Marin et al., 2010; Samorì et al., 2013). In particular, nutrient removal capability of microalgae from unsterilized municipal wastewater in semi-continuous and continuous modes has been investigated only in few studies. Among these studies, Li et al. (2013) grew *C.vulgaris* in sterilized municipal effluent and reached removal efficiency of 93.6% for total nitrogen and 91.8% for total phosphorus. Woertz et al. (2009) achieved over 99% removal of ammonium and orthophosphate from primary treated municipal wastewater by mixed cultures. Tercero et al. (2013) compared different urban wastewaters and obtained the best growth rate and final biomass concentration with primary treated wastewater.

2.3. Microalgae

Algae are defined as a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicultural forms (Znad et al., 2012). They use carbon dioxide, energy from the sun and inorganic nutrients to produce oxygen and complex organic compounds including biomass. Algae can be found in a wide range of water habitat, including freshwater, brackish water and marine environment (Kiepper, 2013).

Algae are subdivided into two groups based on their sizes, macroalgae and microalgae. Macroalgae are large and multi-cellular organisms and generally found in ponds. On the other hand, microalgae are microscopic (less than 2 mm in diameter) and unicellular microorganisms and found in both marine and freshwater environments (Znad et al., 2012). Microalgae are amongst the most photosynthetically efficient organisms and they are more productive than land plants and macroalgae (Haiduc et al., 2009). Microalgae produce more than half of the world's primary production of oxygen and consume large amounts of CO_2 (Edberg, 2010).

The number of microalgae species are estimated between 200,000 and 800,000 and just around 35,000 species of microalgae have been studied so far (Arenas et al., 2016). *Cyanophyceae* (blue-green algae), *Chlorophyceae* (green algae), *Bacillariophyceae* (including diatoms) and *Chrysophyceae* (including golden algae) are the most frequently cited microalgae since these have one or more of the desirable features for efficient and economical combination of CO_2 fixation, wastewater treatment and lipid synthesis toward biofuel production (Kumar et al., 2010).

2.3.1. Growth Kinetics

In batch cultures, where food supply is limited and nothing is added or removed (Becker, 1994), algal growth will pass through the following six growth phases (Lee et al., 2015):

1) Lag phase
2) Exponential phase
3) Linear phase
4) Declining growth phase
5) Stationary phase
6) Death phase

These growth phases are illustrated in Figure 2. The individual phases shown in the figure are not always clearly defined, their length and slope may change depending on prevailing culture conditions such as nutrient concentration, light intensity and temperature.

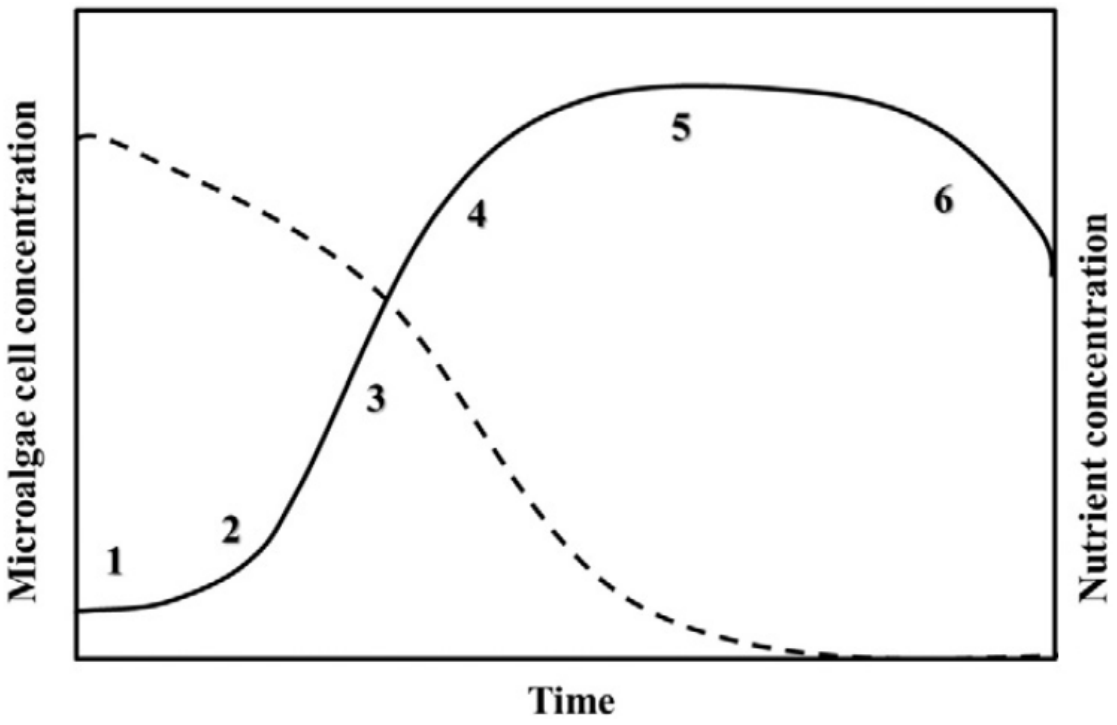


Figure 2 Microalgal growth phases (solid line) and nutrient concentration (dashed line) in batch culture (Lee et al., 2015)

During lag phase, microalgae cells are adapting to change in nutrient concentration or culture conditions. At the end of lag phase, the cells are well adjusted to the new environment and ready to enter exponential growth phase, where algal cells grow and divide as an exponential function of time. In this period, light intensity and nutrients are saturated and do not limit microalgae growth. As it can be seen from the Figure 2, the algal cells divide at a constant rate and the slope of exponential growth phase is called as productivity, *P* which can be calculated by using Equation 1 (Samorì et al., 2013):

$$P\left(\frac{mg}{L.day}\right) = \frac{dN}{dt} = \frac{X_2 - X_1}{t_2 - t_1} \qquad \text{(Equation 1)}$$

In this equation;

X_1 is the biomass concentration at the beginning of selected time interval (t_1)

X_2 is the biomass concentration at the end of selected time interval (t_2)

Biomass productivity (*P*) is an important parameter that should be considered in microalgae cultivation, since it shows the capacity of a reactor to produce biomass under specific operating conditions (Mennaa et al., 2015).

The plot of the log algal biomass concentration values versus time yields a straight line and its slope gives the specific growth rate. Its value can be determined as in Equation 2 (Becker, 1994):

$$\mu(d^{-1}) = \frac{dN}{Ndt} \qquad \text{(Equation 2)}$$

After an exponential growth, algae cell division slows down or specific growth rate tends to decrease and increase in biomass becomes almost linear since light becomes limiting. This phase is called as linear growth phase.

In the declining growth phase, cell division rate reduces due to limiting factors, such as nutrients, carbon dioxide, and others.

In stationary phase, there is an equilibrium between growth rate and death rate, thus growth rate remains constant. Maximum biomass concentration that can be reached is observed in this phase.

Decrease in the concentrations of nutrients, overheating, pH disturbance, or contamination are the factors leading to death phase.

2.3.2. Algal Photosynthesis

Photosynthesis is a complex biological process. Algae can convert CO_2 into carbohydrates and produce oxygen by using energy contained in sunlight and water. H_2O provides electrons necessary for reduction of CO_2 to sugars (Costa et al., 2011). Light energy is required for breaking up of water and, pigments like chlorophyll is used to absorb solar

energy (Ringsmuth et al., 2016). The overall photosynthesis reaction is given below (Zhu, 2010):

$$6CO_2 + 12H_2O + photons \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$$

Algal photosynthesis involves many different types of organisms including green algae, diatoms and cyanobacteria. However, many of the major photosynthetic pathways are similar in all algae species. In general, photosynthesis can be divided into two stages namely light-dependent reactions and Calvin cycle (Zhao et al., 2014). Light depended reactions are oxidative processes and require light for splitting of water. Energy carrier molecules such as ATP and NADPH are produced in this stage. In the Calvin cycle, energy carrier molecules are utilized to convert CO_2 and water into carbohydrates (Moroney, 2009). General scheme of the photosynthesis is given in Figure 3.

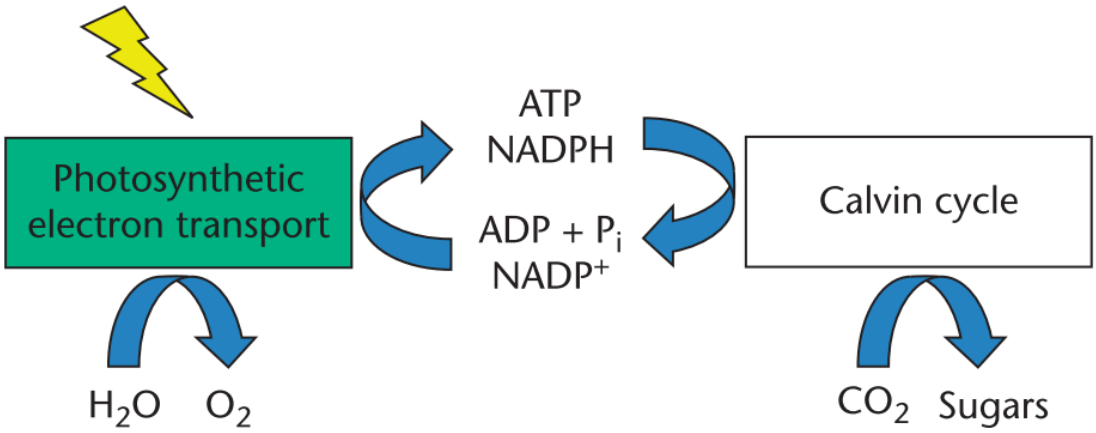


Figure 3 General scheme of photosynthesis (Moroney, 2009)

2.3.3. Environmental Factors Affecting Microalgal Growth

There are some abiotic, biotic and operational factors influencing algal growth. The effects of these factors on the algal growth are explained in the next sections.

2.3.3.1. Nutrients

Nitrogen and phosphorus are the two essential nutrients for microalgae growth and contribute about 10–20% of microalgal biomass (Zhu, 2010). Both nutrient deficiency and excess nutrients can negatively affect physiology and morphology of microalgae (Rowley, 2010). Therefore, sufficient amounts of nutrients must be provided in growth medium (Wang et al., 2008).

Nitrogen is required for the growth and biomass synthesis. Nitrate (NO_3^-), nitrite (NO_2^-), and ammonia (NH_4^+-N) are the chemical forms of nitrogen that can be directly assimilated by all eukaryotic algae. On the other hand, urea had to be hydrolyzed to NH_4^+-N before its assimilation. It should be noted that NH_4^+-N is the most readily taken up and assimilated form of nitrogen.

Phosphate is responsible for the energy transfer of the cells and the formation of cell membranes and nucleic acids (Ding et al., 2014). Phosphorus is preferentially assimilated as inorganic phosphates in the form of dihydrogen phosphate ($H_2PO_4^-$) and hydrogen phosphate (HPO_4^{-2}). It is mainly supplied in the form of orthophosphate (PO_4^{3-}) in the cultivation of microalgae.

Moreover, inorganic N:P ratio is also important in terms of growth. General stoichiometric formula of an average algal cell is $C_{106}H_{181}O_{45}N_{16}P$ and the elements should be available in these proportions in nutrient medium to achieve optimum growth (Larsdotter, 2006). Composition with N:P ratios between 3:5 and 38:1 have been reported in literature for different species (Boelee et al., 2012). The optimum N:P ratio for microalgae growth was stated to be in the range of 6.8-10 (Cheah et al., 2016). High N:P ratios like 30:1 indicates P limitation while low ratios of N:P like 5:1 indicates N limitation (Larsdotter, 2006).

Both domestic and industrial wastewaters can also be used as a source of nitrogen and phosphorus for algae cultivation since they contain large quantities of different forms of nitrogen and phosphorus sources. The N:P ratio is between 4 and 5 for most wastewater

(Cheah et al., 2016). Therefore, it should be noted that domestic and industrial wastewaters generally have lower N:P ratios compared to typical ratios in rapidly-growing microalgae and, nitrogen is the limiting nutrient in many case (Tchobanoglous et al., 2003).

2.3.3.2. Carbon dioxide

Carbon dioxide (CO_2) is the usual carbon source for microalgae and the CO_2 demand is about 1.8 g CO_2/g biomass, considering algal biomass contains roughly 50% carbon by dry weight (Hulatt et al., 2011b). Three different sources of CO_2 for microalgae are CO_2 in the atmosphere, CO_2 in discharge gases from industries, and CO_2 chemically fixed in the form of soluble carbonates (e.g. $NaHCO_3$ and Na_2CO_3) (Singh et al., 2014).

Microalgae can directly utilize CO_2 and often bicarbonate (Sims et al., 2016). Concentrations of the inorganic carbon forms depend on pH and temperature of the medium. At pH values greater than 9, inorganic carbon is in the form of carbonate form, which cannot be directly utilized by microalgae (Larsdotter, 2006).

Atmospheric CO_2 is provided to microalgal cultivation systems via aeration. However, since CO_2 concentration in the atmosphere is very low (0.033%) to meet the need of optimum algal growth, addition of extra CO_2 to the air supply might be necessary. This is why most previous studies have been performed providing the cultures with air enriched with 1 to 5% CO_2 (Larsdotter, 2006).

Regarding maximum CO_2 tolerance, some microalgae species can survive with very high CO_2 concentrations. However, lower CO_2 concentration is required for their maximum growth. For example, maximum tolerance and optimum CO_2 concentrations for *Chlorella sp.* were found to be 40% and 10%, respectively, while *Scenedesmus sp.* could grow under 80% CO_2 conditions but the maximum cell mass was observed at 10% - 20% CO_2 concentrations (Hanagata et al., 1992).

2.3.3.3. pH

Microalgae growth can be affected by variations in pH in different ways. Besides distribution of carbonate species, pH has a strong impact on solubility, bioavailability and toxicity of the nutrients and heavy metals present in the medium (Weisse et al., 2006). pH values above 9 induce the precipitation of phosphorus in the form of calcium phosphate (Laliberté et al., 1997). High pH conditions (10.5 – 11.5) result in ammonia stripping and cause high rates of ammonia removal from the medium (Kim et al., 2003). Physiological effects are also likely to be observed at extreme pH values (Chen et al., 1994).

In most cases, microalgae prefer pH values between 7 and 9 (Ruiz-Martinez et al., 2012). Optimum productivity of *Anabaena variabilis* was observed at pH 8.2 and 8.4 (Fontes et al., 1987). On the other hand, optimum pH for C. *reinhardtii* was found to be around 7.5 (Kong et al., 2010). The optimal pH for the growth and lipid accumulation of the microalga *Chlorella vulgaris* was found to be 7.5 (Sakarika et al., 2016).

In a previous study, the growth of two freshwater species, namely *Scenedesmus obliquus* (Turp.) Kutz. and *Chlorella vulgaris* Beij, at pH values between 7.7 and 10.6 have been investigated. Both species could grow up to pH 10.6 although *C.vulgaris* was more adversely affected by alkaline pH than was *Scenedesmus obliquus* (Goldman et al., 1982).

2.3.3.4. Temperature

Temperature has direct effect on cellular chemical composition, nutrient uptake, CO_2 fixation and growth rate of algae (Cassidy, 2011). Increase in temperature enhances growth up to an optimum temperature is reached (Becker, 1994).

The optimal temperature for microalgae cultures vary with the composition of the culture medium, the species and strain cultured. Generally, optimum temperature is in the range of 20 ^{o}C and 30 ^{o}C for ideal microalgae growth (Chisti, 2007; Zhu, 2010). Temperatures higher than 35 ^{o}C may cause cell damage or death and temperatures lower than 16 ^{o}C will slow down growth (Bitog et al., 2011). At low temperatures, microalgae are more

sensitive to bright light and it can be an operational problem for outdoor microalgae cultivation systems in cold climate (Larsdotter, 2006).

In a previous study, the effect of temperature on the growth of *Chlorella vulgaris* with CO_2-enriched air has been investigated. The growth parameters were the highest at 30 °C, slightly higher than at 25 °C (Bamba et al., 2015). At 35 °C, growth was negatively affected. In another study, the growth of *Chlorella vulgaris* with ambient level of CO_2 at different temperatures (30, 40 and 50 ^oC) has been compared. Results indicated that the highest growth was at 30°C and, no growth was observed at 50 ^oC (Chinnasamy et al., 2009). Some other *Chlorella* species also grew successfully between 26 ^oC and 36 ^oC (Kessler, 1985).

2.3.3.5. Light

Light intensity greatly affects the growth, composition and content of lipid in microalgae. The growth rate of microalgae increases with rising light intensity, before reaching the stage of photo-inhibition, i.e., inhibition of photosynthesis by increased light intensity (B. Zhao et al., 2014). A research indicated that high light intensity stimulated the accumulation of lipid in *Chlorella sp.* L1 and *M. dybowskii* Y2 and the content of protein, carbohydrate and Chlorophyll-a decreased (He et al., 2015). Conversely, at low light intensities, Chlorophyll-a concentration increases due to the reduced light absorption (Torzillo et al., 2013).

Previous studies also depicted that saturation irradiance varies between 30 and 280 µmol m^{-2} s^{-1} for aquatic photosynthetic microorganisms with an average of 100±50 µmol m^{-2} s^{-1} (Bohutskyi et al., 2016). Generally, light intensities between 100 and 200 µmol m^{-2} s^{-1} are used in lab-scale studies and this corresponds to 5-10% of full daylight (Barsanti et al., 2006). Illumination necessary for algal cultivation can be provided naturally, artificially or by both. Artificial illumination is provided by florescent lamps in the laboratory settings.

Photoperiod has an important role in the gases exchange pattern. Microalgae consume organic carbon via heterotrophic metabolism and, consume O_2 and release CO_2 during

dark periods. It has been observed that biomass productivity reduces with the increased dark period (Pires et al.,2012). Different light/dark regimes, such as 16:8, 12:12, 8:16 (h:h) and continuous illumination, are also used for the lab-scale photobioreactors. In most studies, 12h:12h photoperiod has been used to more closely mimic natural solar day-night cycle (Su et al., 2012).

Photoperiod is also an important factor affecting nutrient removal. Nutrient and organic carbon removal of heterotrophic *Chlorella kessleri* under 12h:12h and continuous lighting have been investigated. The removal efficiency of organic carbon and phosphorus was greater under a 12h light/12h dark lighting scheme than that under continuous lighting, while nitrate removal efficiency under continuous lighting was greater than that under 12h:12h photoperiod (Lee et al., 2001).

The effects of light intensity and photoperiod cycle have been investigated in many studies. For example, microalgae growth has been compared at three photoperiods (8h:16h, 12h:12h and 16h:8h light/dark cycles) and light intensities (37.5, 62.5 and 100 µmol m^{-2} s^{-1}). Maximum biomass production has been observed at 62.5 µmol m^{-2} s^{-1} light intensity and 16h:8h photoperiod (Khoeyi et al., 2012). On the other hand, it was found that biomass production increased in parallel with the increasing light period duration (Jacob-Lopes et al., 2009). Furthermore, *Chlorella* species achieved higher biomass production under continuous illumination compared to cyclic light/dark conditions (Ogbonna et al., 1996).

2.3.4. Microalgal Cultivation Systems

Microalgae cultivation can be conducted either in open or closed systems. In the following sections, basic properties of open and closed systems are explained.

2.3.4.1. Open Systems

Shallow big ponds, raceway or high rate ponds (HRP) (Figure 4), circular ponds and tanks are commonly used open systems for cultivation of microalgae (Zhang, 2015). The HRP is the mostly used and cheapest system for commercial algae production (de Vree et al., 2015). Although open systems are economical and easily scalable, they have

disadvantages of high evaporation, poor control of culture conditions such as temperature and pH and low photosynthetic efficiency, high CO_2 loss, low biomass productivity and large area requirement (Harun et al., 2010).

Figure 4 Photograph of a pilot scale raceway pond in AlgaePARC, Wageningen University, the Netherlands (Cakirlar, 2014)

2.3.4.2. Closed Systems

Closed systems or photobioreactors (PBR) allow better control of cultivation conditions such as temperature and pH compared to open systems. Besides, various designs, applications and operation methods of closed systems have been developed to overcome the problems with open systems described above.

Proper photobioreactor design is important for achieving maximum biomass production. The most important design parameters influencing growth in closed systems are light penetration, gas injection and mixing speed. Light penetration directly affects biomass composition, growth rate and end products (Bitog et al., 2011). Aeration with CO_2 enriched air can meet the CO_2 demand of microalgae and provides mixing. It has been reported that 5% or 10% (v/v) CO_2 enriched air at rates of 0.025–1 vvm (volume of

air/medium/time) is cost effective for cultivation (Zhang et al., 2002). Generally, flue gases from combustion processes contain 5% - 15% (v/v) CO_2 depending on the fuel type and therefore, they can be used to provide sufficient amounts of CO_2 for large-scale production of microalgae (Van Den Hende, 2012). Sufficient mixing is essential for ensuring light intensity distribution, providing sufficient CO_2 transfer and maintaining uniform pH (Bitog et al., 2011). Furthermore, illumination surface area per unit volume, high mass transfer of CO_2 and O_2, energy requirement for illumination and mixing should be considered for appropriate design (Harun et al., 2010). Flat plate, tubular and vertical column photobioreactor configurations are widely used for both laboratory scale and large scale cultivation of microalgae.

Flat-plate photobioreactors have large illumination surface area that provides high biomass productivity and photosynthetic efficiency. Flat-plate configuration is suitable for outdoor cultures and, typically flat plate photobioreactors are made of transparent materials in order to utilize solar energy as efficiently as possible. Furthermore, operational flexibility, ease of cleaning and low oxygen build-up and low cost are the advantages offered by flat plate photobioreactors (Ugwu et al., 2008). Main disadvantages of flat-plate reactors are the scaling-up problems, poor culture temperature control, the possibility of algal wall growth, and the incompatibility with some microalgae species (Sierra et al., 2008).

Tubular photobioreactors are very suitable for outdoor cultivation and consist of straight, coiled or looped glass or plastic tubing arranged in various ways in order to increase illumination area and to maximize solar energy utilization (Grima et al., 1999). Beside high illumination area, low investment cost and suitability for outdoor cultivation are main advantages of tubular photobioreactors. Limitations are large area requirement and non-uniform pH, dissolved oxygen and carbon-dioxide distribution along the tubes (Ugwu et al., 2008). Tubular photobioreactors may have different configurations as shown in Figure 5.

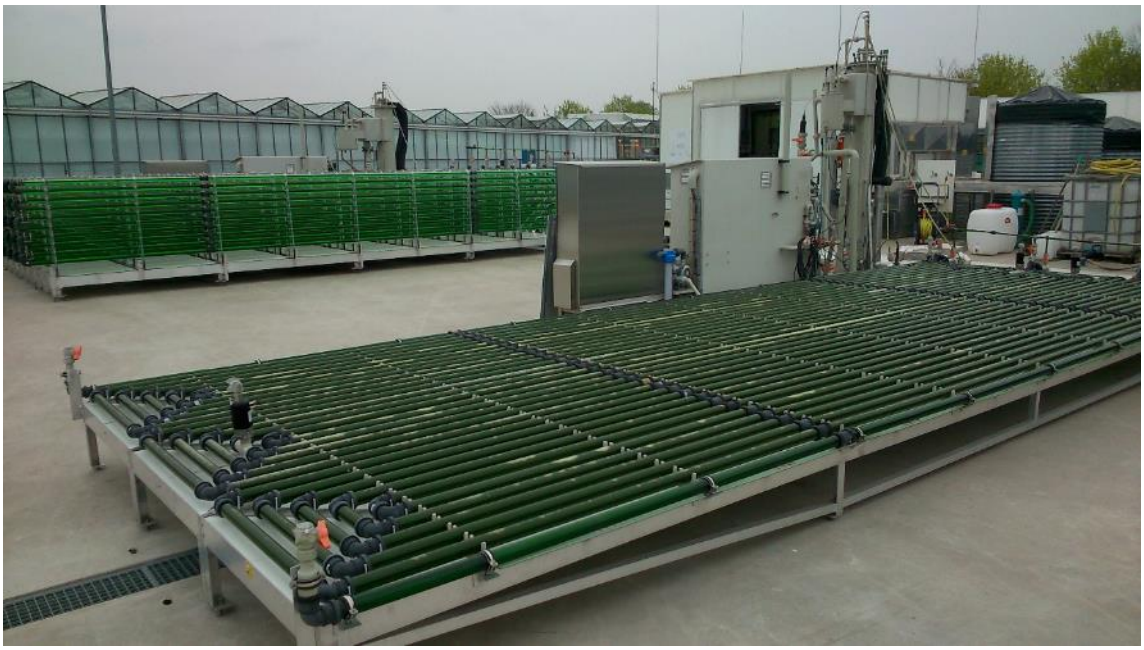


Figure 5 Photograph of pilot scale horizontal and vertical tubular PBRs in AlgaePARC, Wageningen University, the Netherlands (Cakirlar, 2014)

In addition to being suitable for large scale algae cultivation, **vertical-column photobioreactors** are compact, cost efficient, and easily operated reactors (Mirón et al., 2002). Furthermore, other advantages include high mass transfer, good mixing, low photoinhibition and photo oxidation risk, low energy consumption and ease of sterilization. Low illumination area and requirement of sophisticated construction materials are basic disadvantages of vertical-column photobioreactors (Ugwu et al., 2008).

Bubble column and airlift photobioreactors, which are configurations of vertical-column photobioreactors, have been widely studied for the microalgae cultivation. A typical vertical column photobioreactor consist of a glass or plastic column with an air inlet at the bottom. Air bubbling from bottom provides good mixing, enhance CO_2 utilization and provide optimal O_2 removal (Castellanos, 2013). Air pumps or preferably air lift systems are used to provide aeration and mixing (Ugwu et al., 2008). Bubble column

photobioreactors have the highest gas hold-ups rates and consequently the best mass transfer compared to other systems (Znad et al., 2012).

2.3.4.3. Cultivation System Operation Modes

Photobioreactors can be operated in batch, semi-continuous or continuous mode. However, using continuous mode has several advantages as opposed to the batch mode including providing a higher degree of control, regulating and maintaining of the growth rate for extended time periods, controlling biomass concentration by varying the dilution rate and achieving more reliable and easily reproducible results (Mata et al., 2010). Table 1 summarizes the advantages and disadvantages of the batch and continuous operation modes.

Table 1 Comparison of cultivation modes (Zhu, 2015)

Culture Mode	Advantages	Disadvantages
Batch	• Easy set up and operate • Efficient nutrient removal • Easy to set specific conditions for the accumulation of desired end-products in biomass • Lower contamination risk • Popular in lab-scale studies	• Long cultivation periods • A limited capacity and ability to treat wastewater • Lower volumetric biomass productivity • Expensive set-up
Semi-Continuous and Continuous	• No time requirement for the preparation of a culture system in the middle of operation • Steady-state conditions can be maintained • High volumetric biomass productivity • High capacity and ability to treat wastewater • Easy to scale up • Higher automation	• Technical and complex installation • Composition of nutrient supply might affect the growth and end products in biomass • Uncertain stability for long term operation performances • Contamination is a high risk • The installation is more expensive

2.4. Greenhouse Gases and Global Warming

The atmosphere traps some of the Sun's energy, heats the Earth’s surface to support life and, Earth reflects some of the sunlight into space as heat. Greenhouse gases (GHGs) in the atmosphere allow sunlight to enter the atmosphere and to reach a planetary surface. GHGs tend to absorb reflected infrared radiation from Earth’s surface and heats both the atmosphere and the planetary surface (Condie, 2015). This natural process is called as greenhouse effect.

Global warming refers to the rise in the average temperature of atmosphere and in turn, change in climate, which is caused by produced greenhouse gases by human activities (Ramanan et al., 2010). Increase in the average temperatures will result in changes in the amount and distribution of the precipitation, reduction in food production, glacial melting, rise of the ocean level and species extinction (Pires et al., 2012). Studies also suggest that global warming will increase the negative consequences of man-made eutrophication mainly due to the higher water temperatures in lakes, estuaries and coastal rivers. Rising temperature will also increase the nutrient inputs to water bodies as a result of increased rate of nutrient release from soils (Moss et al., 2011). Figure 6 illustrates that how global warming accelerate the eutrophication in lakes, estuaries and coastal rivers.

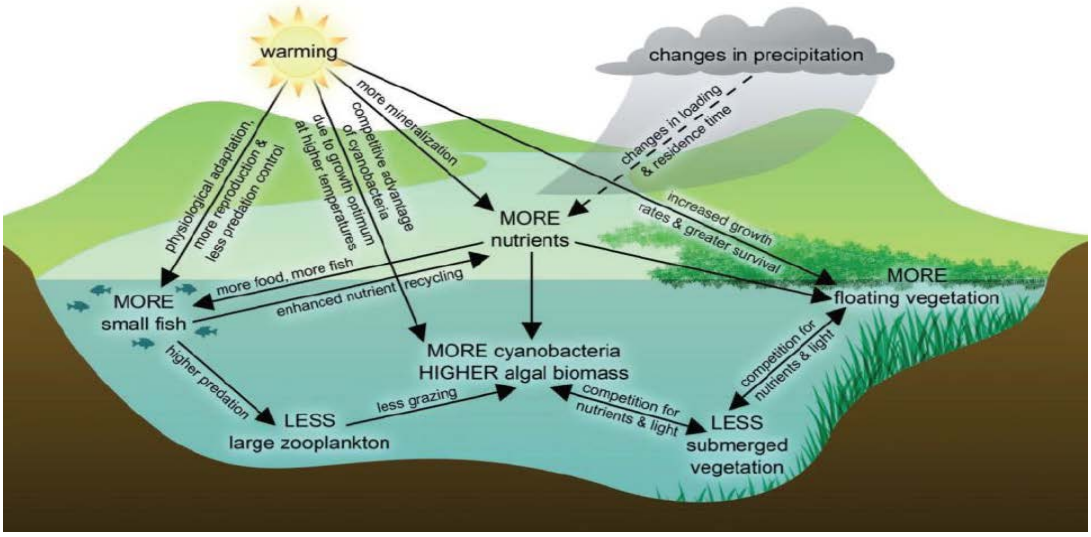


Figure 6 Effects of global warming on eutrophication (Moss et al., 2011)

GHGs include carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), sulfur oxides (SO_2), ozone (O_3), water vapor and halogenated organic compounds such as chlorofluorocarbons (CFCs), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs) and sulfurhexafluoride (SF_6). Since industrial revolution, the atmospheric concentrations of GHGs have been increasing primarily due to human activities. CO_2, which makes up 68% of the total greenhouse gas emissions, is the principle gas causing global warming (Harrington and Foster, 1999). CO_2 concentration has increased by about 25 percent since pre-industrialization and it is rising at about 0.5 percent per year. In addition, atmospheric lifetime of CO2 is between 50 and 200 years which means that its atmospheric concentration responds slowly to changes in emission rates (Hammitt et al., 1996).

Combustion of fossil fuels such as coal, natural gas, and oil for energy production and transportation is the main human activity emitting CO_2. Combustion of fossil fuels represents about 75 percent of total anthropogenic greenhouse gas emissions (López et al., 2009). Average carbon emission factors from electricity generation for selected fuel sources are presented in Table 2.

Table 2 CO_2 emissions from generation of electricity from different sources (Word Nuclear Association, 2013)

Fuel Source	g CO_2/kWh
Natural Gas	499
Lignite	1054
Hard Coal	888
Fuel-oil	733

Beside combustion processes for energy production, certain industrial processes including refining, cement production and iron and steel industry cause large amounts of CO_2 (Jansson et al., 2010). Table 3 shows the contribution of different industrial activities to the total CO_2 emissions.

Table 3 Worldwide large stationary CO_2 sources (Metz et al., 2005)

CO_2 Sources	Contribution (%)	Emissions (Mt CO_2 $year^{-1}$)
Power	78.3	10,539
Cement Production	6.9	932
Refineries	5.9	798
Iron and Steel Industry	4.8	646
Petrochemical Industry	2.8	379
Oil and Gas Processing	0.4	50
Other Sources	0.3	33
Biomass (Bioethanol and bioenergy)	0.7	91
Total	**100**	**13,468**

Researchers have been studying on development of energy efficient technologies and utilization of renewable energy to lessen consumption of fossil fuels. On the other hand,

usage of coal and other fossil fuels will continue in the electric power industry in the near future and, carbon mitigation for coal-fired power plants is prerequisite for climate change strategy (Zhu, 2010).

Municipal wastewater treatment is one of major contributors to the increase in the some GHG emissions, namely N_2O, CH_4 and CO_2. Wastewater treatment was the fifth largest source of worldwide anthropogenic CH_4 emissions with 9% and the sixth largest contributor to worldwide anthropogenic N_2O emissions with 3% in 2000. It is expected that global CH_4 emissions and N_2O emissions will grow by approximately 20% and 13%, respectively, between 2005 and 2020. It should be also noted that Turkey is among the countries with high GHG emissions (Gupta et al., 2012).

The emission rates per treated wastewater ranged from 0.005 kg CO_2-equivalent for primary treatment facilities, 0.26 kg CO_2-equivalent for conventional activated sludge with anaerobic sludge digestion to over 0.8 kg CO_2-equivalent/m^3 for extended aeration with aerobic digestion (Maclean et al., 2005). In A/O and SBR wastewater treatment systems, GHG emissions were 0.405 kg CO_2-equivalent/m^3 wastewater and 0.865 kg CO_2-equivalent/m^3 wastewater, respectively (Bao et al., 2016).

Two main conceptual methods to reduce CO_2 in atmosphere are physicochemical methods and biological methods. Many physicochemical methods have been developed for CO_2 mitigation in recent years. Carbon dioxide Capture and Storage (CCS) is a set of technologies and techniques that separate CO_2 from centralized industrial and energy-related emission sources, compress, transport to store the extracted gas in non-atmospheric reservoirs. CCS allows long-term isolation of CO_2 from the atmosphere however there are some limitations including high capital cost, difficulty in locating suitable carbon reservoirs, potential risks of aquifer contamination, and leakage and corrosion (Muhs et al.,2009). Physicochemical techniques, such as wet absorption or dry adsorption, membrane separation and cryogenic fractioning, are applied to capture CO_2 from large emission source points however they are far away from being sustainable due to high cost, high energy consumption and also some disposal problems (Znad et al.,

2012). Therefore, it is necessary and valuable to develop cost-effective and sustainable alternatives to directly remove or fix CO_2 from the flue gas (Wang et al., 2008).

Biological CO_2 mitigation can be achieved by terrestrial plants and photosynthetic microalgae. Since terrestrial plants have slow growth rates, it has been estimated that only 3-6% fossil fuel emissions can be removed in agriculture by plants (Skjånes et al., 2007). Unlike terrestrial plants, microalgae have fast growth rate. Microalgae are able to fix CO_2 using solar energy with efficiency 10 to 50 times greater than that of terrestrial plants (Mata et al., 2010; Wang et al., 2008). Therefore, it can be said that biological capture of CO_2 using microalgae is a promising technology.

2.4.1. CO_2 Sequestration by Microalgae

The concept of using flue gas from power generation and industrial processes for the large-scale algae production has been studying since the 1960s (Benemann et al., 2002). Considering that a typical flue gas has CO_2 concentrations of 2 to 20% (volume), flue gases from power plants and industry are favorable CO_2 sources for microalgae cultivation. Moreover, it has been shown that higher algal growth rate and CO_2 fixation rate can be achieved when flue gas is used as carbon source than that of systems using simulated air (Douskova et al., 2009). Therefore, utilization of microalgae is becoming an appealing option for CO_2 mitigation since they have ability to utilize concentrated forms of CO_2.

Many laboratory-scale studies have been published regarding the use of flue gas as a carbon source for microalgae cultivation (Doucha et al., 2005). Besides, several pilot-scale projects have been conducted in USA, Germany, Austria, Israel, China, India and South Africa to investigate the feasibility and benefits of supplying flue gas to algae cultures (Zhang, 2015). On the other hand, in Turkey, only a limited number of pilot scale projects have been conducted so far. In Akcansa Cement Factory located in Çanakkale, Turkey, *Nannochloropsis* has been cultivated in pilot scale raceway, plate and tubular PBRs using the flue gas. An annual CO_2 reduction of 25,360 kg and daily microalgae production of 5 kg are aimed at the pilot plant (Akcansa, 2015).

Among the companies investing in large scale microalgae cultivation to produce biofuel (Usher et al., 2014), Algenol Biofuel Inc. has already started commercial ethanol, gasoline, diesel, and jet fuel production using CO_2 emissions from industry as the carbon source. There are also some commercial microalgae production plants in Hawaii (Cyanotech Corporation) and Israel (Seambiotic) using flue gas from power plants (Jacob et al., 2015; Van Den Hende et al., 2012).

It is important to note that flue gases of coal fired power plants and iron and steel factories have high concentrations of NO_x and SO_x (Table 4) which may inhibit algal growth. Tolerance of microalgae species to these pollutants are different and varies as shown in Table 5 and therefore treatment of the flue gases is needed before being injected to the microalgae cultivation systems. Flue gases have also very high temperatures and low pressures which must be taken into account when designing such systems and they must be cooled to the temperature range of 25-35 ^oC prior to feeding to the photobioreactors (Zhao et al., 2014).

Table 4 Composition of the flue gases from energy production and iron and steel industry

Source of Flue Gas	**Temperature (^OC)**	**CO_2 (%)**	**NO_x (ppm)**	**SO_x (ppm)**	**Reference**
Coal fired Power Plant	120	13	150	10	(Kumar et al., 2011)
Coal fired Power Plant	316	10-15	40-100	N.A.	(Cassidy, 2011)
Natural Gas fired Combined Cycle Power Plant	100-120	1-2.5	250-300	0	(Packer, 2009)
Gas Turbine Combustor	-	5.7	30.6	0	(Olaizola et al., 2004)

Table 4 (continued)

Coke Oven	N.A.	18	150	200	(Li et al., 2011a)
Coke Oven	N.A.	23±5	78±4	87±9	(Chiu et al., 2011)
Blast Furnace	120-160	22.11	N.A.	N.A.	(Gielen, 2003)

Table 5 Temperature and flue gas tolerance of various algal species (Kumar et al., 2011)

Algal Species	**Max Temperature Tolerance (^{O}C)**	**Max CO_2 Tolerance (%)**	**Max NO_x Tolerance (ppm)**	**Max SO_x Tolerance (ppm)**
Cyanidium caldarium	60	100	-	-
Scenedesmus sp.	30	80	-	-
Chlorella sp.	45	40	-	-
Monoraphidium minutum	25	13.6	150	200
Tetraselmis sp.	-	14	125	185
Nonnochloris sp.	25	15	100	-

The advantages of using microalgal-based CO_2 fixation system are as follows:

I. High purity CO_2 gas is not required so that flue gas can be directly introduced into the microalgal cultivation systems, and the microalgae can transform the CO_2 in the flue gas into microalgal biomass. Therefore, the cost of CO_2 separation and purification from flue gas in CCS can be eliminated (Li et al., 2011a; Doucha et al., 2005).

II. In addition to CO_2, microalgae can use other combustion products, such as NO_x or SO_x, as nutrients for growth (Hauck et al.,1996). It has been reported that direct blowing of flue gas will not negatively affect the growth of the two selected microalgae compared with the group that is supplied with pure CO_2 (Negoro et al., 1993).

III. Although the percentages vary with the type of algae, all algae contain proteins, carbohydrates, lipids and nucleic acids that could be extracted and converted into high valuable commercial products which can offset the capital and the running costs of microalgal cultivation operations. Human food, animal feed mainly for aquaculture, cosmetics, medical drugs, fertilizers, biomolecules for specific applications and biofuels can be produced from microalgae (Pires et al., 2012). Regarding biofuel production, microalgae has many advantages over other conventional energy crops including high lipid content when compared with the conventional feedstocks (Table 6), and less land and water requirement.

Table 6 Oil yield of biodiesel sources (Chisti, 2007)

Biodiesel Source	**Yield of oil (L $ha^{-1}yr^{-1}$)**
Corn	172
Soyabean	446
Canola	1,190
Jatropha	1,892
Coconut	2,689
Oil palm	5,950
Microalgae (30% oil by weight)	58,700

IV. Industrial and domestic wastewater can be used in microalgae cultivation to provide nutrients. It is important to note that combination of microalgae cultivation with wastewater treatment will significantly enhance the

environmental benefits of microalgal CO_2 bio-mitigation strategy (Wang et al., 2008).

V. For regular processing industry, using flue gas as a carbon source can greatly reduce the algal system cost since CO_2 cost plays a crucial role in overall economics (Kadam, 1997; Mata et al., 2010).

2.4.2. Flue Gases for Microalgae Cultivation

Iron and steel factories have many processes which have different flue gas compositions and their flue gases may have high concentrations of CO, PM, NO_x and SO_x depending on the process. Therefore, it is required to add NO_x, SO_2 and dust removal systems before injecting the flue gas to the microalgae cultivation systems.

Conversely, flue gas from natural gas fired power plants has no high SO_x and PM emissions that can affect algal growth negatively. Only NO_x emissions are needed to be considered in the design of full scale systems. New gas turbines have little NO_x and CO emissions and flue gas of these plants is promising to be used for microalgae cultivation. CO_2 concentrations in their flue gas is at about 2-4% which is found to be the optimal for algal growth in the previous studies (Chae et al., 2006; Taher et al., 2015). Therefore, flue gases from natural gas fired power plants are suitable in terms of their composition.

Beside the presence of impurities, high temperatures and low pressures of the flue gases are the main concerns limiting the usage of algal based CO_2 sequestration technologies. Flue gases whether from power plants or iron and steel mills are needed to be cooled and pressurized to provide suitable growth conditions for microalgae. Installing heat exchangers may be necessary in order to cool the water from the temperatures around 120 ^oC to the the 20-30 ^oC (Kumar et al., 2011). The waste heat can be used for drying the algal biomass (Zhu, 2010).

Based on these requirements, a simple microalgae cultivation system for CO_2 removal from a fossil fuel-fired combustion system is illustrated in Figure 7.

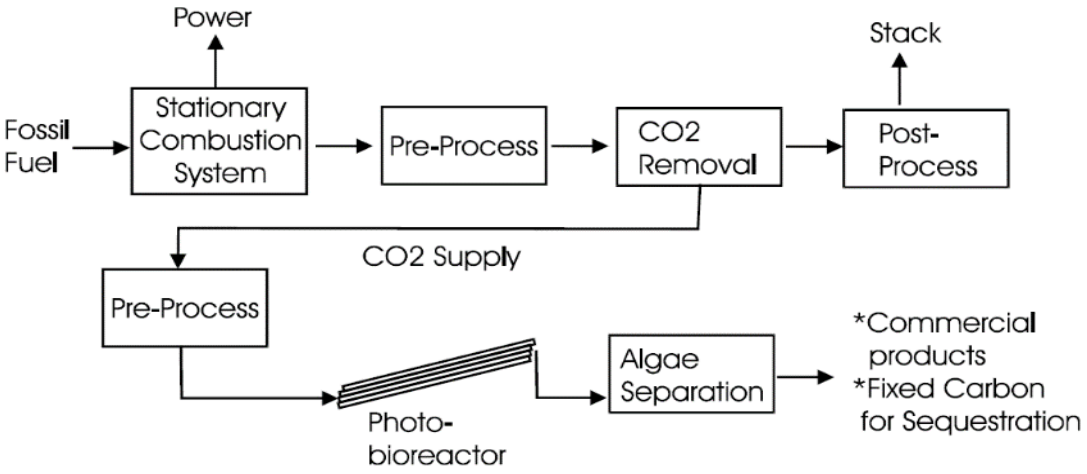


Figure 7 A proposed microalgae cultivation coupled with sequestration of CO_2 in flue gas of a stationary combustion system (Olaizola et al., 2004)

2.5. Studies on Wastewater Treatment and CO_2 Sequestration using Microalgae

In numerous previous batch studies, it has been demonstrated that microalgae have a great potential for the removal of nitrogen and phosphorus from both synthetic and real wastewater (Aslan and Kapdan, 2006; Ji et al., 2014; Ruiz-Marin et al., 2010). While batch studies are critical for determining specific growth parameters, steady-state (i.e. semi-continuous and continuous) studies are necessary to develop commercially viable microalgae-based production systems for biofuel production integrating with wastewater treatment and CO_2 sequestration. (Tang et al., 2012).

Hydraulic Retention Time (HRT) is an important process parameter that affects both biomass growth and nutrient removal. While short HRT values result in washout of biomass, long HRT values allow slow algae growth due to nutrient limitation and increased internal shading (Larsdotter, 2006). HRT depends on the type of wastewater and therefore it should be pre-investigated before the implementation of a larger scale application (Wang et al., 2010). Although, some researchers have investigated semi-continuous cultivation of different monocultures of microalgae, this type of systems have not been well-studied for mixed microalgae cultures and there is a lack of scientific data

on the effect of HRT on biomass growth and productivity as well as nutrient removal efficiencies (Li et al., 2013; Woertz et al., 2009). Particularly, studies on nutrient removal from unsterilized municipal and industrial wastewaters by mixed microalgae cultures are very limited. Besides, available studies have evaluated only the quality of the final effluent, a few have utilized semi-continuous mixed microalgae cultures for integrated nutrient removal and CO_2 sequestration. The relevant research studies have investigated CO_2 fixation abilities of microalgae however most of them have utilized monocultures, batch mode operation and artificial wastewater as the cultivation medium (de Morais et al., 2007; Gómez-Villa et al., 2005; Jiang et al., 2013; Ramaraj et al. 2015). This study, unlike other relevant studies, aims to present the effects of hydraulic retention time and N:P ratio on the growth, nutrient removal and CO_2 fixation of mixed microalgae culture grown in different types of wastewater.

Several pilot-scale demonstration projects utilizing flue gas and/or wastewater have also been implemented. In some of the previous pilot-scale operations, flue gases from coal and natural gas fired power plants and from industrial facilities such as coke oven, aluminium and cement factory have been introduced into open ponds and photobioreactors of different configurations (Chae et al., 2006; Kumar et al., 2010; Pires et al., 2012; White et al., 2015). In the pilot demonstrations, different types of wastewaters including municipal wastewater, dairy and fish farm wastewater and anaerobic digestates have been used as the cultivation medium (White et al., 2015).

In Appendix J, a summary of lab-scale and pilot-scale research performed in various photobioreactor configurations using different microalgae species is given.

CHAPTER 3

MATERIALS AND METHODS

The following sections cover the analytical methods, inocula used and experimental sets and procedures followed throughout this study in which the experimental design can be divided into two main groups:

(1) Microalgal biomass production and nutrient removal studies, which aim investigation of the growth of microalgae in batch and semi-continuous PBRs in the primary treated domestic wastewater under ambient air supply;

(2) Microalgal biomass production, nutrient removal and CO_2 sequestration studies, which aim determination of biomass production, nutrient removal and CO_2 sequestration potential of the microalgae grown in batch and semi-continuous PBRs using the mixtures of industrial and domestic wastewaters under sparging with CO_2-enriched air.

Experimental sets and procedures followed in this study are tabulated in Table 7. The details of the experiments are given in detail in Section 3.6.

Table 7 Experimental summary table

Experiment	Aim	Inoculum	Cultivation Media	CO_2 Source	Reactors	Section
Batch Study	To determine suitable inoculum source (R3 PBR vs. R4 PBR) and inoculum volume (50 mL vs. 100 mL)	Mixed cultures grown in Basal Medium **(R3 and R4 microalgae cultivation PBRs)**	Primary Settling Tank Effluent	Ambient Air	1-L Test PBRs named as B3-50, B3-100, B4-50 and B4-100	3.6.1.1.

Table 7 (continued)

Semi-Continuous Study	To determine optimum HRT for the treatment of primary treated domestic wastewater	Mixed culture acclimated to the primary settling tank effluent in the Batch Study (Section 3.6.1.1) **(B4-100 PBR)**	Primary Settling Tank Effluent	Ambient Air	1-L Test PBRs named as Y1, Y2 and Y3 with HRTs of 2, 4 and 8 days, respectively.	3.6.1.2.
Kinetic Study	To determine growth and nutrient removal kinetic parameters of the mixed microalgae culture	The last withdrawal made during the operation of semi-continuous PBR with 2-day HRT (Section 3.6.1.2) **(Y1 PBR)**	Primary Settling Tank Effluent	Ambient Air	1-L Test PBR named as YB-1	3.6.1.3.
Batch Study	To investigate the effects of ($TAN:PO_4$) (g/g) ratio on microalgae growth, nutrient removal and CO_2 fixation efficiencies	Mixed culture grown in Basal Medium **(R4 microalgae cultivation PBR)**	Coke Plant wastewater diluted with sludge thickener supernatant	%4 CO_2 Enriched air	1-L Test PBRs named as DB6, DB8 and DB10 for TAN/PO_4 ratios of 6, 8 and 10, respectively.	3.6.2.1.
Semi-Continuous Study	To determine optimum HRT for efficient nutrient removal from diluted coke plant wastewater and CO_2 sequestration	Mixed culture acclimated to the mixture of Coke Plant Wastewater and Thickener Supernatant in the Batch Study (Section 3.6.2.1) **(DB6 PBR)**	Coke Plant wastewater diluted with sludge thickener supernatant with a TAN/PO_4 ratio of 6	%4 CO_2 Enriched air	1-L Test PBRs named as D5, D8 and D12 with HRTs of 5, 8 and 12, respectively.	3.6.2.2.

3.1. Inocula

3.1.1. Microalgae

The algal culture was collected from Araç Creek in the vicinity of Karabük University Campus in Karabük Province. Araç Creek has a flowrate of 18.7 m^3/sec and a total length of 150 km and, it is one of the main surface water resources of Karabük Province together with Soğanlı Creek. These two creeks merge into Yenice River within the borders of Karabük Province (Figure 8).

In Karabük Province, domestic wastewaters from all the district and town municipalities are discharged into Soğanlı and Araç Creeks except the central district municipality and municipality of Safranbolu (Uncer et al., 2012). The main source of pollution in these creeks is untreated domestic wastewater discharges. Besides, some industrial facilities such as KARDEMIR Integrated Iron and Steel Factory and Karabük Small Organized Industrial District discharge their treated wastewaters to Soğanlı Creek.

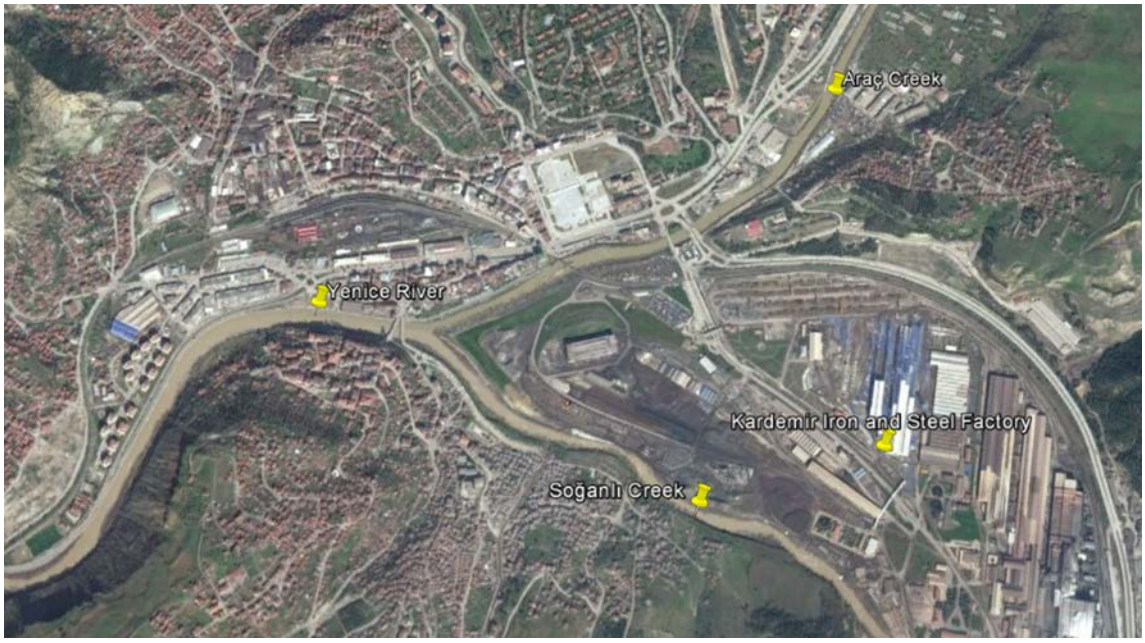


Figure 8 Aerial view of Araç Creek, Soğanlı Creek and KARDEMIR Integrated Iron and Steel Factory (Google Earth, 2017)

The analyses performed by using a microscope (Section 3.2.16) revealed that inoculum samples contained a mixture of green algae. Dominant microalgae in the inoculum samples was *Chroccoccus turgidius*. Besides, *Kirchneriella sp.* and *Cryptomanas sp*. were also observed. Photographs of microscopic analyses are given in Appendix D.

3.2. Analytical Methods

3.2.1. pH

pH values were measured for all the batch and semi-continuous photobioreactor experiments using a pH meter (Eutech, CyberScan pH510, Nijkerk, The Netherlands) and pH probe (Sensorex, p350, Garden Grove, CA, USA). The pH meter was always rinsed off distilled water before measuring. It was calibrated daily with pH 4, pH 7 and pH 11 solutions.

3.2.2. Temperature

Temperature measurements for the batch and semi-continuous photobioreactors were taken directly by using a submerged thermometer (Sensorex, p350, Garden Grove, CA, USA).

3.2.3. Light Intensity

Light intensity in the batch and semi-continuous cultures was measured using a light meter (Li-Cor, 250 A, Lincoln, Nebraska, USA) having a quantum sensor. The sensor was inserted in the reactors to determine the light intensity with the unit of μmol m^{-2} s^{-1}.

3.2.4. Optical Density

Optical density of any sample was measured using a spectrophotometer (HACH, DR 2800, Berlin, Germany) at 685 nm wavelength which was found to reveal the highest absorbance value within the range of 450 to 800 nm wavelength range as shown in Figure 9 (Calicioglu, 2013). This wavelength has been also used by several other researchers (He et al., 2013; Ho et al., 2010; Li et al., 2011a).

Since absorbance values below 0.1 and above 1 are not reliable, each sample was diluted to give an absorbance in the range of 0.1 and 1.0. Macro-cuvettes 10-mm path length with a sample holding capacity of 4 mL was used to hold the samples.

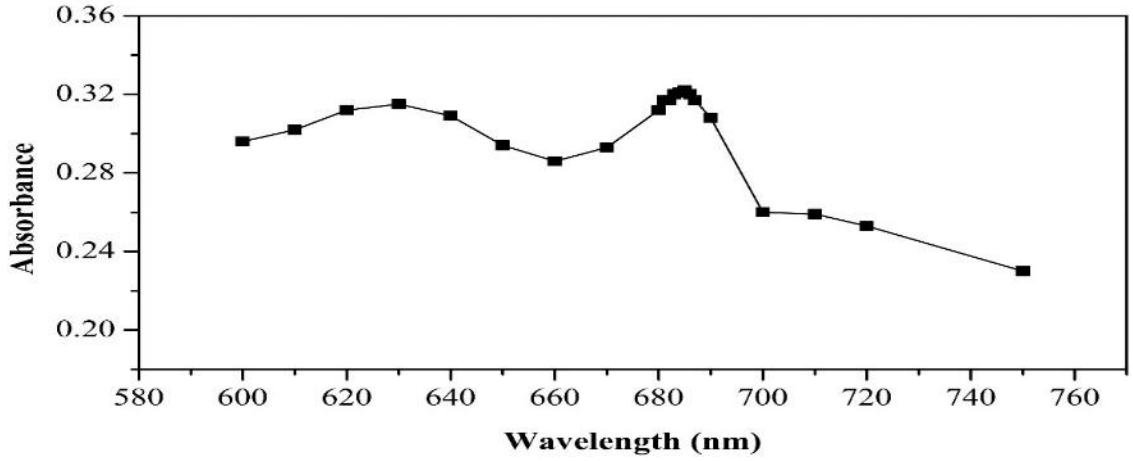


Figure 9 Wavelength vs. Absorbance curve for *Chlorella vulgaris* (Calicioglu, 2013)

The O.D. measurement is a quick method to monitor algal growth. However, dead and alive cells cannot be distinguished in the O.D. measurements and cellular conglomerates can cause faulty readings (Brooker, 2011).

3.2.5. Solids

TS, VS, TSS and VSS values were determined according to Standard Methods 2540 (APHA et al., 2005) by using a 5 mL algae suspension from the batch and semi-continuous reactors. The suspended fraction of any sample was obtained by membrane filtration with 0.45 mm size glass fiber filters (Millipore, AP40, Billerica, MA, USA) using a vacuum filtration unit (Millipore, WP8 11 2250, MA, USA).

3.2.6. Total Chemical Oxygen Demand

Standard Method 5220 B (APHA et al., 2005) was used to determine tCOD concentration in the wastewater samples.

3.2.7. Soluble Chemical Oxygen Demand

The soluble chemical oxygen demand (sCOD) determinations were carried out according to Calicioglu (2013). First, the samples were filtered with 0.45 µm cellulose acetate membrane filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250, Billerica, MA, USA). Then, sCOD of the samples were determined by Micro-COD method by using medium-range (0 – 1500 mg/L COD) and low-range (0-150 mg/L COD) test kit vials (Lovibond, Aqualytic, Dortmund, Germany). Finally, the vials were heated up to the temperature of 150^{o} C, digested for 120 minutes and cooled down to room temperature before sCOD value detection using a spectrophotometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany).

3.2.8. TKN and Organic Nitrogen

TKN concentrations in the domestic and industrial wastewaters were determined by applying Standard Method (APHA et al., 2005) Macro Kjeldahl Method 4500-N_{org}. The content of organic-N was determined by subtracting the TAN (NH_4^+-N + NH_3-N) from the TKN values.

3.2.9. Total Soluble Nitrogen

TN measurements were made by first filtrating the samples with 0.45 µm cellulose acetate membrane filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250, Billerica, MA, USA).

Soluble TN values were then determined using test kit vials (Lovibond, Vario 535560, Aqualytic, Dortmund, Germany). Finally, soluble TN values were detected using a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany).

3.2.10. Total Ammonia Nitrogen

TAN (NH_4^+-N + NH_3-N) measurements were made by first filtrating the samples with 0.45 µm cellulose acetate membrane filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250, Billerica, MA, USA).

TAN concentrations were then determined using test kit vials (Lovibond, Vario 535600, Aqualytic, Dortmund, Germany). Finally, TAN values were detected using a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany).

3.2.11. Nitrate-N

Nitrate (NO_3^--N) measurements were made by first filtrating the samples with 0.45 µm cellulose acetate membrane filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250, Billerica, MA, USA).

NO_3^- -N concentrations were then determined using test kit vials (Lovibond Vario NitraX 535580, Aqualytic, Dortmund, Germany). Finally, soluble NO_3^- -N concentrations in the samples were detected using a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany).

3.2.12. Nitrite-N

Nitrite (NO_2^--N) measurements were made by first filtrating the samples with 0.45 µm cellulose acetate membrane filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250, Billerica, MA, USA).

NO_2^--N concentrations were then determined using test kit vials (Lovibond Vario Nitri 3 530980, Aqualytic, Dortmund, Germany). Finally, soluble NO_2^--N concentrations in the samples were detected using a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany).

3.2.13. Orthophosphate

Orthophosphate (PO_4^{3-}-P) measurements were made by first filtrating the samples with 0.45 µm cellulose acetate membrane filter (Sartorius Stedim, 1110647-N, Goettingen, Germany) using a filtration unit (Millipore, WP8 11 2250, Billerica, MA, USA).

PO_4^{3-}-P values were then determined using Lovibond phosphorus tablet pack (Lovibond, Vario 515810, Aqualytic, Dortmund, Germany). Finally, PO_4^{3-}-P concentrations in the

samples were detected using a photometer (PC Multinet Autoset photometer, Aqualytic, Dortmund, Germany).

3.2.14. Chlorophyll-a

Chlorophyll-a measurements were done according to the Standard Method 10200H (APHA et al., 2005). A known volume of algae samples was poured in plastic tubes and centrifuged at 5000 rpm for 20 min. The pellets were re-suspended and extracted with 90% v/v acetone during 24 h at 4 ^{o}C and in darkness. $MgCO_3$ was used to prevent degradation of chlorophyll during the measurements. Chlorophyll-a concentration in the extract was estimated by measuring absorbance before and after acidification using the spectrophotometric Equation 3:

$$Chlorophyll\ a, (\frac{mg}{L}) = \frac{26.7\ (E_{664} - E_{665}) \times V_1}{V_2 \times L \times 1000} \quad \text{(Equation 3)}$$

Where:

E_{664} = (Optical density of filtrate at 664 nm) – (Optical density of filtrate at 750 nm)

E_{665} = (Optical density of acidified filtrate at 665 nm) – (Optical density of acidified filtrate at 750 nm)

V_1 = volume of extract in L

V_2 = volume of sample filtered in m^3

L = light path length or width of cuvette in cm

3.2.15. Elemental Analysis

Minimum 10 mg dry microalgae biomass was necessary for the elemental analysis. The cultures were put in plastic tubes and centrifuged for 5 min, 5000 rpm, washed with distilled water and dried at 80 ^{o}C. In order to determine C, H and N weight percentages, LECO Elemental Analyzer, CHNS-932 was used in order to determine weight percentages of C, H and N elements. The results of the CHN analyses were given in Appendix F.

3.2.16. Visual Algae Identification and Imaging

Algae inolcula was collected from Araç Creek in Karabük. Visual identification and imaging of microalgae species were performed by using a Lecia DM 2500 microscope. An aliquot of microbial from the reactors was placed on a slide glass and covered with a cover slip (Choi et al., 2010).

3.2.17. CO_2 Analysis

Evaluation of CO_2 removal was conducted by taking samples from gas inlet and outlet of the semi-continuously operated bubble-column reactors during steady-state growth period and then measuring gaseous CO_2 concentrations by using a Gas Chromatography (GC). Air samples from the air inlet and outlet of the reactors were collected into 1-L plastic bags. Then, a sample volume of 10 µL was taken from the bags and injected to the GC by using a 1-mL gastight manual injection syringe (Reno, Nevada, USA). The Agilent 6890N, CA, USA Gas Chromatography (GC) equipped with a thermal conductivity detector and capillary column CP-Sil 8 (CP8752, Varian) was used to analyze gaseous CO_2 concentration. Temperature of the injector and detector and oven were 50 ^oC, 80 ^oC and 35 ^oC, respectively. Helium was used as the carrier gas and controlled at a temperature of and at a pressure of 100 Pa.

A calibration curve was prepared by injecting different CO_2 concentrations and corresponding area readings. Four standardized CO_2 solutions (100, 200, 400 and 800 ppm CO_2) were used. The actual inlet and outlet CO_2 concentrations were calculated using the area obtained by chromatogram and comparing the area with the calibration curve. Calibration curve for the CO_2 analysis can be found in Appendix C.

3.3. Preparation of the Acid and Base Solutions

During the semi-continuous studies, pH of the cultures was adjusted with diluted 5 N H_2SO_4, 1 M HCl and 1 M NaOH aqueous solutions. Preparation procedures are given below:

- A 1 L of 5 N sulphuric acid (H_2SO_4) solution was prepared by using 135.85 mL 98% sulphuric acid stock solution and 864.15 distilled water.
- In order to prepare a 0.5 L of 1 M hydrochloric acid (HCI) solution, 41.75 mL of 37% hydrochloric acid stock solution and 458.25 mL distilled water were used.
- In order to get a 0.5 L 1 M sodium hydroxide (NaOH) solution, 20 g of sodium hydroxide was added to enough distilled water and the final volume of the solution was made 0.5 L.

3.4. Characterization of Cultivation Medium and Wastewaters

3.4.1. Cultivation Medium

Mixed culture collected from Araç Creek, Karabük, has been cultivated with Bold's Basal medium enhanced with 3-Fold Nitrogen and Vitamins (3N-BBM+V) as reported by Bilanovic et al. (2009). The formulation is given in Table 8.

Table 8 3N-BBM + V constituents (Bilanovic et al., 2009)

Constituents	Concentration (g/L)
$NaNO_3$	0.750
$CaCl_2.2H_2O$	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
$FeCl_3.6H_2O$	$58.4x10^{-5}$
$MnCl_2.4H_2O$	$24.6x10^{-5}$
$ZnCl_2$	$3x10^{-5}$
$CoCl_2.6H_2O$	$1.2x10^{-5}$
$Na_2MoO_4.2H_2O$	$2.4x10^{-5}$
Vitamin B1	$12x10^{-5}$
Vitamin B12	$1.2x10^{-5}$

3.4.2. Wastewaters Obtained from Ankara Tatlar WWTP Cultivation Medium

Two different types of wastewaters were obtained from Tatlar Wastewater Treatment Plant (WWTP), located in Ankara, Turkey for this study. These wastewaters were supernatants of the primary sedimentation tanks and sludge thickening tanks. A simplified scheme of the WWTP and sampling points are shown in Figure 10.

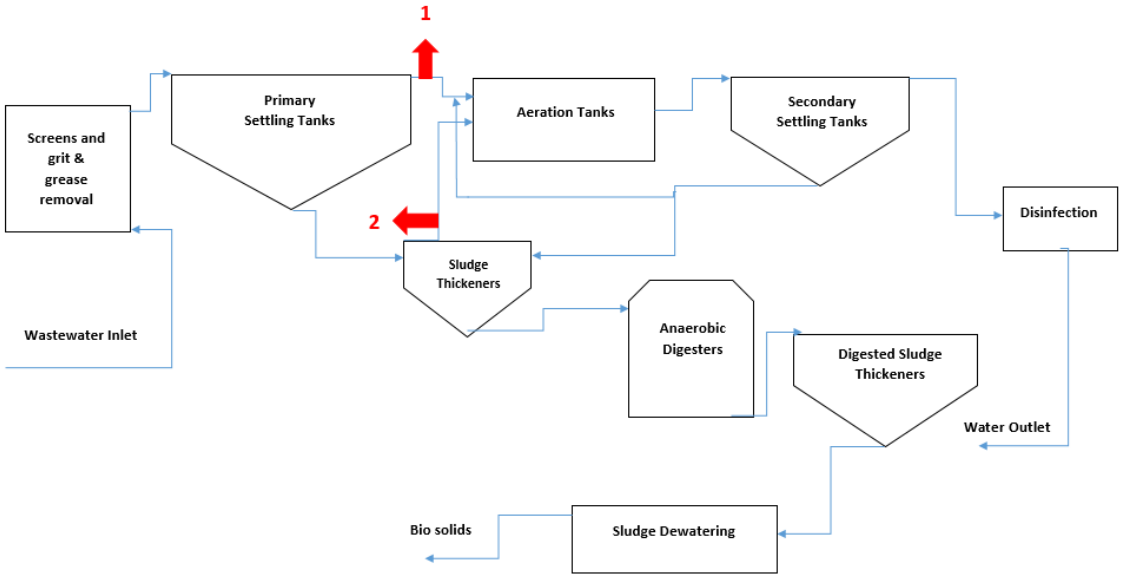


Figure 10 Simplified scheme of Ankara Tatlar WWTP and wastewaters used in experiments (1-primary settling tank supernatant; 2-sludge thickener tank supernatant) (Ankara Water and Sewerage Authority, 2017)

3.4.2.1. Supernatant of Primary Settling Tanks

Supernatant of the primary settling tanks was used in the batch and semi-continuous studies conducted to observe nutrient removal from domestic wastewaters by microalgae culture.

Collected wastewater was passed through a sieve with pore size of 0.3 mm in order to remove larger particles and was not sterilized. The wastewater was stored in a refrigerator at 4°C and analyzed twice a week to monitor its nutrient and sCOD concentrations. The characterization of the supernatant effluent of primary settling tank is given in Table 9.

Table 9 Characteristics of primary settling tank supernatant

Parameter	Value
Optical Density @ 685 nm	0.09 ± 0
TS (mg/L)	413 ± 17

Table 9 (continued)

VS (mg/L)	269 ± 17
TS (%VS)	65
pH	7.95
tCOD (mg/L)	254 ± 2.5
sCOD (mg/L)	78.5 ± 0.3
TN (mg/L)	42.1 ± 2.1
TKN (mg/L)	42 ± 5.9
Organic-N (mg/L)	11.5
TAN (mg/L)	30.5 ± 1.2
NO_3-N (mg/L)	< 1
NO_2-N (mg/L)	< 0.01
PO_4^{3-}-P (mg/L)	4.9 ± 0.3

3.4.2.2. Supernatant of Sludge Thickening Tanks

Supernatant of the sludge thickening tanks was used to dilute the industrial wastewater which had a high ammonia concentration but low levels of phosphate. Collected thickener wastewater was passed through a sieve with pore size of 0.3 mm in order to remove larger particles prior to storage at 4°C at dark.

As shown in Table 10, supernatant waters of thickening tanks had a high PO_4^{3-}-P content which was essential for the support of the microalgal growth during the studies conducted with industrial wastewater. It should be noted that the use of high phosphate containing wastewater for this purpose is particularly important when side-stream wastewaters of the sludge treatment facilities are considered (Wang et al., 2013).

Table 10 Characteristics of sludge thickener supernatant

Parameter	Value
TS (mg/L)	880 ± 42
TVS (mg/L)	488 ± 20
TVS (%TS)	55
tCOD (mg/L)	587 ± 3.6
sCOD (mg/L)	328 ± 6.2
TN (mg/L)	47.2 ± 1.5
TAN (mg/L)	41.7 ± 2.1
NO_3-N (mg/L)	< 0.1
NO_2-N (mg/L)	< 0.01
Organic-N (mg/L)	5.48
PO_4^{3-}-P (mg/L)	19.9 ± 0.2

3.4.3. Industrial Wastewater

Industrial wastewater used in the experiments was obtained from the junction point which connects wastewater channel of KARDEMIR Coke Plant to the main channel collecting different types wastewaters from different units in the factory. The production flow chart of the factory is given in Figure 11.

The coke plant wastewater was passed through a sieve with pore size of 0.3 mm and stored at 4 ^oC in a refrigerator as well. No sterilization has been carried out for the industrial wastewater.

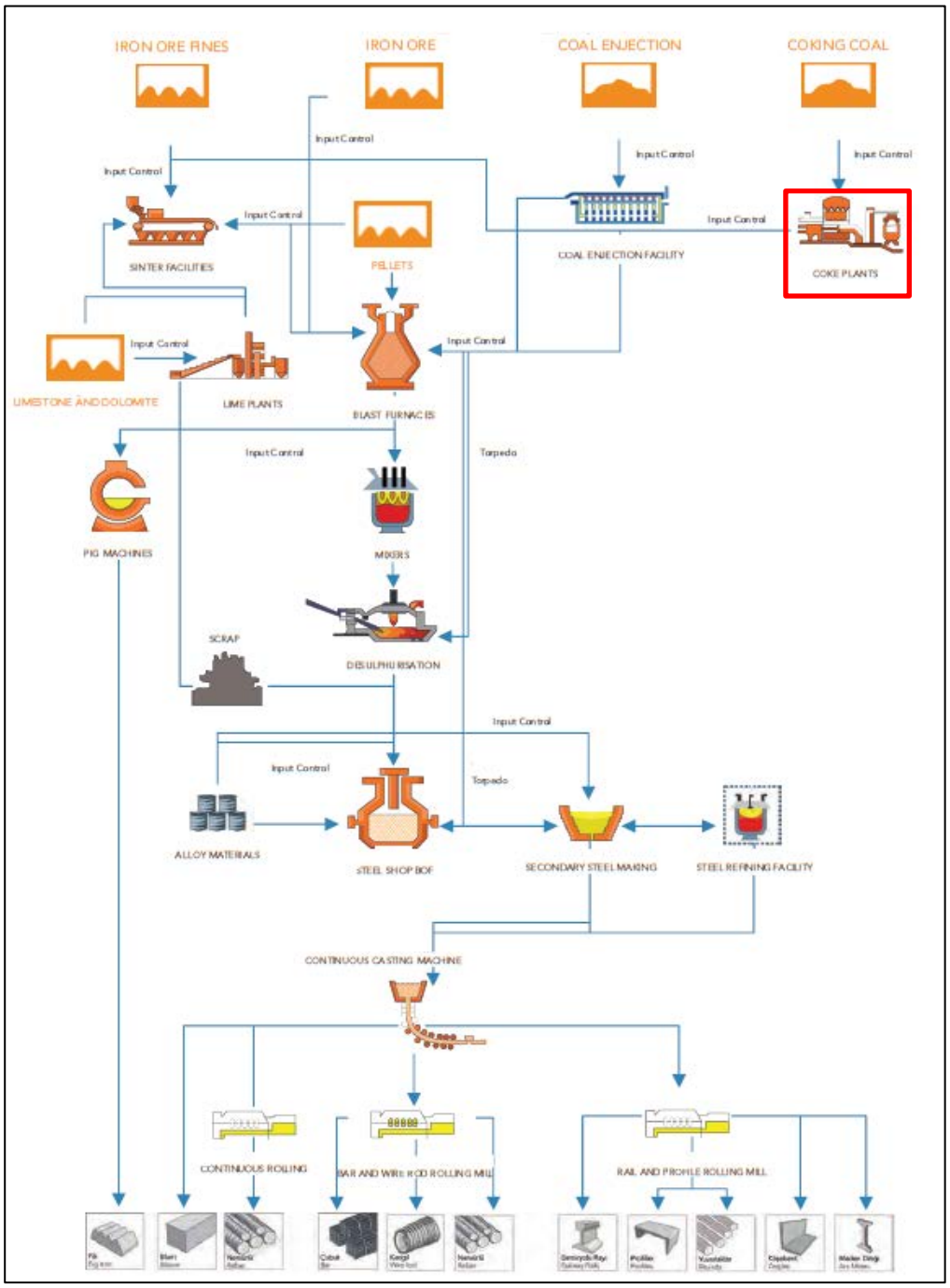


Figure 11 Production flow chart of KARDEMIR Integrated Iron and Steel Factory (Kardemir Product Catalog, 2016)

The characterization of the wastewater is given in Table 11. Sulfate, phenol and heavy metal analyses were conducted by a commercial accredited laboratory. Related standards methods, namely SM-4110 B, SM-4500-CN A and E, SM-5530 A, B and C, were used for the analysis of sulfate, cyanide and phenol in the wastewater. EPA 200.7 method was used for the determination of other constituents (heavy metals). The analysis results and the methods are also given in Appendix E.

Table 11 Characteristics of the coke plant wastewater

Parameters	**Value**
TS (mg/L)	8471 ± 311
TVS (mg/L)	136 ± 4
TS (%TVS)	2
Chlorophyll-a (mg/L)	N.D.
tCOD (mg/L)	11,827 ± 150
sCOD (mg/L)	10,225 ± 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
PO_4^{3-}-P (mg/L)	1 ± 0.1
Sulphate (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
Total Chrome (µg/L)	<1.8

As it can be seen from Table 11, industrial wastewater had very high TAN concentration but very low PO_4^{3-}-P concentration. Moreover, it contained high levels of heavy metals and phenols, which might have affected biological activity of microalgae negatively. Therefore, it was a good opportunity to investigate whether there were any negative effects of these pollutants on microalgae growth.

3.5. Design of Photobioreactors

Two different photobioreactors (PBR) were used during the experiments: microalgae cultivation PBRs and test PBRs. 3-L reactors were used to cultivate mixed microalgae culture, which was collected from Araç Creek in Karabük (Turkey), in the Bold's Basal Medium (3N-BBM+V). On the other hand, glass gas wash bottles having a 1-L working volume were used in the batch studies, kinetic studies and semi-continuous studies.

3.5.1. Microalgae Cultivation Photobioreactors

Two identical 3-L glass reactors, namely R3 and R4, were used for the cultivation of mixed microalgae culture (Figure 12). The reactors were made by 0.3 cm thick glass. They had an internal diameter of 9 cm, and a total height of 40 cm. The cultivation photobioreactors were capped with a glass cap allowing gas entrance and exit. An aeration tube with inner diameter of 0.5 cm submerged into the reactors was connected to an air pump (RESUN AC-9602, China). Gas inlet and outlet of the reactors were equipped with 0.2 µm syringe filters (Hydrofobic Minisart Syringe Filter) to prevent contamination from the outer laboratory environment. The reactors were operated semi-continuously with 10 days of HRT. In other words, in 10-days cycles, 10 percent volume of the mixed microalgae culture from the reactors were withdrawn and same volume of Bold's Basal Medium (3N-BBM+V) stored at 4 ^{o}C was added to the reactors. The temperature was maintained at 30±2˚C and continuous illumination at 120 µmol m^{-2} s^{-1} using T8 fluorescent tubes (OSRAM L18/840) was provided to the PBRs.

Figure 12 3-L Microalgae cultivation PBRs (R3 and R4)

3.5.2. Test Photobioreactors

Test Photobioreactors which were used for the batch, kinetic and semi-continuous experiments had a diameter of 8 cm, a height of 24 cm and a working volume of 1-L (Figure 13). As in the microalgae cultivation photobioreactors, aeration and mixing was provided with the air pumps (RESUN AC-9602, China). The reactors were operated under continuous illumination at 120 μmol m^{-2} s^{-1} using T8 fluorescent tubes (OSRAM L18/840) as well.

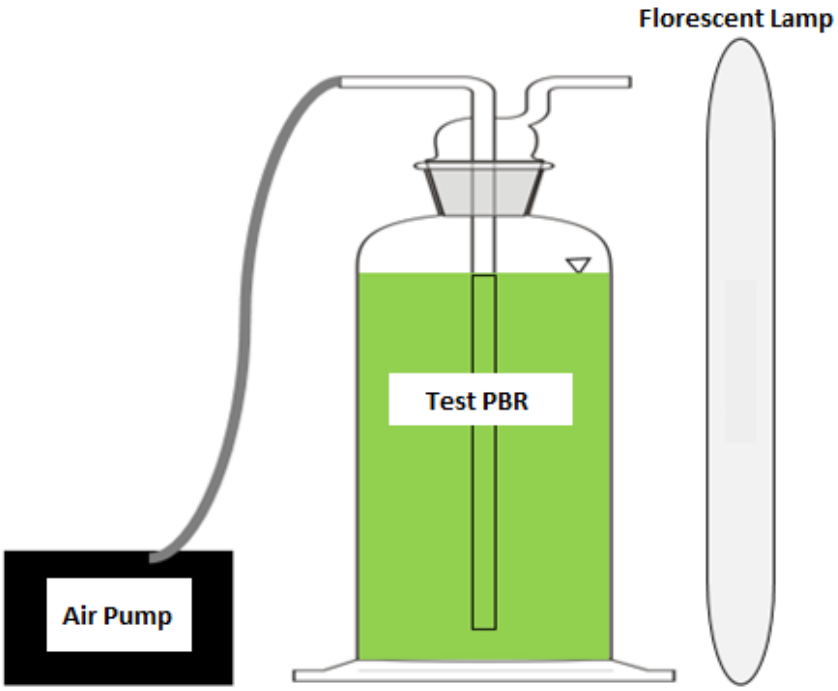


Figure 13 Test PBRs

3.5.3. Control Reactor

A 1-L control photobioreactor was operated for 2 days to examine the abiotic removal of CO_2. To this aim, the control reactor was not inoculated with microalgae and only filled with diluted coke plant wastewater. The reactor was operated semi-continuously with a HRT of 12 days.

The control reactor had the same diameter and height as the test photobioreactors. Aeration and mixing was provided with the air pumps (RESUN AC-9602, China). The reactor was operated under continuous illumination at 120 μmol m^{-2} s^{-1} using T8 fluorescent tubes (OSRAM L18/840) and aeration with 4% CO_2 at 0.5 vvm.

3.6. Experimental Sets and Procedures

3.6.1. Studies with Primary Settling Tank Effluent

3.6.1.1. Batch Study

A batch study was conducted in order to determine the most suitable cultivation reactor to be used as inoculum source and inoculum volume for the semi-continuous study which has been performed with primary treated domestic wastewater (Section 3.6.1.2). This batch study was also essential for the adaptation of mixed microalgae culture to the primary treated domestic wastewater since the mixed cultures have been cultivated using a synthetic medium (i.e. Bold's Basal Medium) in two separate semi-continuously operated cultivation reactors, namely R3 and R4 (Section 3.5.1).

Batch reactors were inoculated with 50 mL and 100 mL microalgae from R3 and R4 microalgae cultivation reactors. Then, 950 mL and 900 mL primary settling tank effluent was added to achieve a microalgae-wastewater mixture volume of 1 L. Corresponding names of the reactors were determined as B3-50, B3-100, B4-50 and B4-100. Summary of the batch experiments conducted using the inoculums from cultivation reactors and domestic wastewater is given in Table 12.

Table 12 Components of the batch reactors

Reactor	Inoculum source	Inoculum volume (mL)	Wastewater volume (mL)	Total working volume (mL)
B3-50	R3	50	950	1000
B3-100	R3	100	900	1000
B4-50	R4	50	950	1000
B4-100	R4	100	900	1000

The reactors were operated for 4 days at 0.5 vvm air and 30±2˚C with 120 μmol m^{-2} s^{-1} continuous illumination using T8 fluorescent tubes (OSRAM L18/840). 50 mL sample was collected daily from the reactors in order to measure the O.D., TAN and PO_4^{3-}-P concentrations.

3.6.1.2. Semi-Continuous Study

The main goal of this semi-continuous experiment was to determine optimum HRT value for the treatment of non-sterilized primary effluent from Ankara Tatlar WWTP by measuring and comparing algal growth (biomass concentration) and nutrient consumption (nutrient removal efficiencies) in the PBRs during steady-growth conditions. In order to determine optimum HRT, three semi-continuous reactors with 2 (Y1 PBR), 4 (Y2 PBR) and 8 (Y3 PBR) days of HRT were operated. These values were chosen based on the reported HRT values in the literature for microalgal municipal wastewater treatment (Garcia et al., 2000; Larsdotter, 2006; Woertz et al., 2009). The microalgae growth and nutrient removal performance of the reactors were investigated.

The output of B4-100 PRB that was adapted to the primary domestic wastewater during the previous batch study (Section 3.6.1.1) was used as inoculum. Algae were inoculated at 10% ($v_{inolculation}/v_{total}$), was determined as the optimum inoculum volume/reactor volume in the batch study (Section 3.6.1.1), in 1-L bubble column reactors containing 900 mL primary treated domestic wastewater. In 24 hour cycles a predetermined volume of the mixed liquor from reactors were withdrawn and same volume of domestic wastewater stored at 4 ^{o}C was added to the reactors. For example, for the reactors with 4 days of HRT, the daily replaced volume was 250 mL. Table 13 summarizes the experimental procedures followed.

Table 13 Summary of the semi-continuous operation with primary treated domestic wastewater

Reactor	Inoculum source	Inoculum volume (mL)	Wastewater volume (mL)	Total working volume (mL)	HRT (days)	Daily replaced volume (mL)
Y1	B4-100	100	900	1000	2	500
Y2	B4-100	100	900	1000	4	250
Y3	B4-100	100	900	1000	8	125

Optical density, TN, TAN, PO_4^{3-}-P, sCOD and Chlorophyll-a analyses were conducted on the mixed liquor withdrawn from the reactors. pH of each reactor was adjusted to 6 with diluted H_2SO_4, HCI and NaOH solutions daily, after the reactors were feed with domestic wastewater. The reactors were operated at 30±2˚C under 120 µmol m^{-2} s^{-1} for 24 hours a day and aerated with ambient air at 0.5 vvm (Figure 14).

Figure 14 A photo from the experimental setup of the semi-continuous study with primary treated domestic wastewater

3.6.1.3. Kinetic Study

In order to investigate the growth and nutrient removal kinetics of microalgae, a 72-hour kinetic study was conducted with mixed microalgae culture that was acclimated to domestic wastewater during the batch (Section 3.6.1.1) and semi-continuous (Section 3.6.1.2) studies. Algae inoculum used in this batch study was the last withdrawal made during the operation of Y1 PBR. Algae were inoculated at 10% ($v_{inolculation}/v_{total}$) in 1-L bubble column reactor containing 900 mL primary treated domestic wastewater as summarized in Table 14. The reactor was operated at 30±2˚C with 120 µmol m^{-2} s^{-1}

continuous illumination and 0.5 vvm aeration. 50-100 mL sample was collected periodically from the reactor in order to measure O.D., TAN and PO_4^{3-}-P concentrations.

Table 14 Initial conditions in the kinetic study conducted with the domestic wastewater

Reactor	Inoculum source	Inoculum volume (mL)	Wastewater volume (mL)	Total working volume (mL)
Kinetic Study Reactor	YB1	100	900	1000

3.6.2. Studies with Coke Plant Wastewater Diluted with Primary Thickener Supernatant

3.6.2.1. Batch Study

The effects of different N:P ratios on microalgae growth and biomass yield, nutrient removal and CO_2 fixation was studied in this part of batch study. With the aim of evaluating the effects of N:P ratio, mixed algal culture was grown with TAN:PO_4^{3-}-P ratios of 6, 8 and 10. In order to get these ratios, coke plant wastewater and sludge thickener supernatant were mixed at different volumes as shown in Table 15.

Table 15 Volume of domestic and industrial wastewater used to prepare different TAN: PO_4^{3-}-P Ratios

TAN/ PO_4^{3-}-P	Coke Plant Wastewater (mL)	Sludge Thickener Supernatant (mL)	Total working volume (mL)
6	23	977	1000
8	34	966	1000
10	45	955	1000

During cultivation, temperature was maintained at 28±2 ^oC and light intensity was supplied continuously at 120 µmol m^{-2} s^{-1}. All reactors were bubbled with %4 CO_2-

enriched air at an aeration rate of 0.5 L/min (vvm). 50-100 mL sample was collected periodically from the reactors for the analyses. A schematic diagram of the experimental setup is shown in Figure 15.

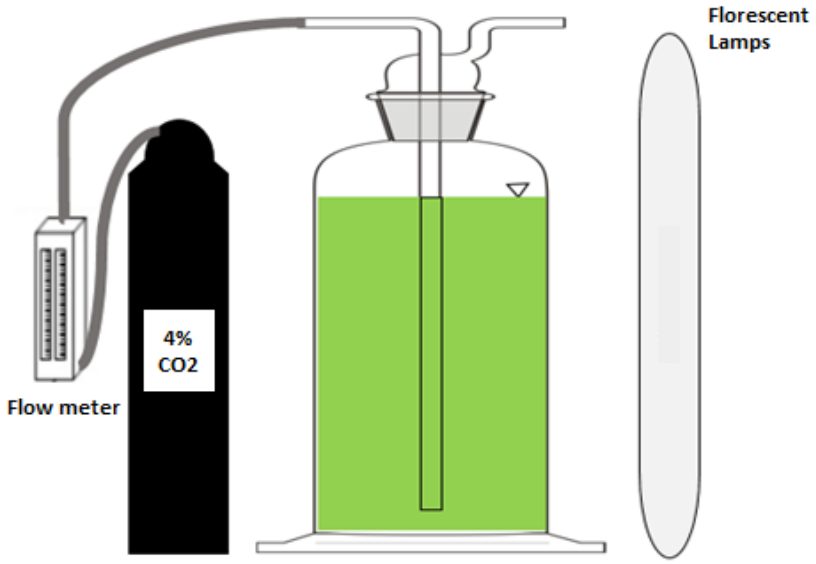


Figure 15 Schematic diagram of the batch study with diluted industrial wastewater

Table 16 provides the summary of the batch experiments with diluted industrial wastewater.

Table 16 Summary of the batch operation with diluted industrial wastewater

Reactor	Inoculum source	Inoculum volume (mL)	TAN / PO_4^{3-}-P ratio of the diluted wastewater	Diluted wastewater volume (mL)	Total working volume (mL)
DB6	R4	100	6	900	1000
DB8	R4	100	8	900	1000
DB10	R4	100	10	900	1000

3.6.2.2. Semi-Continuous Study

The objective of this study was to determine optimum HRT for nutrient removal coupled with CO_2 sequestration by using mixed microalgae culture. For this purpose, three PBRs were operated in semi-continuous mode by using KARDEMIR coke plant wastewater diluted with sludge thickener supernatant obtained from Ankara Tatlar WWTP with a TAN/PO_4^{3-}-P ratio of 6 (g/g). This ratio was determined as the optimum inlet TAN/PO_4^{3-}-P supporting microalgal growth and nutrient removal in the previous batch study (Section 3.6.2.1). The HRT of the PBRs were selected as 5, 8 and 12 days considering the HRT values reported by the researchers who have studied with nutrient-rich wastewaters such as dairy manures and mixtures of settled swine and sewage (Travieso et al. (2006); Wang et al., (2010)) The HRT of the PBRs was adjusted by varying the volume of wastewater added per day as previously described in Section 3.6.1.2 for the semi-continuous study with primary treated domestic wastewater. In 24 hour cycles, a predetermined volume of the mixed liquor from reactors were withdrawn and same volume of wastewater mixture was added to the reactors.

All PBRs were supplied with CO_2-enriched air (4%). To achieve 4% CO_2 concentration, 20% CO_2 enriched air was mixed with ambient air. Flow rates of the ambient air and 20% CO_2 enriched air entering a photobioreactor were regulated by two gas flow meters to achieve a total 0.5 vvm inlet airstream with 4% CO_2 concentration. Table 17 shows the experimental procedures followed during the semi-continuous operation using diluted industrial wastewater and 4% CO_2 enriched air.

Table 17 Summary of the semi-continuous operation with diluted industrial wastewater

Reactor	Inoculum source	Inoculum volume (mL)	Diluted wastewater volume (mL)	TAN/PO_4-P ratio of the diluted wastewater	Total working volume (mL)	HRT (days)	Daily replaced volume (mL)	CO_2 (%)
D5	DB6	100	900	6	1000	5	200	4
D8	DB6	100	900	6	1000	8	125	4
D12	DB6	100	900	6	1000	12	83.3	4

Optical density, TN, TAN, PO_4^{3-}-P, sCOD and Chlorophyll-a analyses were conducted on the mixed liquor withdrawn from the reactors. Mixed microalgae culture grown in DB6 PBR was used as inoculum at a ratio of 1:10 ($v_{inoclumum}/v_{total}$) for all PBRs which were operated at 28±2 ^{o}C with 120 µmol m^{-2} s^{-1} continuous illumination and aerated with 4% CO_2 enriched at a constant rate of 0.5 vvm. During steady-state conditions, CO_2 concentrations in inlet and outlet gas streams were conducted for each PBR. A schematic diagram and a photo of the experimental setup for the abovementioned semi-continuous study are shown in Figure 16.

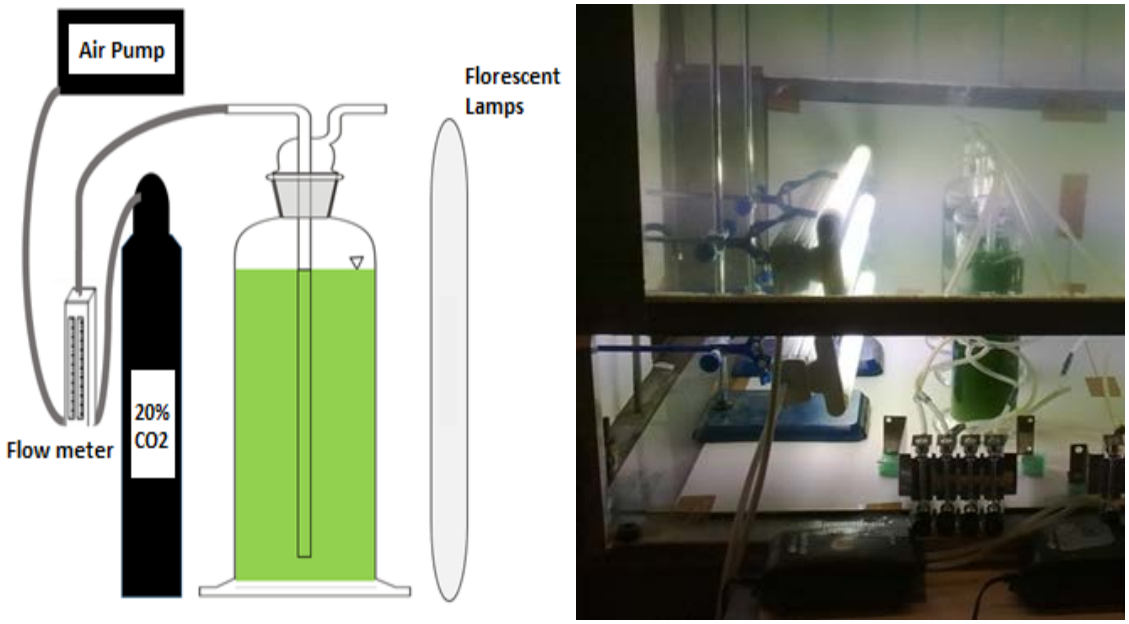


Figure 16 Schematic diagram and a photo of the experimental setup of the semi-continuous study performed with diluted industrial wastewater

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Microalgae Cultivation in Primary Treated Domestic Wastewater

4.1.1. Batch Operation

In this batch study, suitable inoculum source (microalgae cultivation PBRs; R3 vs. R4) and inoculum volume (5% (v/v) vs. 10% (v/v)) were investigated. This was necessary to achieve efficient microalgal growth and nutrient removal in the upcoming batch and semi-continuous experiments (Sections 4.1.2, 4.1.3, 4.2.1 and 4.2.2). As explained in the Section 3.6.1.1, 50 mL or 5% (v/v) and 100 mL or 10% (v/v) inoculums were transferred to four 1-L test PBRs (i.e. B3-50, B3-100, B4-50 and B4-100) from the microalgae cultivation reactors, namely R3 and R4. Remaining volume of the test PBRs were filled with primary treated domestic wastewater. The reactors were operated in batch mode for four days under continuous illumination (120 μmol m^{-2} s^{-1}) and aerated with ambient air (0.5 vvm). Microalgal growth and nutrient removal were followed by daily measurements of O.D. and nutrient (TAN and PO_4^{3-}-P) concentrations. The results of this batch study are given in Figure 17 and Figure 18.

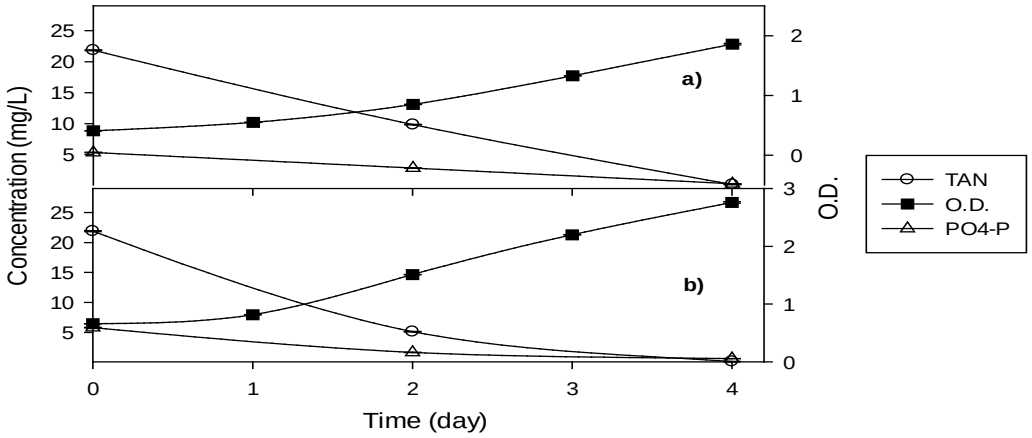


Figure 17 The change in Microalgal Biomass (O.D)., TAN and PO_4^{3-}-P concentrations in (a) B3-50 and (b) B4-50 PBRs

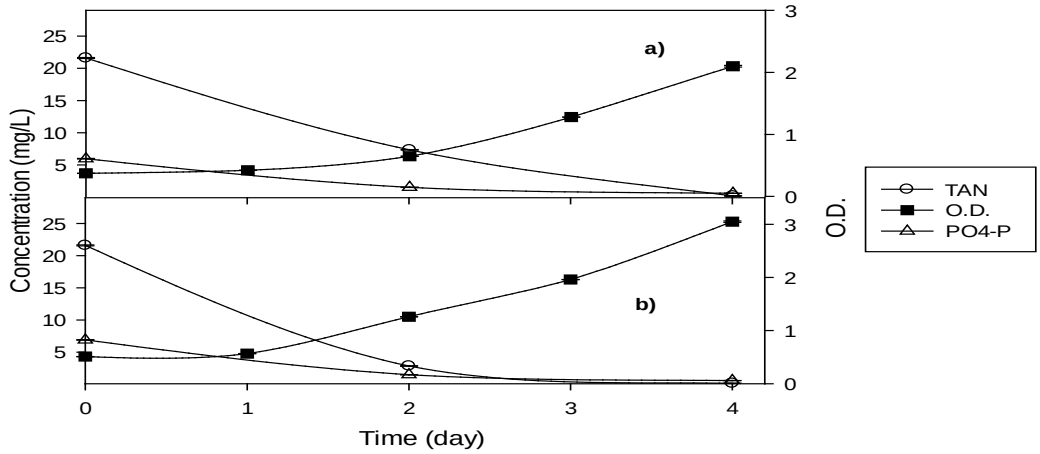


Figure 18 The change in Microalgal biomass (O.D.), TAN and PO_4^{3-}-P concentrations in (a) B4-50 and (b) B4-100 PBRs

At the beginning of the cultivation, a short acclimation period was observed and logarithmic growth phase started on the 1st day in all reactors as can be seen from the O.D. data in Figure 17 and Figure 18. The specific growth rates were calculated by taking the natural log of the biomass concentration (as ln(O.D.)) and plotting it over time (Brooker, 2011; Woertz, 2007). The specific growth rates were obtained as 0.38, 0.40, 0.46 and 0.48

abs/day for B3-50, B3-100, B4-50 and B4-100, respectively (Appendix A). Therefore, it can be concluded that in the reactors with 100 mL inoculum volume, higher biomass concentrations and higher growth rates were achieved than those with 50 mL inoculum volume within 4 days of cultivation.

Nutrient removal rate was another important factor in deciding the suitable inoculum source. Both nitrogen (TAN) and phosphorus (PO_4^{3-}-P) removal rates were calculated by using the Equation 4 (Ding et al., 2014).

$$R\left(\frac{mg}{L.d}\right) = \frac{S_0 - S_i}{t_i - t_0} \quad \text{(Equation 4)}$$

Where;

R is the removal rate of nutrient (mg (TAN or PO_4^{3-}-P) /L.d)
S_0 is the nutrient concentration (mg/L) at the beginning of the cultivation t_0 (d)
S_i is the nutrient concentration (mg/L) at the end of the cultivation (d)

Based on the results, it can be stated that nutrient removal rates demonstrated a parallel relationship with the growth rates. The highest TAN removal rates were achieved as 5.36 and 5.25 mg/L.d in B4-100 and B3-100, respectively. TAN removal rates obtained in other two reactors were 5 mg/L.d for B3-50 and 5.1 mg/L.d for B4-50. PO_4^{3-}-P removal rates in B3-50, B3-100, B4-50 reactors were 1.26, 1.29, 1.34 mg/L.d, respectively and, was highest in B4-100 with 1.58 mg/L.d. The growth-limiting nutrient is generally nitrogen for the domestic wastewater as discussed in Section 2.3.3.1. Based on the TAN and PO_4^{3-}-P data in Figure 17a ,b and Figure 18a and b, TAN removal rates were higher than PO_4^{3-}-P removal rates and microalgae could almost completely (>99%) removed TAN from the domestic wastewater in the all PBRs in 3 days. As expected, limiting nutrient was nitrogen for the microalgae grown in the domestic wastewater. The summary of the results is given in Table 18.

Table 18 Summary of the batch experiment results

Reactor	O.D.	Specific Growth Rate (d^{-1})	TAN Removal (%)	TAN Removal Rate (mg/L.d)	PO_4^{3-}-P Removal (%)	PO_4^{3-}-P Removal Rate (mg/L.d)
B3-50	1.7	0.38	99	5.00	94	1.26
B3-100	2.76	0.40	99	5.25	89	1.29
B4-50	2.1	0.46	99	5.10	90	1.34
B4-100	3.05	0.48	99	5.36	91	1.58

Regarding the selection of microalgae cultivation reactor to be used as the inoculum source in the semi-continuous study (and further experiments), the results have indicated that R4 microalgae cultivation PBR was more suitable than R3 PBR in terms of growth rate and nutrient uptake (Table 18). Moreover, it was observed that growth rates and nutrient removal performances were higher in the reactors inoculated with 100 mL (10% (v/v)) microalgae culture than the reactors inoculated with 50 mL microalgae (5% (v/v)) (Table 18) and inoculation with 10% basis was used throughout the experiments (Sections 4.1.2, 4.1.3, 4.2.1 and 4.2.2).

In the literature, inoculum sizes used for batch mode operations generally vary between 3% v/v and 10% v/v (Ojo et al., 2014; Wang et al., 2013; Yun et al., 1997). Higher inoculum sizes of 20% v/v and 37% v/v were also reported (Aslan and Kapdan, 2006; Hongyang et al., 2011). Although the effect of the initial inoculum size and volume on the microalgal biomass concentration is apparent (Lau et al., 1995), there are only few studies about the influence of inoculum volume on the microalgal growth and nutrient removal from wastewater. One of the related studies indicated the importance of the inoculum size on achieved biomass densities and productivities (Bohutskyi et al., 2016). Their study suggested that increasing the algal inoculum has a significant effect on relations between microalgae and wastewater-borne bacteria, and allows microalgae to compete successfully with wastewater bacteria for nutrients and organic carbon in the

wastewater. The study also notes that high initial microalgae concentration has been found to be beneficial for their survival in the presence toxic compounds, which may be present in wastewater. On the other hand, in another study, it has been stated that the competition between microalgae for nutrients is also expected to increase at high inoculum volumes, which may cause a slower growth (Ale et al., 2014). The results of their study have showed that high inoculum volume result in higher biomass concentration however biomass concentration is not always proportional to the initial size of the inoculum and optimal balance between inoculum size and microalgal density should be defined. This was also true for the current batch study. The amount of inoculum was 2 times higher in the B3-100 and B4-100 reactors as compared to the low inoculum volume in B3-50 and B4-50 reactors. On the other hand, both optical density and nutrient removal rate in these two reactors were much less than 2 times at the end of the cultivation (Table 18).

As can be seen in Table 19, complete removal of TAN and PO_4^{3-}-P from the primary treated domestic wastewater has been achieved in 4 days by the mixed culture. Longer time periods (Lau et al., 1995; Martinez et al., 2000) were required in some relevant studies to remove the nitrogen and phosphorus in the primary treated municipal wastewater. On the other hand, Ruiz-Marin et al. (2010) could achieve a significant nutrient removal in 2 days in the batch cultivation of *S.obliquus.*

Table 19 Reported nutrient removal efficiencies for batch cultures

Microalgae Specie	Nutrient Source	Cultivation Period (days)	TAN Removal (%)	PO_4^{3-}-P Removal (%)	Reference
C. Vulgaris	Primary treated sewage	10	74.1-99.9 depending on inoculum size	68.8-92.8 depending on inoculum size	(Lau et al., 1995)
Scenedesmus obliquus	Municipal wastewater	7.8	100	98 (TP)	(Martinez, et al., 2000)
C.vulgaris and *S.obliquus*	Urban wastewater (Secondary)	2	60.1-80 for *C.vulgaris* and 100-96.6 for *S.obliquus*	53.3-80.3 for *C.vulgaris* and 55.2-83.3 for *S.obliquus*	(Ruiz-Marin et al., 2010)
Mixed culture	Primary treated wastewater	4	100	100	This study

4.1.2. Semi Continuous Operation

As stated in Section 3.6.1.2, the main goal of this semi-continuous study was to determine optimum HRT for the treatment of the non-sterilized primary treated domestic wastewater. The outcome of this study would help make a comparison between the steady-state biomass concentrations, effluent concentrations and nutrient removal efficiencies in the PBRs operated at the HRT of 2 days (Y1), 4 days (Y2) and 8 days (Y3). These values were selected based on the previously reported values for microalgal domestic wastewater treatment (Larsdotter, 2006). The change in pH, O.D., solids, Chlorophyll-a, TN, TAN, PO_4^{3-}-P and sCOD concentrations during the semi-continuous operation of the Y1, Y2 and Y3 PBRs are shown in Figure 19, Figure 20 and Figure 21, respectively.

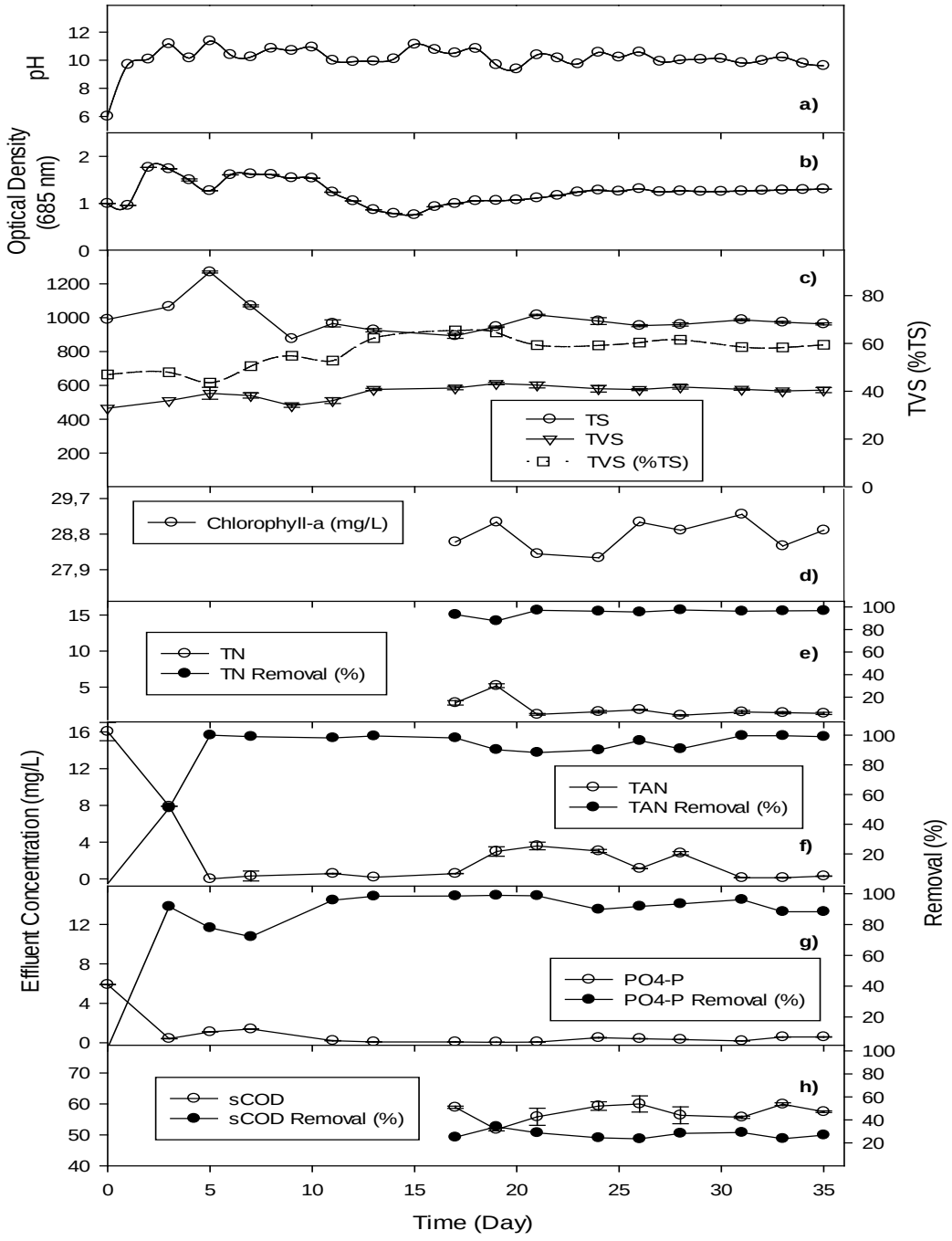


Figure 19 The change in a) pH, b) Optical Density, c) TS, TVS, TVS (%TS), d) Chlorophyll-a, e) TN, f) TAN, g) PO_4^{3-}-P, h) sCOD in the photobioreactor with 2-day HRT (Y1 PBR)

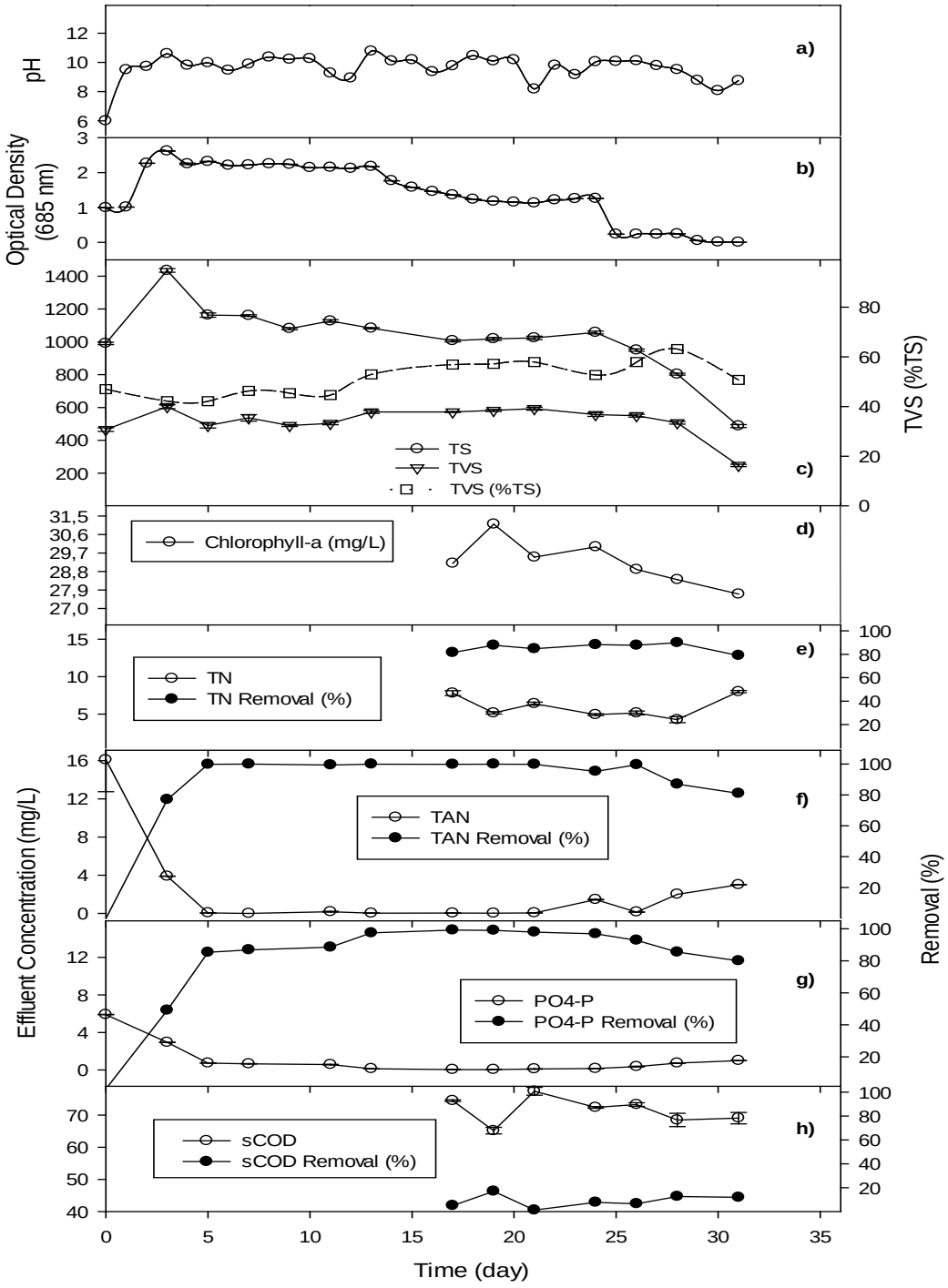


Figure 20 The change in a) pH, b) Optical Density, c) TS, TVS, TVS (%TS), d) Chlorophyll-a, e) TN, f) TAN, g) PO_4^{3-}-P, h) sCOD in the photobioreactor with 4-day HRT (Y2 PBR)

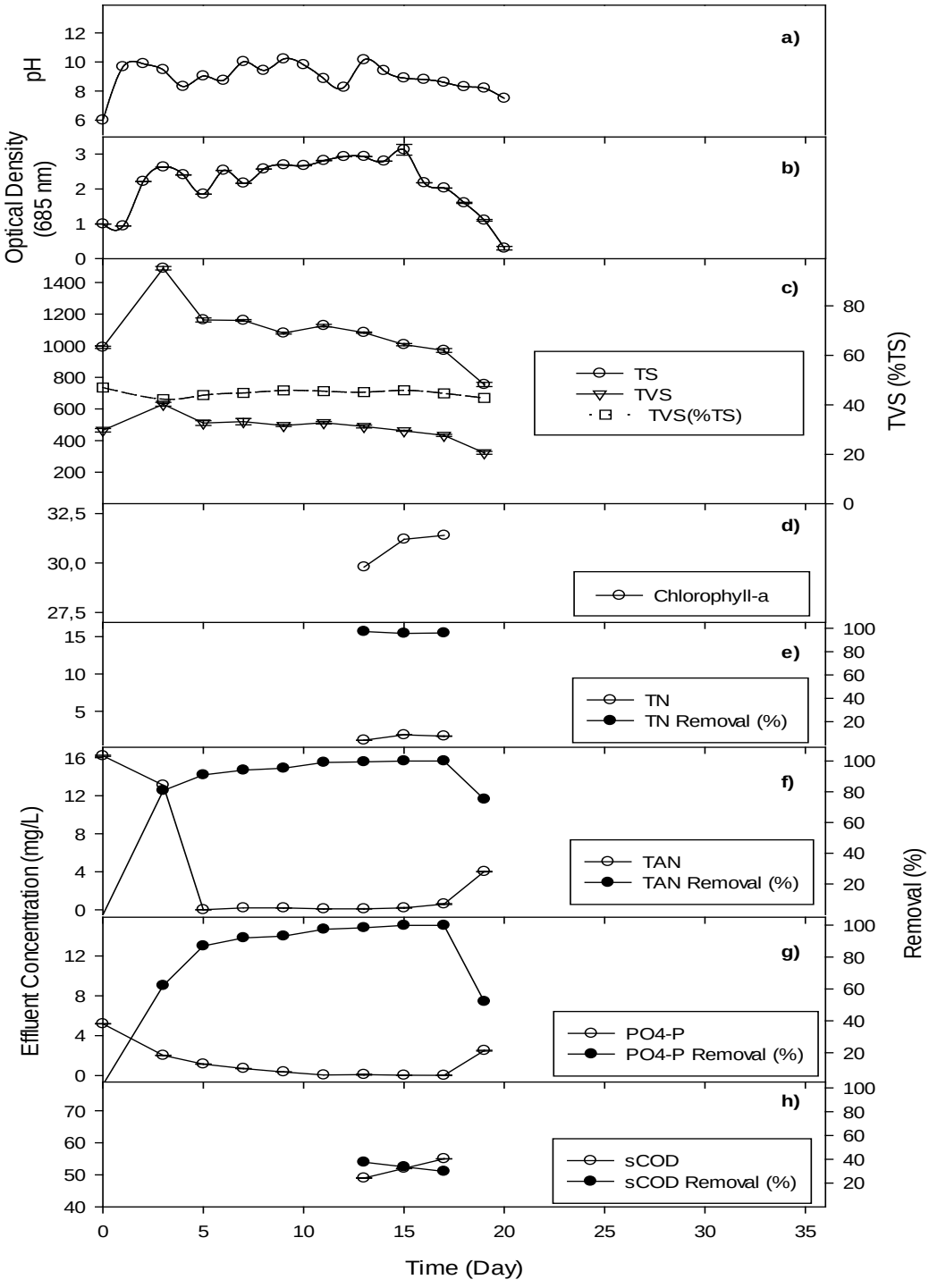


Figure 21 The change in a) pH, b) Optical Density, c) TS, TVS, TVS (%TS), d) Chlorophyll-a, e) TN, f) TAN, g) PO_4^{3-}-P, h) sCOD in the photobioreactor with 8-day HRT (Y3 PBR)

4.1.2.1. The Effect of HRT on Biomass Production

The pH was controlled to maintain the culture at an optimum range for growth. To this aim, pH of the cultures was adjusted to 6 with diluted H_2SO_4, HCI and NaOH solutions (Section 3.3) after addition of the domestic wastewater to the reactors during the operation period of the PBRs. pH in the reactors was observed in the range of 8 to 11 in the following day as a result of the microalgal photosynthetic activity (Figure 19a, Figure 20a and Figure 21a). This is consistent with a previous study that continuous illumination caused high photosynthetic activity which resulted in high pH values (Su et al., 2012).

During the semi-continuous runs on treatment of domestic wastewater, optical density (O.D.) at 685 nm, total solids (TS) and total volatile solids (TVS) parameters were monitored periodically to quantify algal biomass concentrations in the reactors. Besides, Chlorophyll-a was monitored during the steady-state conditions.

In the reactor with 2-day HRT (Y1), O.D. value increased from 0.95 to 1.8 after 3 days (Figure 19b). TS and TVS concentrations showed a similar trend with respect to O.D (Figure 19c). Then, a reduction was observed in the algal biomass due to the low HRT value. However, the culture could be adopted to the operating conditions and, the O.D. and solids concentrations were stabilized and, reached steady-state after day 17 as can be seen in Figure 19b and c. During the steady operation phase, O.D. ranged between 1.1 and 1.3. The average TS and TVS concentrations obtained at steady growth were 963 mg/L and 584 mg/L, respectively (Table 20).

In the reactor with 4-day HRT (Y2), O.D. increased sharply to above 2.5 in 3 days and then slightly decreased to the range of 2.1-2.3, where it was stable for about 10 days (Figure 20b). During this steady-state period (Figure 20c), average TS and TVS concentrations were 1123 and 519 mg/L, respectively. Then, O.D. and solids concentrations decreased gradually between days 14-26. At day 26, O.D. value dropped to 0.01 level (Figure 20b) which indicated the wash-out of the algae culture from the reactor. The operation of the reactor was ended on day 32.

In the reactor with 8-day HRT (Y3), O.D. value rapidly increased to 2.63 after 3 days and it varied between 1.85 and 3.1 following 12 days of the operation (Figure 21b). During this period, average TS and TVS concentrations were in the range of 1159 mg/L and 517 mg/L, respectively (Table 20). A sharp decline in O.D. (Figure 21b) and solids concentrations (Figure 21c) was observed after day 17. Then, a change in biomass color from bright green to yellow was observed on day 20 and O.D. value decreased nearly to zero (Figure 21b). Thus, the operation was ended on day 20 in this PBR. The average of the measured OD, TS and TVS concentrations under steady-state conditions are given with their standard deviations (±) in Table 20. The average of measured Chlorophyll-a (Chl-a) concentrations were calculated using the whole data obtained during the operation of the PBRs. The number of samples used to calculate the average concentrations are given in brackets.

Table 20 Average steady state biomass concentrations in Y1, Y2 and Y3 PBRs

PBR&HRT	OD at 685 nm	TS (mg/L)	TVS (mg/L)	Chl-a (mg/L)
Y1 (2 days)	1.17±0.15 (21)	963±33.7 (9)	584±14.4 (9)	28.76±0.4 (9)
Y2 (4 days)	2.20±0.06 (10)	1123±40.1 (5)	519±36.0 (5)	29.26±1.1 (7)
Y3 (8 days)	2.81±0.18 (13)	1159±155.8 (7)	517±53.4 (7)	30.8±0.9 (3)

Although higher steady-state biomass concentrations were achieved in the PBRs with 4 and 8 days of HRTs than those achieved with 2 days of HRT (Table 20), steady-state conditions could not be maintained at these HRTs. The biomass data show that an HRT of 2 days is not limiting the growth for the studied domestic wastewater under the conditions of this experiment.

The steady-state O.D. and solids concentrations obtained in this study are consistent with the results of the relevant studies. Li et al. (2013) studied indoor semi-continuous cultivation of *C.vulgaris* in municipal wastewater using cultures with 2-4 day HRTs. Solid concentrations were in the range of 425-550 mg TSS/L and O.D. values were in the range of 1.1-1.426 in their work. Woertz et al. (2009) reported steady state biomass

concentrations between 300 and 800 mg VSS/L during the cultivation of mixture of green algae and diatoms using municipal wastewater at HRTs of 2 to 4 days. Tercero et al. (2013) could achieve a steady-state biomass concentration of 470 mg TS/L during semi-continuous cultivation of *C.prototheco*ides fed with non-sterilized primary treated municipal wastewater and with a shorter residence time of 1.26 day. On the other hand, Wang et al. (2010) used a longer HRT of 5 day for the semi-continuous cultivation of *C.vulgaris* in undigested dairy manure due to the high organic and nutrient content of the wastewater. They achieved biomass concentrations between 1000 and 1380 mg TSS/L.

It should be noted that biomass concentrations obtained in this study are lower than those reported by Park et al. (2010) and Hulatt et al. (2011a). Park et al. (2010) could obtain maximum biomass concentrations between 2 and 2.4 g/L using the ammonia-rich anaerobic digestion effluent at a 10-day HRT. Hulatt et al. (2011a) obtained dry weight values between 1.5 g/L and 3.5 g/L with a mean value of 2.45 g/L in the semi-continuous cultivation of *Scenedesmus obliquus* in a well-designed 500-L horizontal tubular PBR (outdoor) using Jaworski medium. They used pure CO_2 to stabilize pH at 7 and variable HRT to maintain the nitrate concentration higher than 2 mmol/L.

In addition to O.D. and solids concentrations, Chlorophyll-a content also provided quantitative information on the algal biomass. In Y1 reactor, it was in the range of 27.9-29.8 mg/L. As a result of higher biomass produced, Chlorophyll-a contents were slightly higher in reactors Y2 and Y3 at steady-state conditions. Chlorophyll-a concentrations in these reactors ranged between 26.1-31.2 mg/L and 28.1-32.7 mg/L, respectively. These results are slightly higher than those reported by Li et al. (2013), who treated sterilized municipal wastewater by using *Chlorella vulgaris* at semi-continuous mode and obtained Chlorophyll-a concentrations around 20 mg/L at steady-state growth period. The lower concentrations can be explained by low light intensity and different photoperiod that they used in their study.

4.1.2.2. The Effect of HRT on Nutrient Removal

In this semi-continuous study, concentrations of Total Nitrogen (TN), Total Ammonia Nitrogen (TAN), Orthophosphate (PO_4^{3-}-P) and soluble Chemical Oxygen Demand (sCOD) in the effluent of PBRs were monitored periodically to determine nutrient removal efficiencies in the reactors. The average of measured effluent TAN, PO_4^{3-}-P and TN concentrations under steady-state conditions and corresponding nutrient removal efficiencies are given with their standard deviations (±) in Table 21. It should be noted that whole TN data obtained during the operation of the PBRs were used for the calculation of average TN concentrations and average TN removal efficiencies. The number of samples used to calculate the average concentrations are given in brackets.

Table 21 Average steady-state effluent concentrations and nutrient removal rates in the Y1, Y2 and Y3 PBRs

PBR&HRT	Effluent TAN (mg/L)	TAN Removal (%)	Effluent PO_4^{3-}-P (mg/L)	PO_4^{3-}-P Removal (%)	Effluent TN (mg/L)	TN Removal (%)
Y1 (2 days)	1.6±1.4 (9)	94.7±4.7 (9)	0.3±0.2 (9)	93.8±4.5 (9)	2.0±1.3 (9)	95.2±3.0 (9)
Y2 (4 days)	0.05±0.05 (4)	99.8±0.2 (4)	0.3±0.3 (4)	93.5±6.3 (4)	6.5±1.3 (9)	84.7±3.1 (9)
Y3 (8 days)	0.16±0.06 (6)	96.5±6.8 (6)	0.25±0.3 (6)	96.2±3.4 (6)	1.55±0.4 (3)	96.3±0.9 (3)

In the reactor with 2-day HRT (Y1), effluent TN, TAN and PO_4^{3-}-P concentrations decreased rapidly within 3 days and remained constant through most of the experiment which indicates steady growth of the mixed culture. The removal rates of TN and TAN were 88-97% and 88-100%, respectively (Figure 19e and f). In addition to TN and TAN, a high removal performance was also observed for PO_4^{3-}-P (90-98 %) as it can be seen from Figure 19g.

In the reactor with 4-day HRT (Y2), significantly high nutrient removal performance was achieved until day 25. Namely, 90-95% for TN, 95-100% for TAN and 85-99% for PO_4^{3-}-P were obtained during steady-state operation. However, the nutrient removal efficiencies decreased significantly after day 25 since the algal cells have started to washed out from the reactor (Figure 20e, f and g).

In the reactor with 8-day HRT (Y3), almost complete removal of nutrients was observed after day 10 till day 17 as shown in Figure 21e, f and g. TAN and PO_4^{3-}-P between days 5-15 was in the range of 91-100% and 87-99%, respectively. TN removal was between 95.6% and 97.3% on the last 4 days of steady state operation (Figure 21e). Similar to Y2, a significant decrease in nutrient removal efficiencies was observed after beginning of washout of algal cells in the reactor. Ruiz-Marin et al. (2010) has also observed that the semi-continuous culture of *S.obliquus* collapsed after four cycles of steady state operation. This was attributed to a protein synthesis limiting step which might have caused a decreasing amount of protein in the algae cells for every increasing number of cycles in semi-continuous systems.

In this study, mixed microalgae culture could efficiently remove TN, TAN and PO_4^{3-}-P from the primary treated domestic wastewater even at a short HRT of 2 day. Nutrient removal efficiencies obtained in the PBRs (Table 21) suggest that nitrogen is the limiting nutrient and that it starts to become limiting due to increasing HRT.

The experimental results of the study show that higher biomass concentrations and lower effluent concentrations can be achieved by increasing HRT. However, domestic wastewater tested 2 day-HRT is more suitable than HRTs of 4 and 8 days since washout of algal biomass was observed which in turn affected nutrient removal efficiencies and Chlorophyll-a concentrations in Y2 and Y3 reactors. Considering high nutrient removal performance of the reactor operated with 2 days of HRT (Y1), washout observed in Y2 and Y3 reactors was mainly due to nutrient limitation.

It should be noted that ammonia-stripping affects nitrogen removal from wastewaters in addition to direct utilization by microalgae. It has been reported that alkaline conditions,

elevated air and water temperatures and the existence of abundant urea result in ammonia-stripping (Li et al., 2011b; Su et al., 2012). Although the pH in the reactors was observed in the range of 8 to 11 as a result of the photosynthetic activity, air temperature was kept at 30±2˚C and urea was not dominant in the domestic wastewater. TAN removal trends matched with growth curves (Figure 19b and f, Figure 20b and f, Figure 21b and f), which suggest that main nitrogen removal mechanism was absorption by microalgae as previously reported by Boonchai et al. (2012). A statistical analysis was also performed using the algal growth and nutrient data obtained in the kinetic study conducted under the same experimental conditions (Section 4.1.3). The statistical analysis results showed that main nutrient removal mechanism is algal uptake since nutrient removal trends well fitted with the algal growth (Chl-a) (Appendix H and I). Some researchers have performed nitrogen balances to ensure that algal uptake is the main mechanism for nitrogen removal during their experiments. For example, Woertz et al. (2009) has reported that ammonia volatilization was minor (<7% of the total influent nitrogen) in the cultivation of algae in municipal wastewater. They measured pH values around 10.3 during the semi-continuous operation of the 3-day HRT photobioreactor without CO_2 sparging. They carried out a nitrogen balance for the determination of Volatilized-N concentration by measuring influent and effluent Ammonium-N, Nitrite-N, Nitrate-N and Organic-N concentrations. Moreover, Su et al. (2012) have also reported that algal uptake was the main nitrogen removal mechanism during the cultivation of mixed algal culture in municipal wastewater and removal by ammonia stripping and denitrification accounted for 7.6%-10.1% of the total nitrogen. For this study, nitrogen balance could not be made since only TAN and TN concentrations were measured during the operation of the PBRs.

Nutrient removal efficiencies achieved in this semi-continuous study are in consistency with relevant studies (Table 22). Woertz et al. (2009) has reported 84% removal of ammonium and over 99% orthophosphate removal from primary treated municipal wastewater in their semi-continuous experiment under ambient air supply. Wang et al. (2010) obtained 99.7% ammonium, 89.5% total nitrogen and 92.0% total phosphorus removal in the cultivation of *Chlorella vulgaris* (UTEX 2714) using 20x diluted

undigested dairy manure with 2% CO_2 supplementation to stabilize pH. In the semi-continuous study conducted by Lee et al. (2013) using mixed culture and 2nd lagoon effluent, the highest nutrient removal efficiencies (about 80% total nitrogen and 90% total phosphorus) were obtained in the case with 3-day HRT and 0.5 mg/L P addition to achieve a balanced N:P ratio. However, Ruiz-Martinez et al. (2012) reported relatively lower ammonia removal efficiency during the semi-continuous cultivation of a mixed microalgae in a phosphorus-limited effluent of a submerged anaerobic membrane bioreactor.

Table 22 Nutrient removal efficiencies by algae reported in literature

Microalgae Specie	**Nutrient Source**	**HRT (days)**	**TAN Removal (%)**	**PO_4^{3-}-P Removal (%)**	**Reference**
Mixed Culture	Domestic wastewater	3 days	84	99	(Woertz et al., 2009)
Chlorella vulgaris	Undigested dairy manure	5 days	99.7	92 (TP)	(Wang et al., 2010)
Mixed Culture	Anaerobic process effluent	2 days	67.2	97.8	(Ruiz-Martinez et al., 2012)
Mixed Culture	Domestic wastewater	3 days	80 (TN)	90 (TP)	(Lee et al., 2013)
Mixed Culture	Domestic wastewater	2 days	94.7	93.8	This Study

Characteristic of the specific type of wastewater utilized and the microalgal species or microbial consortia involved, illumination cycle, CO_2 bubbling are the factors affecting COD removal efficiency (Hu et al., 2012; Olguín, 2012; Tercero et al., 2013). Due to the

operation under continuous illumination, the aerobic respiration of algal biomass was minimal and photosynthesis was the dominating activity of algal cultures. However, a partial COD removal could also be obtained in the reactors (Figure 19g, Figure 20g and Figure 21g). Among the three PBRs, highest sCOD removal efficiencies (30-40%) were measured in Y1 reactor. This suggests that the mixed culture used in this study could utilize different organic compounds as carbon sources besides CO_2 which was discussed in a previous work (Li et al., 2011b). On the other hand, Tercero et al. (2013) has reported that COD was not consumed during their semi-continuous studies conducted under continuous light at 100 μmol m^{-2} s^{-1}. This was explained by the preinoculum grown under sparging with CO_2-enriched air (5%) which affected the capability of *C. protothecoides* to use organic carbon in their studies.

4.1.3. Kinetic Study

This 72-hour kinetic study aimed at determination of growth and nutrient removal kinetic parameters of the mixed microalgae culture which was previously acclimated to domestic wastewater during the semi-continuous study (Section 4.1.2). As also described in detail in Section 3.6.1.3, this batch study was conducted in a 1-L Test PBR (presented schematically in Figure 13) using the mixed microalgae culture (100 mL) which was the output of the semi-continuous PBR with 2-day HRT (Y1) and primary treated domestic wastewater (900 mL). The PBR was operated at 30±2˚C under 120 μmol m^{-2} s^{-1} continuous illumination and 0.5 vvm aeration. 50-100 mL sample was collected periodically from the reactor to measure O.D., TAN and PO_4^{3-}-P concentrations in the reactor. Solids concentrations, TN and sCOD were also measured once a day.

The variation in O.D., solid concentrations, Total Nitrogen (TN), Total Ammonia Nitrogen (TAN), Orthophosphate (PO_4^{3-}-P) and soluble Chemical Oxygen Demand (sCOD) concentrations during the 72 hours of batch operation is depicted in Figure 22.

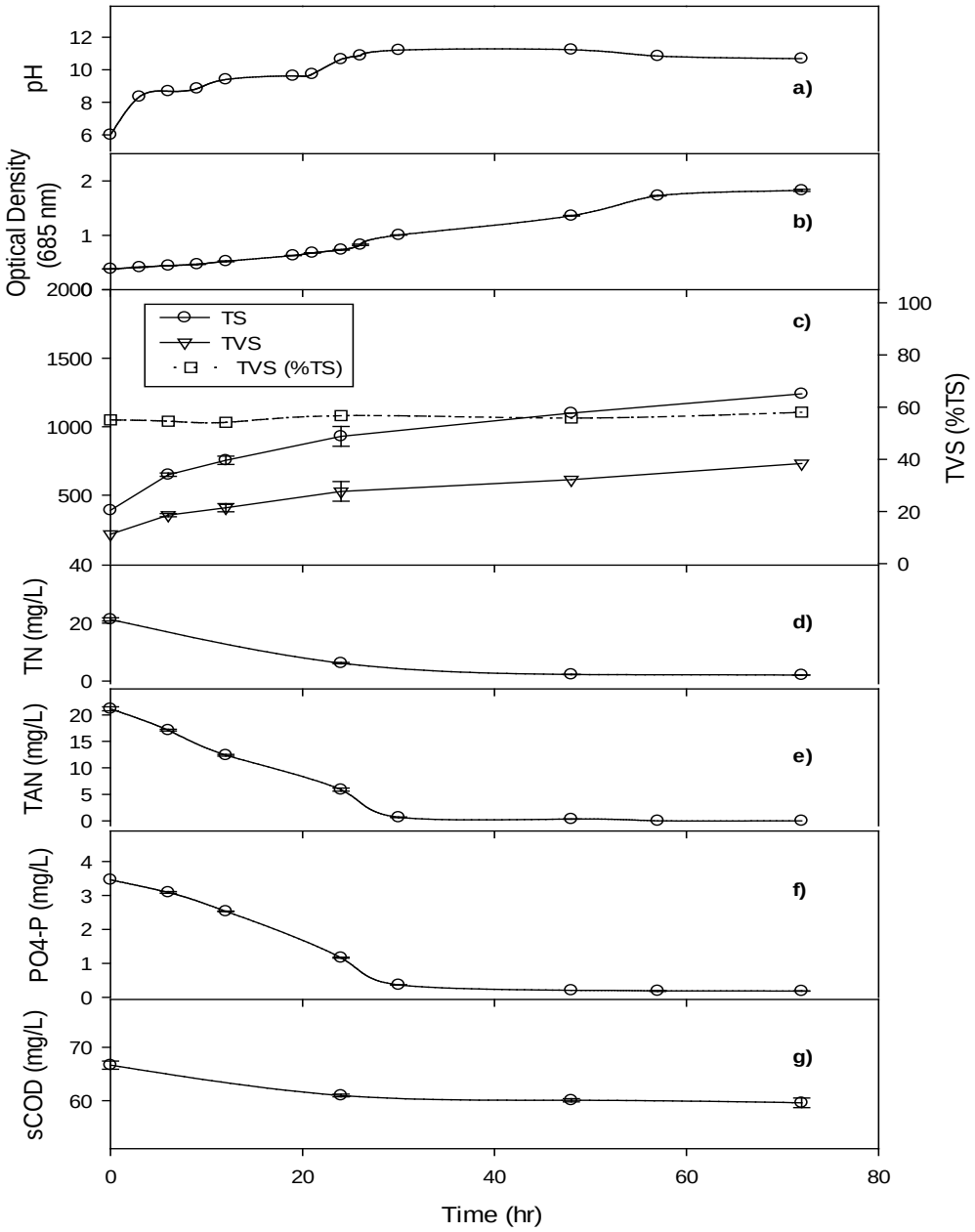


Figure 22 The change in a) pH, b) Optical Density, c) TS, TVS, TVS (%TS), d) TN, e) TAN, f) PO_4^{3-}-P, g) sCOD in YB-1 PBR

4.1.3.1. Change in pH and Biomass Production

Within 72 hours of operation, an increase in pH, from 6 to 10.68, was observed as a result of photosynthetic activity (Figure 22a). This shows that atmospheric air supplied to the culture was not sufficient to provide enough CO_2 to prevent large rise in pH as observed in the study conducted by Ruiz-Marin et al. (2010). Mennaa et al. (2015) has also reported similar final pH values (between 9.7 and 10.3) after a 3-day batch study in which urban wastewater and seven microalgae species were used.

As can be seen from Figure 22b and Figure 22c, the growth curves show no lag or adaptation phase since the stock culture (inoculum) had been already acclimated to the domestic wastewater (Section 4.1.2). Considering O.D. and solids data, it can be said that the growth rate significantly declined after 57 h when O.D. has exceeded 1.0 (Figure 22b). The stationary phase could not be clearly observed in the solids data (Figure 22c), however it can be assumed that it had been reached at 72^{nd} hour by considering the change in O.D. (Figure 22b).

In this study, the specific growth rate was calculated by plotting the natural log of the optical density versus time (black line in Figure 23). The specific growth rate was observed as 0.03 hr^{-1} (0.72 d^{-1}) which is the slope of the trend line (red dashed line in Figure 23) within ln(OD) values between t_1=0 h and t_2=30 h period (red line in Figure 23) with r^2=0.98.

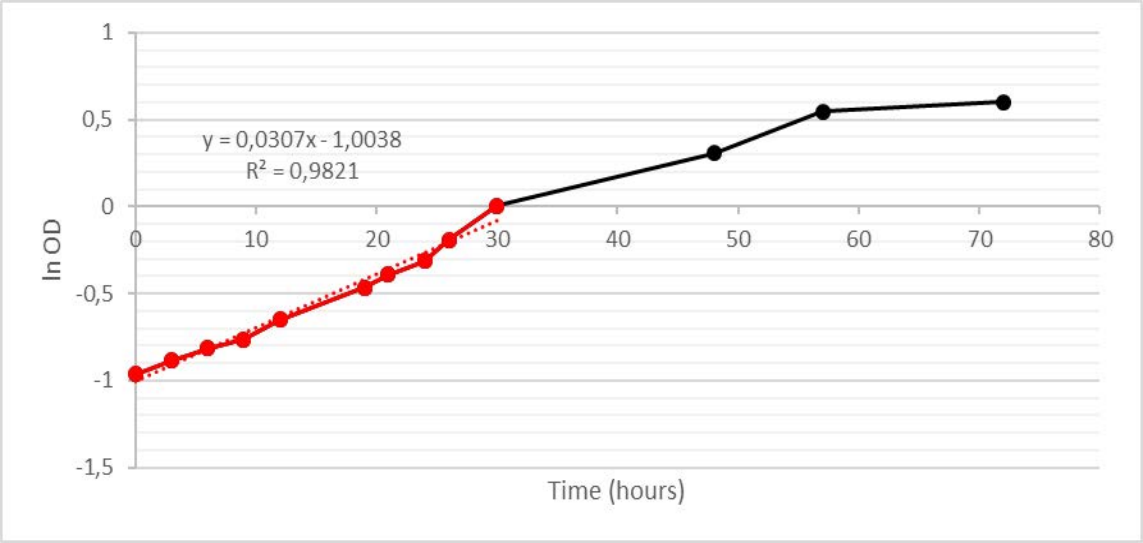


Figure 23 Determination of the specific growth rate

The specific growth rate determined in this study is higher than those obtained in the batch study conducted with unacclimated cultures (Section 4.1.1). This finding shows the importance of the acclimation on the growth (Yun et al., 1997). The obtained growth rate in this study is also higher than those reported in various related studies. For example, Boonchai et al. (2012) achieved a specific growth rate of 0.452 d^{-1} under a light intensity of 50 µmol m^{-2} s^{-1} with the light:dark cycle of 14h:10h in the batch cultivation of *C.vulgaris*. Similarly, Wang et al. (2010) reported a similar specific growth rate of 0.429 d^{-1} when domestic wastewater from primary settling tank was used in the batch cultivation of *Chlorella sp*. under continuous illumination at 200 µmol m^{-2} s^{-1} and at 25±2 ºC. Tam et al. (1990) also reported growth rates in the range between 0.3 d^{-1} and 0.5 d^{-1} for the batch cultivation of *Chlorella pyrenoidosa* in settled and activated sewage under 4000 lux (56 µmol m^{-2} s^{-1}) illumination at 16h:8h light-dark cycles and at a temperature of 20±2 ºC. Li et al. (2013) reported a much lower specific growth rate of 0.30 d^{-1} for the growth of *Chlorella vulgaris* in primary domestic wastewater for 5 days at 25 ºC with an irradiance of 40-60 µmol m^{-2} s^{-1} on a 16h:8h light-dark cycle. Moreover, Lau et al. (1995) reported specific growth rates in the range of 0.2742-0.2771 d^{-1} for the batch cultivation of *Chlorella vulgaris* at 24±1 °C, under continuous illumination of 4300±300 lux (56-62

μmol m^{-2} s^{-1}) at light-dark cycles of 16h:8h. It should be noted that, although domestic wastewater was used in these studies, light intensities and/or photoperiods applied were different than that used in this study. Therefore, it can be concluded that microalgae species, light intensity and the frequency of light/dark cycle strongly influence the growth rate of algal cells.

In this study, TS measurements could have been made five times since each TS measurement (mean of triplicates) required at least a total sample volume of 75 mL. Due to the limited TS data, productivity value was calculated over 72-h period in accordance with Equation 1 (Section 2.3.1). Within the 72-h operation, the initial TS concentration was increased from 0.39 g/L to 1.24 g/L which corresponds to a productivity of 0.283 g/L.d. Similarly, Ji et al. (2014) reported productivity values between 190 and 290 mg/L.day for the batch cultures of *Desmodesmus sp* grown with anaerobic digestion wastewater.

However, Li et al. (2013) reported a lower biomass productivity value of 0.15 g/L.d. with a peak biomass value of 0.76 g/L for the cultivation of *Chlorella vulgaris* in municipal wastewater. Yao et al. (2015) also reported a low productivity value of 0.19 mg/L.d for *Chlorella sorokiniana* grown in a mixture of swine and municipal wastewater. The lower biomass production obtained in their study can be explained by the lower light intensities and different photoperiods used by these researchers.

4.1.3.2. Nutrient Removal

As shown in Figure 22d and Figure 22e, significantly high TN and TAN removal efficiencies were achieved at the end of this kinetic study. TN and TAN contents in the reactor dropped from their initial values of 21.3 mg/L and 21.14 mg/L to less than 2.3 mg/L and 0.35 mg/L after 48 hours, respectively. At the end of 72-hour batch growth, TN removal efficiency and TN removal rate were 90.3% and 6.4 mg TN/L.d, respectively. TAN was completely removed at the end of the operation with a 8.9 mg TAN/L.d removal rate.

PO_4^{3-}-P concentration decreased from 3.46 mg/L to 0.2 mg/L in 48 hours (Figure 22f). The final concentration was obtained as 0.18 mg/L which resulted in a removal efficiency and a removal rate of 94.7% and 1.1 mg PO_4^{3-}-P/L.d, respectively.

In this kinetic study, most of the TAN (72%) and PO_4^{3-}-P (66%) in the wastewater has been removed by microalgae within 24 hours and it has been observed that depletion of nutrients slowed down the growth (Figure 22b and c). When TAN was completely removed from the wastewater (t=57 h), >%5 of the initial PO_4^{3-}-P concentration has not be utilized by the algae. Therefore, growth limiting nutrient was nitrogen in this study as well. Moreover, it can be concluded that N:P ratio of the mixed microalgae culture was similar to that of studied domestic wastewater when very similar nitrogen and phosphorus removal trends are considered (Figure 22e and f).

As stated in Section 4.1.3.1, pH in the PBR has increased from to 6 to the levels higher than 10. Therefore, ammonia-stripping and phosphate precipitation might have indirectly affected the nutrient removal process (Izhar, 2016). In order to verify that the main nutrient removal mechanism was microalgal uptake in the study, the growth data of the microalgae were analyzed using Minitab 17 software. Chl-a data for YB-1 PBR were generated using the correlation between Chlorophyll-a concentration and TVS concentration data obtained during the operation of semi-continuous PBR named Y1 with 2-day HRT (Appendix G). It should be noted the output Y1 PBR has been used as the inoculum source for this kinetic study (Section 4.1.3).

According to the results, there was a strong relationship between Chlorophyll-a concentrations and reduction in TAN (R^2 = - 0.951) and PO_4^{3-}-P (R^2 = - 0.941) concentrations (Appendix H and I) showing that most of the TAN and PO_4^{3-}-P have already been removed by algae before that pH was above 9.5 as discussed by Su et al. (2012).

As shown in Figure 22g, there was a slight decrease in sCOD concentration. It decreased from 66.7 mg/L to 59.6 mg/L in 72 hours and, corresponding removal efficiency was 10.6%. Low sCOD removal in this batch study indicates that the heterotrophy of

microalgae (Section 2.3.3.5), and heterotrophic bacteria was negligible as reported by Yun et al. (1997).

Nutrient removal efficiencies obtained in this batch study were consistent with referenced values. Ruiz-Marin et al., (2010) obtained 100% NH_4^+-N and 83.3% PO_4^{3-}-P removal efficiencies in 50 hours for batch culture of *S.obliquus* growing in urban wastewaters. Li et al. (2013) reported 98.1%, 90.9%, 90% removal efficiencies for NH_4^+-N, TN and TP in 10 days of batch cultivation of *Chlorella vulgaris* in sterilized primary treated municipal wastewater. Soluble N and P removal efficiencies of 95.7% and 96.4% for *Chlorella sp.* and, 93.9% and 96.1% for *Micractinium sp.* were reported at the end of batch cultivation with an urban primary effluent which had similar nutrient concentrations with the wastewater used in this study. On the other hand, sCOD concentrations reduced to 24 mg/L and 25 mg/L from 93 mg/L for *Chlorella vulgaris* and *Micratinium sp.*, respectively, in their study. The high sCOD removal than expected was explained by the growth of heterotrophic algae and bacteria during 8-hours dark periods applied to the cultures (Wang et al., 2013).

Su et al. (2012) reported that mixed culture could treat 98% of NH_4^+-N and %99 of PO_4^{3-}-P in urban wastewater containing 48.9 mg NH_4^+-N/L and 4.0 mg PO_4^{3-}-P. However, in order to achieve these high efficiencies a retention time of 9 days and 7 days for ammonia and orthophosphate, respectively. Therefore, the corresponding nutrient removal rates were only 5.4 mg NH_4^+-N/L /L.d and 0.57 mg PO_4^{3-}-P. The difference in nutrient removal rates between their results and obtained in this study can be explained by the effect of photoperiod and light intensity on nutrient removal. In their study, illumination was 7000 lux (98 µmol m^{-2} s^{-1}) with a period of 12 hours. On the other hand, continuous illumination at 120 µmol m^{-2} s^{-1} was used in this study.

4.2. Microalgae Cultivation in Industrial Wastewater Diluted with Sludge Thickener Supernatant

4.2.1. Batch Operation

In the batch operation, the effects of N:P (TAN:PO_4^{3-}-P) ratio on microalgae growth and biomass yield, nutrient removal and CO_2 fixation efficiency were investigated as described previously in Section 3.6.2.1. With the aim of evaluating the effects of N:P ratio, mixed algal culture was grown in batch mode using unsterilized industrial and domestic wastewater mixtures with TAN: PO_4^{3-}-P (g/g) ratios of 6 (the PBR named DB6), 8 (the PBR named DB8) and 10 (the PBR named DB10). In order to get these ratios, coke plant wastewater taken from KARDEMIR Integrated Iron and Steel Factory was diluted with primary thickener supernatant obtained from Ankara Tatlar WWTP. Table 23 shows the initial (t=0) nutrient concentrations in the DB6, DB8 and DB10 photobioreactors.

Table 23 Initial nutrient concentrations in the batch PBRs

Reactor	Initial TAN (mg/L)	Initial PO_4^{3-}-P (mg/L)	Initial TAN:PO_4^{3-}-P (g/g) Ratio
DB6	148.9	23.6	6.3
DB8	167.2	21.5	7.8
DB10	194.5	20.7	9.4

The batch mode-PBRs were operated at 28±2 ºC under 120 µmol m^{-2} s^{-1} continuous light and 4% CO_2-enriched air. The graphs showing the trends in pH, O.D., TS and TVS, Chlorophyll-a, TAN and PO_4^{3-}-P concentrations in DB6, DB8 and DB10 photobioreactors are given in Figure 24, Figure 25 and Figure 26, respectively.

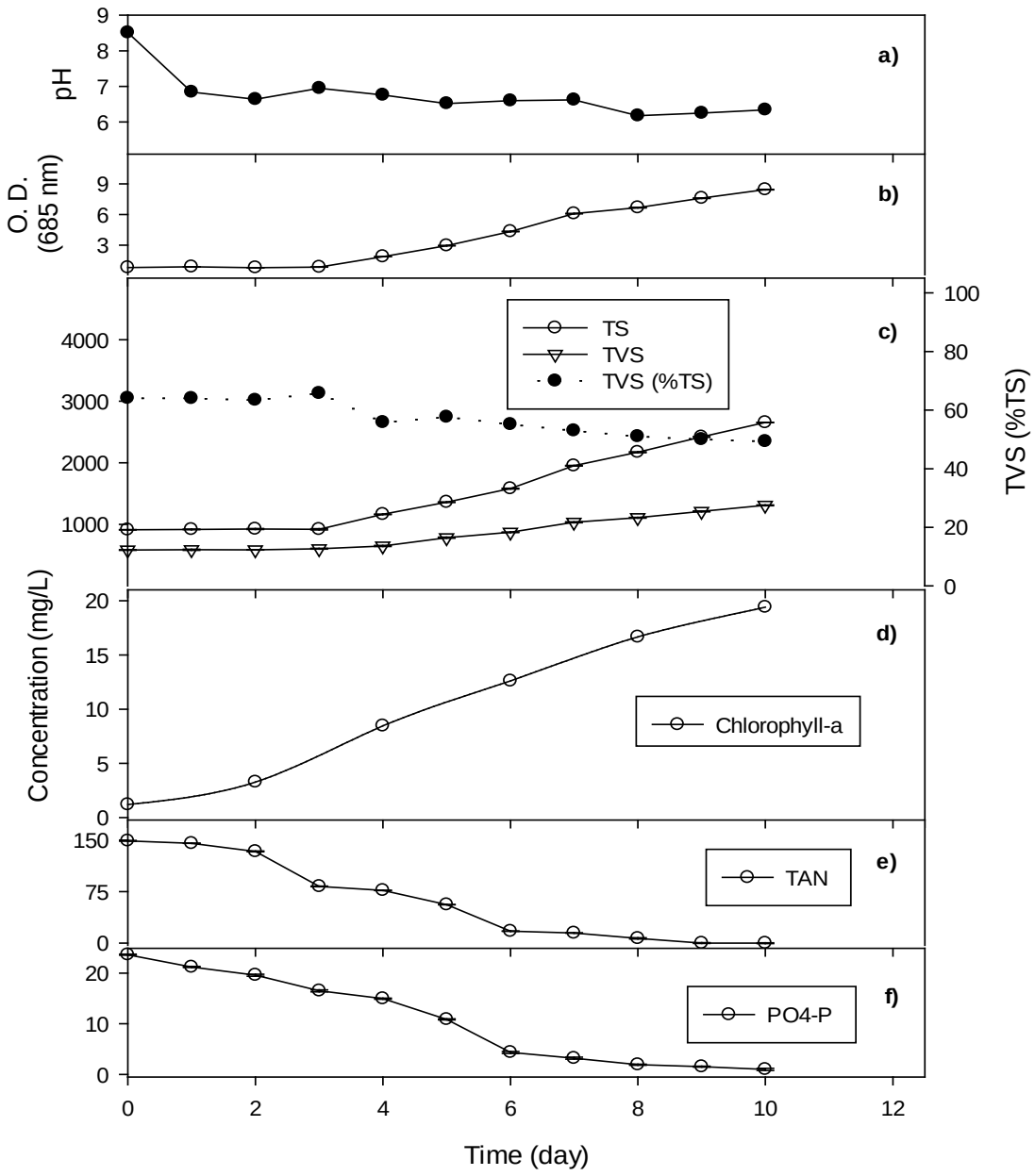


Figure 24 The change in a) pH, b) Optical Density, c) TS, TVS, TVS (%TS), d) Chlorophyll-a, e) TAN, f) PO_4^{3-}-P in DB6 PBR

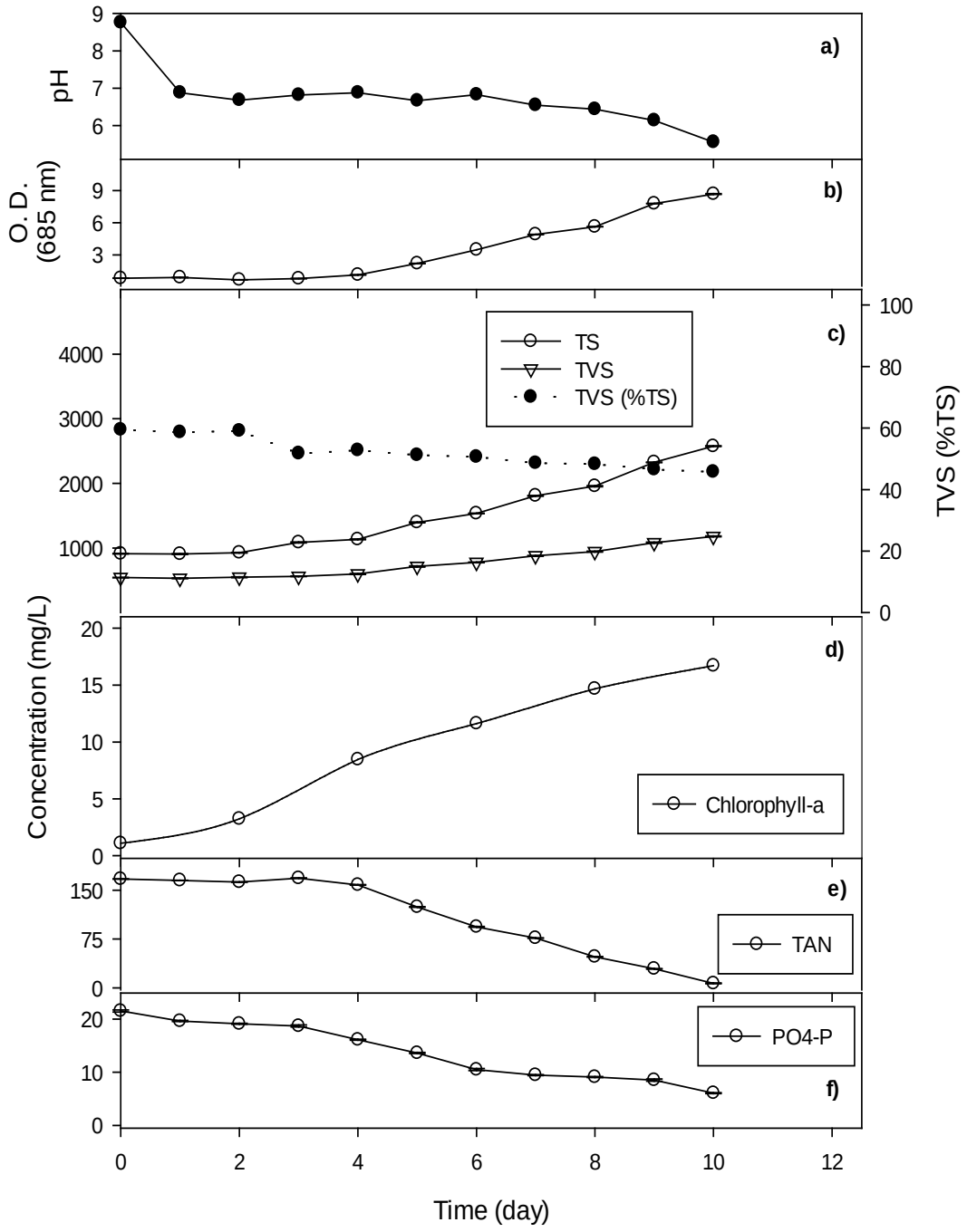


Figure 25 The change in a) pH, b) Optical Density, c) TS, TVS, TVS (%TS), d) Chlorophyll-a, e) TAN, f) PO_4^{3-}-P in DB8 PBR

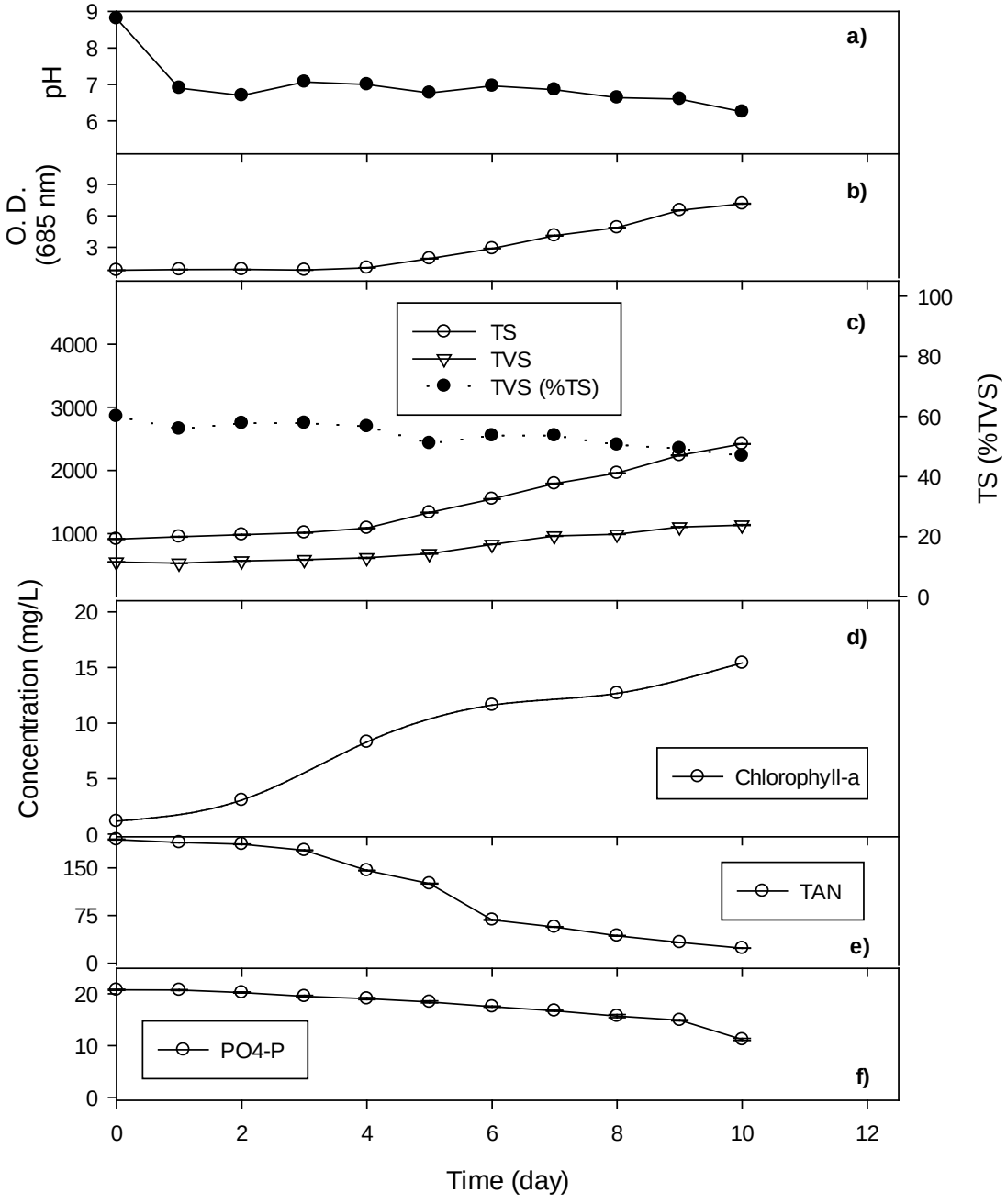


Figure 26 The change in a) pH, b) Optical Density, c) TS, TVS, TVS (%TS), d) Chlorophyll-a, e) TAN, f) PO_4^{3-}-P in DB10 PBR

4.2.1.1.The Effect of N:P Ratio on Biomass Production

The biomass growth in the PBRs was monitored daily by measuring O.D. at 685 nm wavelength, dry weight as Total Solids (TS) and Volatile Solids (TVS) and Chlorophyll-a (Chl-a) concentration. Table 24 provides final pH and biomass concentrations obtained at the end of the experiment.

Table 24 Summary of the results

TAN:PO_4^{3-}-P (N:P) Ratio	Final pH	Final O.D.	Final TS	Specific Growth Rate (d^{-1})	Productivity (mg TS/L.d)	Final Chl-a (mg/L)
6	6.34	8.44	2654	0.476	0.252	19.4
8	5.56	8.67	2576	0.475	0.235	16.7
10	6.25	7.15	2420	0.423	0.222	15.4

As can be seen from Figure 24a, Figure 25a and Figure 26a, pH values in the reactors dropped to the range of 6-7 from 8.5-9 in the first day of operation and then stayed in this range till the end of the experiment (10^{th} day). Figure 24a, Figure 25a and Figure 26a show that CO_2-enriched air supplied to the cultures could prevent increase in pH which was observed in the batch studies conducted with ambient air (Sections 4.1.1 and 4.1.3). Considering that the optimum range is around neutral pH for many microalgae species (Kumar et al., 2010), it can be said that supplied %4 CO_2 had positive effect on microalgae growth in terms of pH buffering.

Mixed microalgae culture showed a lag phase for the all N:P (TAN:PO_4^{3-}-P) ratios (Figure 24b, Figure 25b and Figure 26b) since acclimation of the mixed microalgae culture to the toxic materials such as cyanide originated from the coke plant wastewater (Dash et al., 2009) and to the high nutrient concentrations was necessary. Relatively longer lag phases observed in DB8 and DB10 reactors might have been caused by the larger industrial

wastewater volumes used for the preparation of their inputs (Section 3.6.2.1). It should be also noted that concentrations of hazardous volatile organic contaminants present in the coke plant wastewater might have decreased during the lag period as a result of continuous aeration (El-behlil et al., 2012).

After an initial lag period, optical densities in the PBRs started to increase on 3rd day of the cultivation. At the end of the cultivation period of 10 days, optical densities in DB6, DB8 and DB10 have reached 8.44, 8.67 and 7.15, respectively (Figure 24b, Figure 25b and Figure 26b). In order to determine the specific growth rates, ln(O.D.) versus time were plotted (Appendix B-1). The slopes of the straight line of the plots were equal to specific growth rates. By using this method, logarithmic change in the O.D. values of the cultures between 3rd and 7th days (exponential growth period) was calculated. The resulting growth rates were 0.476 d^{-1}, 0.475 d^{-1} and 0.423 d^{-1} in DB6, DB8 and DB10, respectively. Therefore, it can be postulated that the specific growth rates were similar for the N:P ratios of 6 and 8 and, it was lowest for the N:P ratio of 10 under conditions of this batch experiment.

The growth rate values achieved in this study are comparable with those obtained in other studies. For example, Chiu et al. (2008) obtained a specific growth rate of 0.343 d^{-1} for high density inoculums of *Chlorella sp*. in an 800 mL-photobioreactor at 26±1 ^{o}C under continuous illumination with light intensity of 300 µmol m^{-2} s^{-1}, aerated and mixed with 5% CO_2-enriched air at a rate of 0.25 vvm. In a different study, algal consortium in primary wastewater effluent has showed a specific growth rate of 0.53 d^{-1} at room temperature, 440 µmol m^{-2} s^{-1} with light/dark cycle of 16h:8h and 2% CO_2 in a 1 L-bottle (Samorì et al., 2013). Yet, some researchers reported higher growth rates. For example, Tang et al. (2011) achieved specific growth rates of 0.943 d^{-1} and 0.993 d^{-1} in the cultivation of *S.Obliquus* SJTU-3 and *C.Pyrenoidosa* STJU-2 in 1L Erlenmeyer flasks with BG11 medium under 180 µmol m^{-2} s^{-1} continuous illumination at 25±1 ^{o}C and 5% CO_2. Besides, Filali et al. (2011) achieved a maximum specific growth rate of 1.92 d^{-1} for *Chlorella vulgaris* in a bubble column reactor with a total culture volume of 9.6 L at 25 ^{o}C with 80 µmol m^{-2} s^{-1} continuous illumination and 5% CO_2 supply.

The trend in optical densities was also observed in solids concentrations (Figure 24c, Figure 25c and Figure 26c). Total Solids (TS) and Total Volatile Solids (TVS) concentrations started to increase on 3rd day of the experiments. Then, concentrations of TS and TVS increased almost linearly till 10th day in all reactors. The final TS and TVS concentrations were 0.265 g TS/L - 0.131 g TVS/L, 0.257 g TS/L - 0.118 g TVS/L and, 0.242 g TS/L - 0.114 g TVS/L for DB6, DB8 and DB10, respectively. In parallel with the dry weights, the final Chlorophyll-a concentration was the highest in DB6 and was the lowest in DB10 (Figure 24d, Figure 25d and Figure 26d).

The solid concentrations obtained in this study are lower than what is reported by Hulatt et al. (2011). They studied different power inputs for sparging by batch cultivation of microalgae at 4% CO_2 and achieved higher biomass concentrations, which were in the range of 3.03-3.60 g SS/L.d for *Dunaliella* and in the range of 2.7-3.62 g SS/L.d for *C.vulgaris*. The higher biomass concentrations achieved can be explained by higher incident radiance (350 µmol m^{-2} s^{-1}) used in their study. Yet, Yun et al. (1997) cultivated *C.vulgaris* in raw industrial wastewater at %5 CO_2 and reported biomass concentrations of 1.58 g SS/L when culture was adapted to air and 1.72 g SS/L when the culture was adapted to %5 CO_2. Their study showed that adaptation to the higher CO_2 concentrations could significantly improve the growth of microalgae. Therefore, it can be stated that biomass concentrations obtained in this experiment (Table 24) are promising since no adaptation period to the CO_2-enriched air was applied.

In this study, productivity values were calculated according to the Equation 1 (Section 2.3.1) using the Total Solids (TS) data obtained during the linear growth (Appendix B-2). The productivity values were determined to be 0.252, 0.235 and 0.22 g/L.day in DB6, DB8 and DB10, respectively. These productivity rates are comparable with the reported values in relevant studies. *S.Obliquus* have been cultivated in urban wastewater with %5 CO_2 and, a productivity rate of 0.23 g SS/d.L was achieved (Ruiz et al., 2013). *S.Obliquus* SJTU-3 and *C.Pyrenoidosa* STJU-2 were cultivated in batch mode at %5 CO_2 and productivity values of 0.158 and 0.133 g SS/L.d, respectively, were obtained (Tang et al., 2011). On the other hand, Ryu et al. (2009) cultivated *Chlorella sp.* in Allen medium with

100 µmol m^{-2} s^{-1} continuous illumination at air enriched with 5% CO_2 at a flow rate of 0.2 vvm and, reported 0.34 g SS/L.d productivity.

4.2.1.2.The Effect of N:P Ratio on CO_2 Fixation

In this batch study, all reactors were operated in batch mode under the same experimental conditions and with continuous supply of 4% CO_2. For determination of the CO_2 fixation rates, Equation 5 was used (Jacob-Lopes et al., 2009):

$$R_{CO_2} = C_C \times P \times \frac{M_{CO_2}}{M_C} \qquad \text{(Equation 5)}$$

According to the Equation 5, R_{CO2} is the carbon fixation rate (g CO_2/L.day), C_C is the average carbon content of microalgae, *P* is the productivity, M_{CO2} is the molecular weight of CO_2 (44 g/mol) and M_C is molecular weight of elemental carbon (C). Productivity values were already given in Section 4.2.1.1. The average carbon content of microalgae was obtained as 0.51 g C/g dry cell weight in the elemental analysis conducted by an accredited laboratory (Appendix F). By using these values, CO_2 fixation rates were calculated as 0.477, 0.445 and 0.420 g CO_2/L.d in DB6, DB8 and DB12, respectively. The highest CO_2 fixation rate observed in DB6 since productivity values achieved in these reactors were the determinant in calculation of CO_2 fixation rates.

These results are comparable to the published literature. In the batch study of Hulatt et al. (2011) under %4 CO_2, fixation rates were between 0.43 and 0.51 g CO_2/L.d for *Dunaliella tertiolecta* and, between 0.30 and 0.40 g CO_2/L.d for *Chlorella vulgaris* were obtained. *Synechococcus* PCC7942 was cultivated in an air-lift column at %5 CO_2 and light intensity of 8klx (100 µmol m^{-2} s^{-1}) and CO_2 uptake rate of 0.6 g CO_2/L.d was achieved. However, a higher fixation rate of 0.7 g CO_2/L.d was observed when *Chlorella sp.* AG10002 was cultivated at 5% CO_2. In their study, the effect of flow rate (vvm) on CO_2 utilization efficiency was also investigated and it was found that as aeration rate increased from 0.1 to 0.5, the efficiency decreased. The optimum flow rate was found to be 0.2 vvm in terms of productivity and CO_2 fixation. Furthermore, the carbon content of microalgae used in their study was reported as 0.57 g C/ g biomass (Ryu et al., 2009). Therefore,

higher CO_2 utilization rate achieved at similar experimental conditions can be explained by both appropriate flow rate and microalgae strain used.

4.2.1.3.The Effect of N:P Ratio on Nutrient Removal

It was critical to determine optimum N:P (TAN:PO_4^{3-}-P) ratio for wastewater to be used in order to attain high algae growth and high nutrient removal efficiencies. In parallel with microalgal growth, nutrient removal percentages were different in the reactors. After 10 days of cultivation, TAN and PO_4^{3-}-P removal efficiencies were %100-%95.8, %95.8-%71.5 and %87.8-%46 for DB6, DB8 and DB10, respectively (Figure 24e, f, Figure 25e, f and Figure 26e, f). Average removal efficiencies and rates are given in Table 25. It appears that PO_4^{3-}-P removal was obviously affected by the N:P ratios and TAN could be more efficiently removed than phosphate at N:P ratios of 6, 8 and 10 under the conditions of this study.

Table 25 Nutrient removal efficiencies and rates in the PBRs

Reactor	TAN Removal (%)	TAN Removal Rate (mg/L.d)	PO_4^{3-}-P Removal (%)	PO_4^{3-}-P Removal Rate (mg/L.d)
DB6	100	14.89	95.8	2.96
DB8	95.8	16.02	71.5	1.97
DB10	87.8	17.08	46.0	1.50

It was observed that the nutrient concentrations did not decrease rapidly due to slow growth in the first 3 days of cultivation (Figure 24b, Figure 25b and Figure 26b), while the TAN and PO_4^{3-}-P were almost completely removed within 10 days in reactor DB6. Concentrations of TAN and PO_4^{3-}-P in wastewater were significantly decreased from 148.9 mg/L to less than 1 mg/L and from 23.6 mg/L to 1.1 mg/L, respectively, after 10 days of cultivation. The corresponding nutrient removal rates were 14.89 mg TAN/L.d and 2.96 mg PO_4^{3-}-P/L.d in DB6.

There was also a significant TAN removal in DB8 and DB10 reactors (Figure 25e and Figure 26e). However, 10 days of cultivation period was not sufficient to remove the nutrients completely (Figure 25e, f and Figure 26e, f). TAN concentrations decreased from 167.2 mg/L to 6.9 mg/L in DB8 and, from 194.5 mg/L to 23.7 mg/L in DB 10 and, corresponding TAN removal rates were 16.02 mg.L/d and 17.08 mg.L/d, respectively. These results showed that higher initial ammonia concentration led to over uptake of ammonia by algal cells. Similar observation was made by Wang et al. (2013) and Aslan and Kapdan (2006).

It was observed that PO_4^{3-}-P removal efficiencies and removal rates obviously decreased as N:P ratio increased from 6 to 10 (Figure 24f, Figure 25f and Figure 26f). In DB8 reactor, PO_4^{3-}-P concentration decreased from 21.5 mg/L to 6 mg/L and PO_4^{3-}-P removal rate was calculated as 1.96 mg/L.d. PO_4^{3-}-P removal was the lowest in DB10 among three reactors. PO_4^{3-}-P concentration decreased from 20.76 to 11.2 mg/L and resulting removal rate was only 1.50 mg/L.d.

In this batch study, nutrient-rich wastewater mixtures with N:P (g/g) ratios of 6, 8 and 10 were used to grow mixed microalgae culture. TAN and PO_4^{3-}-P data (Figure 24e, f, Figure 25e, f and Figure 26e, f) showed that microalgae removed TAN faster than PO_4^{3-}-P at the all N:P ratios studied. Although TAN could not be completely removed in the PBRs named DB 8 and DB10 within 10 days (Table 25), the results indicated that nitrogen was the limiting nutrient for algae growth at the all N:P ratios.

Wastewater composition and environmental conditions such as the initial nutrient concentration, N:P ratio, light intensity or algae species directly affect nutrient removal efficiencies. Therefore, reported values vary depending on these factors (Aslan and Kapdan, 2006). The resulting nitrogen removal efficiencies in this batch study are consistent with other studies having similar conditions. For instance, Yun et al. (1997) have reported %100 ammonia-N removal efficiency with a rate of 20.64 mg/L.d. Yet, ammonia-N concentration was only 54.6 mg/L and could be removed in 3 days. In the

present study, ammonia concentrations were between 148.9 and 194.6 mg TAN /L (Table 25).

Higher phosphate removal efficiencies were obtained compared to some of other previously reported values although PO_4^{3-}-P concentrations in the reactors were between 20.7 mg/L and 23.6 mg/L. For example, *Chlamydomonas sp*. was cultivated with industrial wastewater under continuous illumination of 125 µmol m^{-2} s^{-1} and aeration of %5 CO_2/air (v/v) mixture. Microalgae culture could completely remove NH_4^+-N from the wastewater with a consumption rate of 19.2 mg NH_4^+-N/L/d. However, PO_4^{3-}-P removal was only 33% in 10 days with a removal rate of 1.5 PO_4^{3-}-P/L.d (Wu et al., 2012). While, higher phosphate removal efficiencies could also be achieved in some studies. For example, Chinnasamy et al. (2010) cultivated a consortium of 15 native algae in treated and untreated wastewaters containing 85–90% carpet industry effluents with 10–15% municipal sewage by providing continuous illumination at an irradiance of 75–80 µmol m^{-2} s^{-1} and bubbling 6% CO_2–air mixture at a rate of 0.1 L/min. After 72 hours of incubation in treated wastewater, the consortium achieved 99.8% nitrate-N, 100% ammonia-N and 96.6% phosphate-P removal.

The results of this study showed that even at high concentrations, both nitrogen and phosphorus can be efficiently removed provided that N:P ratio of the wastewater is balanced. The results also suggest that N:P (TAN:PO_4^{3-}-P) ratio of 6 is the most adequate ratio to produce the highest amount of biomass and to achieve highest level of nutrient removal within a shorter time (Table 24 and Table 25).

4.2.2. Semi-Continuous Operation

This study investigated the potential for nutrient removal from wastewater coupled with CO_2 sequestration when mixed microalgae culture was grown in semi-continuous PBRs. Within the scope of this study, three semi-continuous PBRs were operated at different HRT. The reactors were fed with coke plant wastewater diluted with sludge thickener effluent. The mixture had a TAN:PO_4^{3-}-P ratio of 6 (g/g), which was found to be the

optimum TAN:PO_4^{3-}-P (g/g) ratio for the studied mixed microalgae culture and experimental conditions (Section 4.2.1).

In order to investigate the effect of the HRT on the biomass production, nutrient removal and CO_2 sequestration potential of the mixed algae culture, HRT of the reactors was adjusted to 5 (the PBR named D5), 8 (the PBR named D8), and 12 (the PBR named D12) days. The conditions of temperature (28±2 ^oC), lighting (continuous, 120 µmol m^{-2} s^{-1}) and CO_2 sparging (4% CO_2-enriched at 0.5 vvm) were the same for all reactors during this study (Section 3.6.2.2).

The biomass production, O.D., TS and TVS, and concentration of nutrients, TAN and PO_4^{3-}-P, in the PBRs were measured from the start-up until the end of operation. Besides, Chlorophyll-a (Chl-a), TN and sCOD parameters were monitored when the reactors at steady-state conditions. The results of the analyses conducted for D5, D8 and D12 PBRs are shown graphically in Figure 27, Figure 28 and Figure 29, respectively.

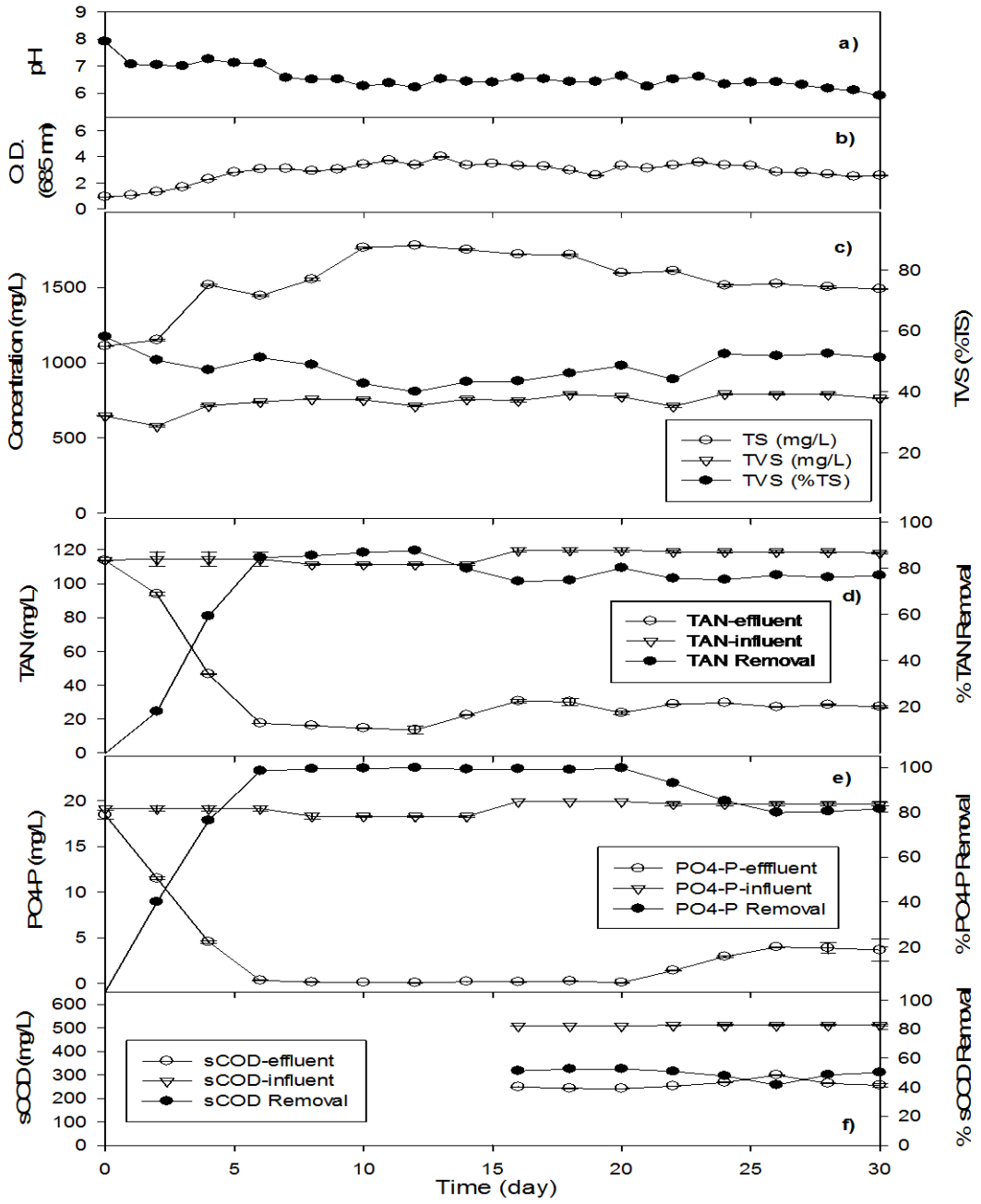


Figure 27 The change in a) pH, b) Optical Density, c) TS, TVS, TVS (%TS), d) TAN, e) PO_4^{3-}-P, f) sCOD in D5 PBR

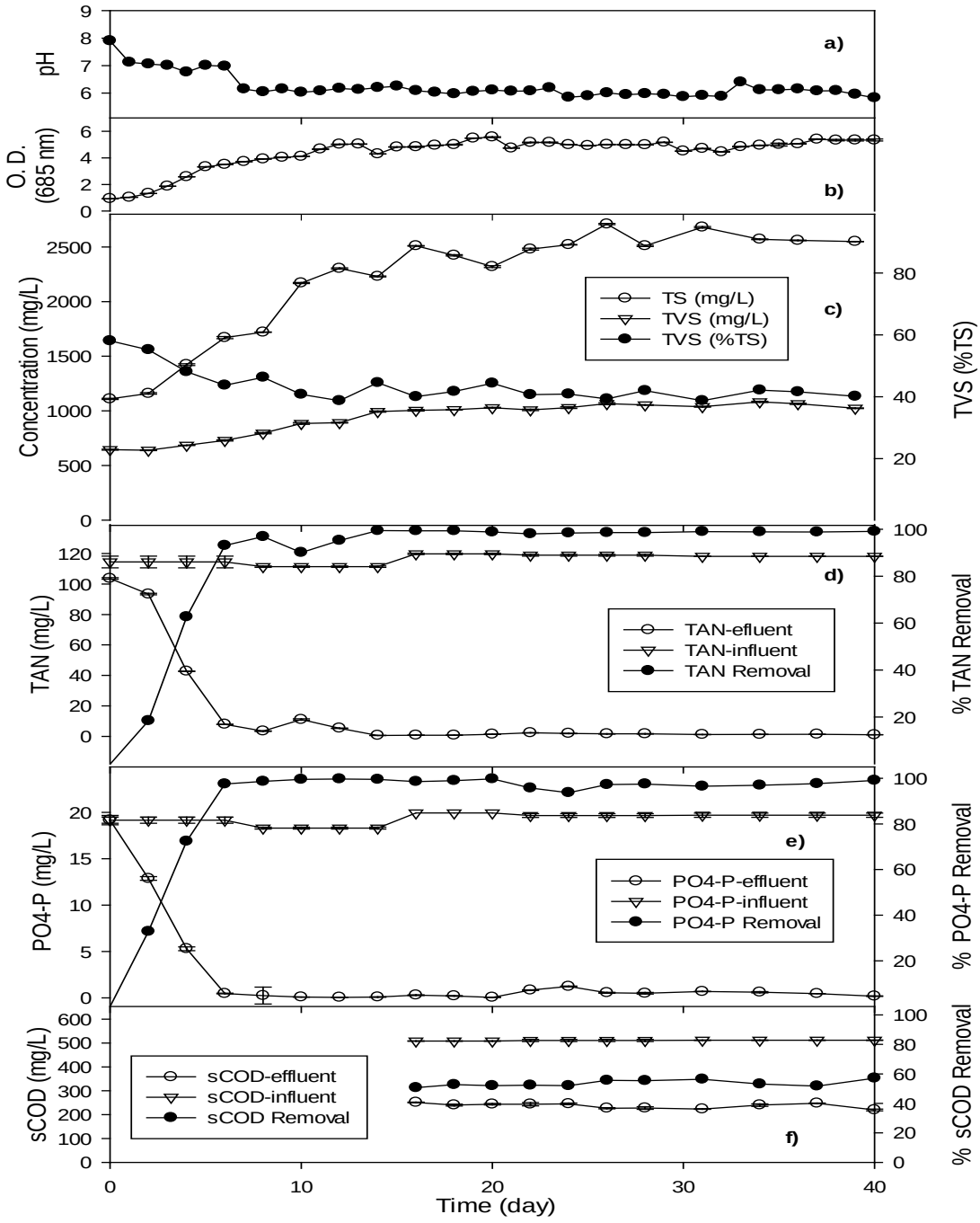


Figure 28 The change in a) pH, b) Optical Density, c) TS, TVS, TVS (%TS), d) TAN, e) PO_4^{3-}-P, f) sCOD in D8 PBR

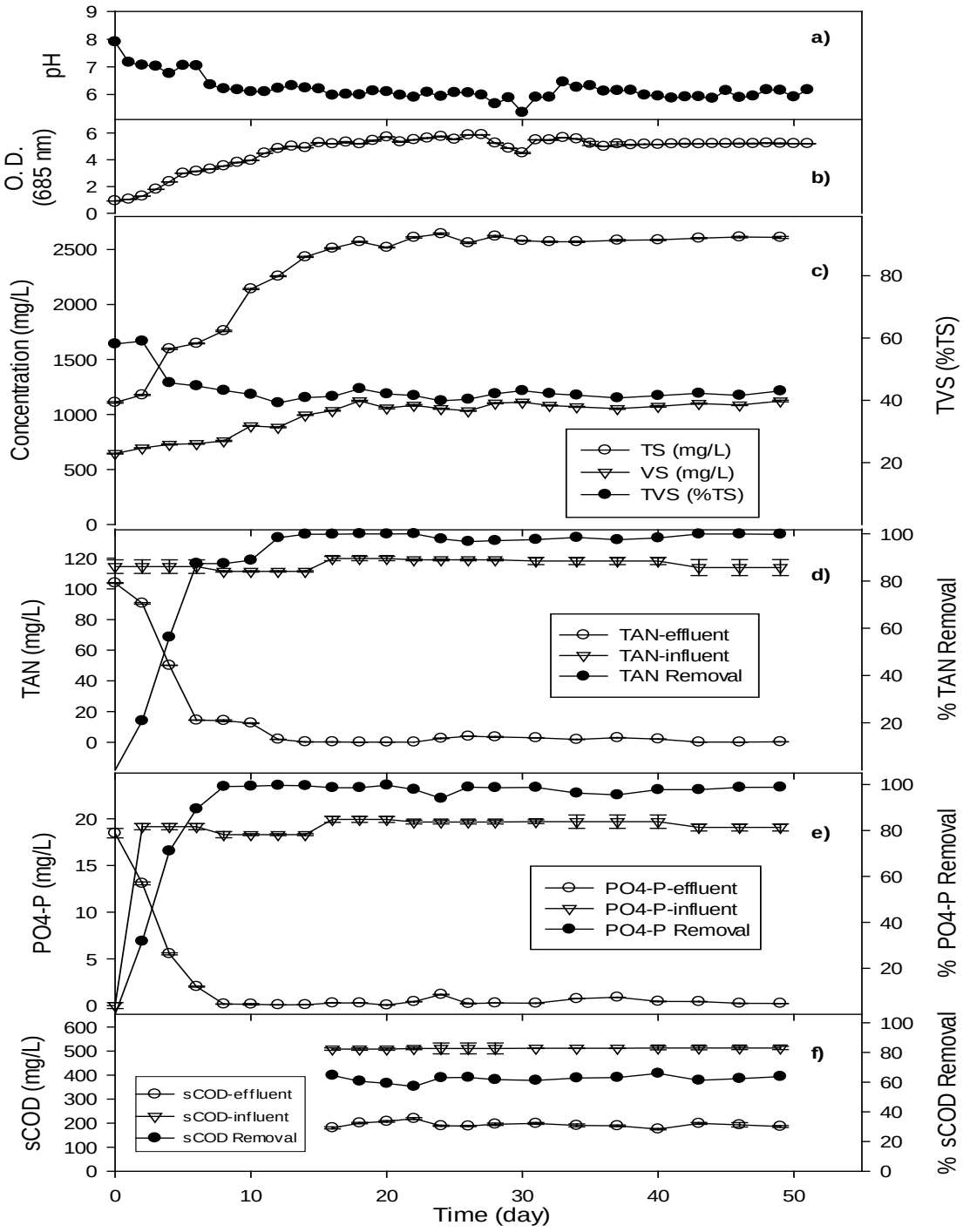


Figure 29 The change in a) pH, b) Optical Density, c) TS, TVS, TVS (%TS), d) TAN, e) PO_4^{3-}-P, f) sCOD in D12 PBR

4.2.2.1. The Effect of HRT on Biomass Production

When the O.D. and solids data of the PBRs are considered, it can be concluded that steady-state growth profile was achieved after 6th, 11th and 12th days of operation in D5, D8 and D12 photobioreactors, respectively (Figure 27b, c, Figure 28b, c and Figure 29b, c). The initial pH in all the reactors was 7.9. The pH values gradually decreased until steady-state conditions were observed (Figure 27a, Figure 28a and Figure 29a). At steady-state, the pH in the PBRs remained stable within the range of 5.85-6.5. Although no pH adjustment was made for the PBRs during their operation, it has been observed that supplied CO_2 provided necessary buffering capacity (measured pH values were between 6.0 and 6.6) for microalgae to grow successfully at all HRTs studied (Figure 27b, Figure 28b and Figure 29b).

The biomass data of the PBRs operated at various HRTs show that the algal cells grew well under continuous supply of 4% CO_2 and without any inhibition caused by the diluted industrial wastewater (Figure 27b, c, Figure 28b, c and Figure 29b, c). The initial OD, TS and TVS values of the PBRs were 0.92, 1110±7 mg/L and 645±4 mg/L, respectively. The average of the measured OD, TS, TVS and Chlorophyll-a (Chl-a) concentrations under steady-state conditions are given with their standard deviations (±) in Table 26. The number of samples used to calculate the average concentrations are given in brackets.

Table 26 Average steady-state biomass concentrations in the D5, D8 and D12 PBRs

PBR&HRT	OD at 685 nm	TS (mg/L)	TVS (mg/L)	Chl-a (mg/L)
D5 (5 days)	3.2±0.4 (25)	1612±118 (13)	760±28 (13)	25.8±0.9 (3)
D8 (8 days)	5.0±0.3 (32)	2489±140 (13)	1023±48 (13)	44.2±1.5 (4)
D12 (12 days)	5.3±0.3 (40)	2555±91 (17)	1063±57 (17)	43.2±1.7 (6)

Based on these results, the biomass production obtained in D12 reactor was the highest and it was comparable with the values achieved in D8 (Table 26). Whereas the biomass production in reactor D5 was significantly lower than these two reactors. In parallel with

the algal biomass concentrations, the lowest steady-state Chlorophyll-a concentration was measured in D5 and, higher and comparable Chlorophyll-a concentrations were obtained in the reactors D8 and D12 (Table 26). From these results, it can be concluded that the steady-state concentration of algal biomass increased when the HRT increased from 5 to 12 under the conditions of this study. When the growth rates are considered, longer HRT values increased the biomass production by introducing higher contact with light and carbon sources (Chae et al., 2006). Similar observation was reported in relevant studies. For example, Tang et al. (2012) reported the highest steady-state biomass concentration at the lowest flow rate (or the highest HRT) during the continuous cultivation of *Dunaliella tertiolecta* in a 6-L PBR operated under continuous light and 4% CO_2 sparging. Similar observation was made by Chae et al. (2006) during the semi-continuous cultivation of *Euglena gracilis* in Cramer-Myers medium and under continuous supply of 10% CO_2 enriched air.

It is important to note that higher biomass concentrations could be achieved in this semi-continuous study than those obtained in the previous semi-continuous study conducted with primary treated domestic wastewater and ambient air supply (Section 4.1.2). Higher biomass production obtained in this semi-continuous study can be explained by the proper N:P ratio which was selected in the batch study (Section 4.2.1), high nutrient concentrations in the feed wastewater which was a mixture of ammonia-rich coke plant wastewater and phosphorus-rich domestic wastewater as well as continuous supply of 4% CO_2.

Biomass concentrations observed in this study are slightly higher than those reported by relevant works. For example, Chae et al. (2006) studied semi-continuous cultivation of *Euglena gracilis* with Cramer–Myers medium, 10% CO_2 and HRT values between 5 and 8 days using a 100-L pilot-scale PBR and they obtained biomass concentrations between 510 mg/L for 5-day HRT and 910 mg/L for 8-day HRT. Tang et al. (2012) reported a biomass concentration of 0.726 g TS/L in the continuous cultivation of *Chlorella minutissima* in a 3-L PBR with continuous light of 50 µmol m^{-2} s^{-1}, 4% CO_2, and at a temperature of 25 ^{o}C. On the other hand, higher biomass concentrations of 5 g/L could be

maintained in the semi-continuous cultivation of *Chlorella sp.* NCTU-2 at HRT values of 2, 3 and 8 days under continuous light at 300 µmol m^{-2} s^{-1} and %5 CO_2 supply (Chiu et al., 2009).

4.2.2.2. The Effect of HRT on Nutrient Removal

The nutrient removal efficiencies were determined based on the nutrient concentrations in the effluent of the PBRs. The average of measured effluent TAN, PO_4^{3-}-P and TN concentrations under steady-state conditions and corresponding nutrient removal efficiencies are given with their standard deviations (±) in Table 27. The number of samples used to calculate the average concentrations are given in brackets.

Table 27 Average effluent concentrations and corresponding removal rates in D5, D8 and D12 PBRs

PBR	Effluent TAN (mg/L)	TAN Removal (%)	Effluent PO_4^{3-}-P (mg/L)	PO_4^{3-}-P Removal (%)	Effluent TN (mg/L)	TN Removal (%)	Effluent sCOD (mg/L)	sCOD Removal (%)
D5	23.8±6.3 (13)	79.6±4.9 (13)	1.3±1.6 (13)	93.3±8.4 (13)	25.8±1.8 (3)	80.3±1.5 (3)	258.4±17.6 (8)	49.3±3.6 (8)
D8	1.6±1.2 (13)	98.6±1.1 (13)	0.44±0.3 (13)	97.7±1.8 (13)	1.3±0.5 (4)	98.9±0.3 (4)	237.2±10.7 (11)	54.0±2.2 (11)
D12	1.42±1.42 (16)	99.0±1.2 (16)	0.4±0.3 (16)	98.1±1.6 (16)	2.1±1.3 (6)	98.4±1.0 (6)	193.8±11.1 (14)	62.0±2.2 (14)

From the results, it can be said that nutrient removal efficiencies had a parallel relationship with the growth data. As previously reported by Kapdan and Aslan (2008) and Lee et al. (2013), a balanced N:P ratio is important to achieve high nitrogen and phosphorus removal efficiencies. In the all PBRs, the nitrogen and phosphorus removal efficiencies were comparable due to the balanced N:P ratio of the influent. Although significant removals were achieved in the PBRs operating with 8 and 12 days of HRT, both TAN and PO_4^{3-}-P in the effluent were not completely removed (Figure 28d, e and Figure 29d, e). Therefore, it can be stated that a limiting factor, probably the light intensity, might have limited further biomass growth and nutrient removal in D8 and D12.

For the D8 and D12 PBRs, the average effluent concentrations of the nutrients were lower than 2 mg/L for TAN and 0.5 mg/L for PO_4^{3-}-P (Table 27), which makes the effluent in compliance with the Turkish Environmental Regulation on Water Pollution and Control (31/12/2004-No.25687) and with the EU directive 98/15/CE on urban wastewater treatment. However, the PBR with 5-day HRT (D5) resulted in slightly lower TN, TAN and PO_4^{3-}-P removal efficiencies than those obtained in the PBRs with 8-day and 12-day HRT (Table 27). This showed that 5-day retention period was not sufficient to achieve an efficient nutrient removal for the studied wastewater.

Besides nitrogen and phosphorus, effluent concentrations of sCOD were followed at steady-state conditions (Figure 27f, Figure 28f and Figure 29f). Although sCOD consumption was not expected due to the continuous supply of light and CO_2 enriched air, at least 49% sCOD removal could be achieved in the PBRs (Table 27). The removal rate was observed as high as 63.7% in PBR with 12-day HRT. As discussed by Delgadillo-Mirquez et al. (2016) microalgae can exhibit different metabolisms including heterotrophic and mixotrophic mode and, they are capable of metabolic shift in response to changes in environmental conditions such as light intensity and photoperiod. Although no dark period has been applied to the cultures, high cell concentrations in the reactors led to limitation in light penetration and increase the self-shading effect in the reactors (Cheah et al. 2014). This resulted in the mixotrophic growth conditions.

Nutrient removal efficiencies obtained in this semi-continuous study are comparable with relevant studies. For example, Woertz et al. (2009) achieved over 99% removal of ammonium and orthophosphate for CO_2-sparged mixed cultures with both 3-day and 4-day HRT. Li et al. (2013) reported that *Chlorella vulgaris* can efficiently remove ammonium and total phosphorus from primary clarifier effluent under 2% CO_2 enriched air by 98.4% and 91.8%, respectively. On the other hand, Ruiz-Martinez et al. (2012) used pure CO_2 to stabilize pH during the semi-continuous cultivation of a mixed microalgae culture in P-limited anaerobic process effluent and reported 67.2% ammonium and 97.8% phosphate removal efficiencies. In the cultivation of mixed culture in 2nd lagoon effluent under 2% CO_2, Lee et al. (2013) could achieve a high phosphorus removal efficiency of

over 80% but a low nitrogen removal efficiency of 30% due to the unbalanced N:P ratio. Tercero et al. (2013) reported 90% ammonia and 70% orthophosphate removal from the primary treated domestic wastewater with a continuously operated culture of *Chlorella protothecoides* at 1.26-day HRT under 5% CO_2.

Table 28 Nutrient removal efficiencies with CO_2 sparging by algae reported in literature

Microalgae Specie	**Nutrient Source**	**CO_2 Supply**	**HRT (days)**	**TAN Removal (%)**	**PO_4^{3-}-P Removal (%)**	**Reference**
Mixed Culture	Domestic wastewater	Pure CO_2 (99.9%) to adjust pH	3 and 4 days	99	99	(Woertz et al., 2009)
Chlorella vulgaris	Undigested dairy manure	2%	5 days	99.7	92 (TP)	(Wang et al., 2010)
Mixed Culture	Anaerobic process effluent	Pure CO_2 (99.9%) to adjust pH	2 days	67.2	97.8	(Ruiz-Martinez et al., 2012)
Chlorella vulgaris	Domestic wastewater	2%	5 days	98.4	91.8 (TP)	(Li et al., 2013)
Chlorella protothecoides	Domestic wastewater	5%	1.26 days	90	70	(Tercero et al, 2013)
Mixed Culture	Domestic wastewater	2%	3 days	80 (TN)	30 (TP)	(Lee et al., 2013)
Mixed Culture	Industrial and domestic wastewater mixture	4%	8 days	98.6	97.7	This Study

4.2.2.3. The Effect of HRT on CO_2 Reduction

Besides nutrient removal, CO_2 removal performance of the PBRs namely, D5, D8 and D12, was investigated in this study. In order to assess the amount of CO_2 consumed by the PBRs, the differences in CO_2 concentrations between inlet and outlet gas loads were monitored during the steady-state growth conditions (Mortezaeikia et al., 2016).

A 1-L control reactor was filled only with the wastewater (without algae inoculation) and operated for 2 days under the same experimental conditions in order to examine the abiotic removal of CO_2 (Chiu et al., 2008). The measurements showed that there was no significant difference between the inlet and outlet CO_2 concentrations (<1%), indicating that there was no abiotic CO_2 removal in the control reactor. A similar observation was made by Chiu et al. (2008) when CO_2-enriched air at different concentrations were given to a control reactor. Also, Keffer et al. (2002) reported that there was no abiotic CO_2 removal in the control reactor.

In this study, the CO_2 removal rates (mass-based) were calculated by the Equation 6 (Reddy, 2002):

$$CO2\ Removal\ Rate\ (\mathrm{g\ CO2/h}) = \frac{P,in - P,out}{R \times T} \times F \times MW \times t \qquad \text{(Equation 6)}$$

Where;

P_{in}=0.039 atm (example for a 3.99% (v/v) inlet CO_2 concentration)
P_{out}=0.036 atm (example for a 3.6% (v/v) outlet CO_2 concentration)
R=0.082057 L.atm/mol.K
T=301 K (28 ^{o}C)
F=Air flow rate (0.5 vvm or 0.5 L_{air}/min.$L_{working\ volume}$)
MW=Molecular weight of CO_2 (44 g/mol)
t=60 min/h

The average of the measured inlet and outlet CO_2 concentrations under steady-state conditions and corresponding CO_2 removal efficiencies (%) and CO_2 removal rates (g

CO_2/h) are given with their standard deviations (±) in Table 29. The number of samples used to calculate the average concentrations are given in brackets.

Table 29 CO_2 removal by the PBRs

PBR	Inlet CO_2 (%)	Outlet CO_2 (%)	Average CO_2 Removal (%)	Inlet CO_2 (g CO_2/h)	Outlet CO_2 (g CO_2/h)	Average CO_2 Removal (g CO_2/h)
D5	3.99±0.05 (3)	3.63±0.01 (3)	8.90±0.97 (3)	2.12±0.03 (3)	1.93±0.01 (3)	0.189±0.023 (3)
D8	4.00±0.03 (7)	3.24±0.02 (7)	19.12±0.56 (7)	2.12±0.02 (7)	1.72±0.01 (7)	0.407±0.014 (7)
D12	4.02±0.03 (10)	3.19±0.07 (10)	20.46±1.63 (10)	2.13±0.02 (10)	1.70±0.03 (10)	0.436±0.004 (10)

In the PBR with 5-day HRT (D5), CO_2 measurements were performed on days 24, 26 and 28 within the steady-state operation period. The lowest CO_2 removal efficiencies were obtained (8.1-10%) in this reactor. The operation of the PBR was stopped on 30th day due to low nutrient (Section 4.2.2.2) and CO_2 removal efficiencies as a result of low microalgae growth in the reactor.

In the reactor with 8-day HRT (D8), the measurements were made on different days 24, 26, 28, 31, 34, 37 and 40 and it has been observed that CO_2 removal efficiency was in the range of 18.4% and 19.9%.

In the reactor with 12-day HRT, the measurements were done on 10 days between days 24 and 49. Although there was no significant difference between performances of the D8 and D12 reactors, the highest CO_2 removal efficiency was measured in D12. The removal efficiencies were in the range of 18.4-23.7%. Figure 30 and Figure 31 show the CO_2 removal efficiencies and CO_2 removal rates based on mass, respectively, obtained during the operation of the D12 photobioreactor.

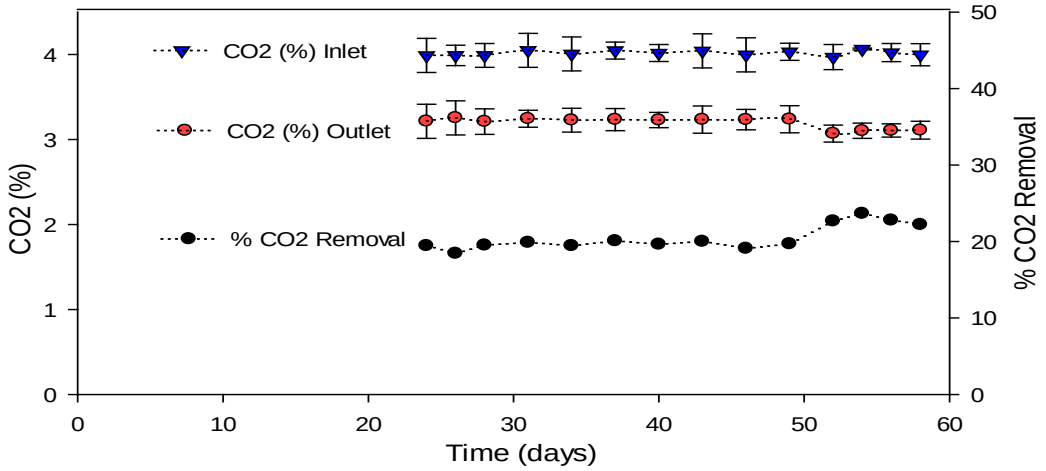


Figure 30 Inlet & outlet CO_2 concentrations and CO_2 removal efficiencies in D12 PBR

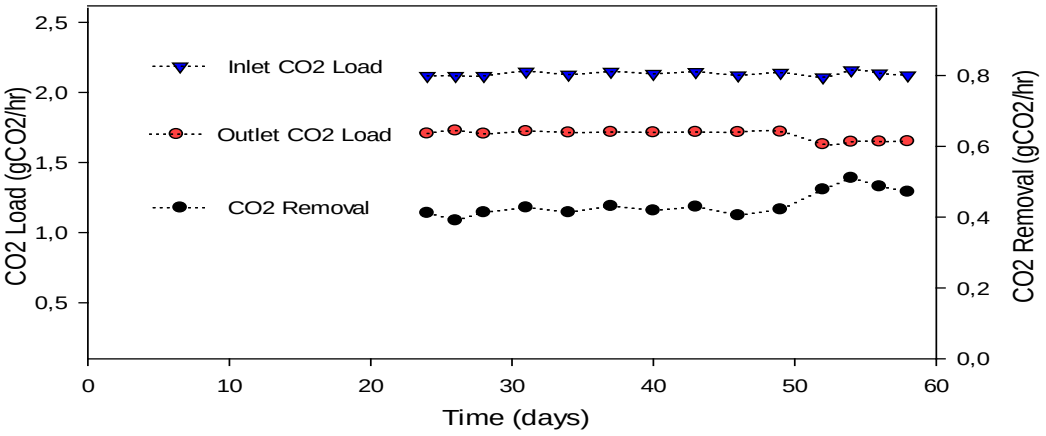


Figure 31 Inlet & outlet CO_2 loads and CO_2 removal efficiencies in D12 PBR

These results indicate that as biomass concentration increases, CO_2 removal rate also increases due to utilization of CO_2 during photosynthesis. It is important to note that CO_2 removal is dependent on various factors including the characteristic of the microalgae strain, cultivation system and reactor type, operating conditions (light intensity, photoperiod and aeration rate etc.) and CO_2 concentration (Cheah et al., 2014; Van Den Hende et al., 2012). Therefore, different values have been reported in the literature for the CO_2 sequestration by algae (Table 30).

The CO_2 removal rates obtained in this study are comparable with those reported by Chiu et al. (2009) and Reddy (2002). Chiu et al. (2009) reported a CO_2 reduction rate of 0.316 g/h in the semi-continuous cultivation of *Chlorella sp*. NCTU-2 with 5% CO_2, 0.25 vvm aeration and continuous illumination in the 4-L bubble column PBR. The results of their study also showed that CO_2 removal efficiency in the porous centric-tube photobioreactor was about 50% higher than those in the bubble column photobioreactor. Moreover, CO_2 removal efficiency could be increased by using high density cultures (5.15 g/L) and lower aeration rates (0.125 vvm). Similarly, Reddy (2002) has reported 0.6 g/h CO_2 removal for mixed algae culture grown in a flat-plate PBR with 5% CO_2 at an aeration rate of 0.132 L/min. On the other hand, Chae et al. (2006) has reported a much lower CO_2 removal rate of 0.003 g/h (74 g/m^3 per day) with L-Shaped Innovative PBR in the semi-continuous cultivation of *Euglena gracilis* at 10% CO_2-enriched air supply. It should be noted that they have calculated the removal rate based on the daily average biomass production value of 113 g/dry cell. Keffer et al. (2002) reported higher CO_2 removal rates between 4.4 g/h and 14.2 g/h with an average of 12.5 g CO_2/h when the *Chlorella vulgaris* culture was exposed to an air stream with 0.185% CO_2. The high CO_2 removals can be explained by the low CO_2 concentration utilized and tubular reactor configuration allowing efficient high light penetration.

Table 30 Reported CO_2 removal efficiencies by microalgae

Reactor Type	Culture	Mode	Light	Inlet CO_2 (%)	CO_2 Removal Rate (g/h)	Reference
Tubular	*C. vulgaris*	Continuous	Continuous	0.185	12.5	(Keffer et al., 2002)
Flat Plate	Mixed	Continuous	Continuous	5	0.6	(Reddy, 2002)
L-Shaped Innovative PBR	*Euglena gracilis*	Semi-cont.	Continuous	10	0.003	(Chae et al., 2006)
Bubble Column PBR	*Chlorella sp.*	Continuous	Continuous	5	0.316	(Chiu et al., 2009)
Test PBR (Bubble Column)	Mixed	Semi-cont.	Continuous	4	0.436	This Study

4.3. Summary of the Results

In this study, biomass production, nutrient removal and CO_2 sequestration potential of batch and semi-continuous mixed microalgae cultures were investigated using the unsterilized wastewaters from Ankara Tatlar WWTP and industrial wastewater from KARDEMIR Coke Plant. Besides, the effects of inoculum volume, HRT, N:P ratio of wastewater and CO_2-enriched air supply on the algal growth and nutrient removal were monitored in different phases of this study.

In the first part of the study, unsterilized primary treated domestic wastewater was used as the cultivation medium. It was shown that the domestic wastewater can provide adequate nitrogen and phosphorus for the growth of microalgae without a supply of additional nutrients. It was also found that competition with endogenous bacteria did not affect the growth of the algae.

In the batch set-up with primary treated domestic wastewater, the effects of inoculum volume on the growth and nutrient removal rates were investigated. It was found that 10% (v/v) was more suitable in terms of higher biomass production and nutrient removal rates achieved within a shorter time. This inoculum volume was used throughout the experiments.

In the semi-continuous operation with domestic wastewater, PBRs were operated with HRTs of 2, 4 and 8 days. It was observed that, even at a low HRT of 2 days, mixed microalgae culture achieved removal efficiencies of 94.7 % and 93.8% for TAN and PO_4^{3-}-P, respectively, at steady-state growth period. The effluent nutrient levels (TN < 2 mg/L and PO_4^{3-}-P < 0.5 mg/L) obtained were lower than the strictest discharge standards in Turkey and EU and, it was proved that the mixed microalgae culture is applicable for treatment of domestic wastewater. The steady-state TS and TVS concentrations in 2-day HRT culture were 0.96 g/L and 0.58 g/L, respectively. However, for the PBRs with HRT of 4 and 8 days, steady-state conditions could not be maintained and the cultures collapsed as a result of nutrient limitation.

After the semi-continuous study, a kinetic study was conducted with the domestic wastewater to determine parameters for microalgal growth and nutrient removal. The mixed culture could completely remove TAN in domestic wastewater within 72 hours with a 8.9 mg/L.d removal rate. PO_4^{3-}-P removal rate was 1.1 mg/L.d in the same study.

In the second part of the study, mixtures of a phosphorus-rich sludge thickener supernatant and nitrogen-rich coke plant wastewater were used as the culture media. The effect of the N:P (TAN/PO_4^{3-}-P (g/g)) ratio of wastewater on the algal growth and nutrient removal rate was investigated by performing a batch study under continuous CO_2-enriched air supply. Within the scope of this batch study, coke plant wastewater was diluted with sludge thickener tank supernatant to obtain different TAN/PO_4^{3-}-P (g/g) ratios. The TAN/PO_4^{3-}-P (g/g) ratio of 6 resulted in the maximum nutrient removal efficiency. This ratio then was used in the upcoming semi-continuous study.

During the semi-continuous experiment with diluted coke plant wastewater, the effect of HRT on nutrient removal was investigated under continuous CO_2 enriched air supply. The HRTs of 5, 8 and 12 were selected for this study. Excellent nutrient removal and high algal biomass concentrations could be achieved in PBRs with 8 and 12 day-HRT at steady-state conditions as a result of nutrient rich cultivation medium with balanced N: P ratio and CO_2 enriched air supplied to the cultures. Results revealed that HRT should be kept 8 days at minimum in order to achieve efficient TAN and PO_4^{3-}-P removal (> 98%) and high steady-state biomass concentrations (> 2.4 mg TS/L) in semi-continuous PBR operation with the studied wastewater under experimental conditions of this study.

Besides nutrient removal, CO_2 removal performance of the PBRs was investigated in the second part. The results showed that CO_2 sequestration rate had a parallel relationship with the biomass concentration and, the highest CO_2 removal was observed at 12-day HRT. The CO_2 removal rates in the PBR with 12 day-HRT was 0.436 g CO_2/h.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

The results obtained in this study are promising. It was shown that primary treated domestic wastewater and coke plant wastewater diluted with thickener supernatant can be successfully used as cultivation medium for mixed microalgae culture. The high productivities obtained in the set-ups also demonstrated the potential of mixed microalgae cultures for integrated CO_2 sequestration and biomass production in unsterilized wastewater mediums. Moreover, the produced algal biomass can be used to produce a broad portfolio of fuels, such as biodiesel, bioethanol, and biogas. However, structure of lipid derived from harvested algae biomass should be examined if it is planned to produce biodiesel from biomass.

The results obtained in the batch and semi-continuous experiments conducted within the scope of this study provided a better understanding of the behavior of the mixed culture in different growth conditions. Most importantly, information obtained on the steady state behavior of the cultures can aid in optimization of process design of future pilot scale applications and then in the development of economically and environmentally sustainable microalgae cultivation systems at larger scale.

Prior to the implementation of a pilot scale facility, lab-scale continuous cultivation operations investigating the light/dark cycles are strongly suggested since continuous illumination was provided to the batch and semi-continuous cultures in this study. Besides, the effect of aeration rate should be deeply investigated in lab-scale studies. The aeration rates lower than 0.5 vvm should be examined. Further research should also be made to understand the effects of impurities contained in real flue gases from thermal power plants, cement plant and iron and steel plants such as NO and SO_3, ash and heavy metals on the growth of mixed microalgae culture.

Lastly, further research is needed for well-designed photobioreactors to achieve higher photosynthetic efficiencies and carbon dioxide fixation capacities. Evaluation of the continuous cultivation of the mixed microalgae culture in well-designed and pilot scale outdoor photobioreactors which increase the contact time between the flue gas and liquid phase is needed to assess the economic feasibility of the microalgae based CO_2 sequestration system at a large scale.

REFERENCES

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

Akçansa. (2015). *Akçansa Cement Company 2014-2015 Sustainability Report*. Available at http://www.akcansa.com.tr/downloads/surdurebilirlik/surdurulebilirlik2015-en.pdf.

Ale, M. T., Pinelo, M., Meyer, A. S. (2014). Assessing effects and interactions among key variables affecting the growth of mixotrophic microalgae: pH, inoculum volume, and growth medium composition. *Preparative Biochemistry and Biotechnology*, *44*(3), 242–256. doi:10.1080/10826068.2013.812562

APHA, AWWA & WPCF. (2005). *Standard Methods for the Examination of Water and Wastewater*, 21th edition. American Public Health Association, Washington DC, USA.

Arenas, E.G., Palacio, M. C. R., Juantorena, A. U., Fernando, S. E. L., Sebastian, P. J. (2016). Microalgae as a potential source for biodiesel production: Techniques, methods, and other challenges. *International Journal of Energy Research*. doi:10.1002/er.3663

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

Bamba, B. S. B., Lozano, P., Adjé, F., Ouattara, A., Vian, M. A., Tranchant, C., & Lozano, Y. (2015). Effects of temperature and other operational parameters on Chlorella vulgaris mass cultivation in a simple and low-cost column photobioreactor. *Applied Biochemistry and Biotechnology*, *177*(2), 389–406. doi:10.1007/s12010-015-1751-7

Barsanti, L., & Gualtieri, P. (2006). *Algae - Anatomy, biochemistry and biotechnology*. Boca Raton, FL: CRC Press.

Becker. E.W. (1994). *Microalgae: Biotechnology and Microbiology*. Cambridge: Cambridge University Press.

Behera, S. N., Sharma, M., Aneja, V. P., & Balasubramanian, R. (2013). Ammonia in the atmosphere: A review on emission sources, atmospheric chemistry and deposition on terrestrial bodies. *Environmental Science and Pollution Research*, *20*(11), 8092–8131. doi:10.1007/s11356-013-2051-9

Benemann, J. R., Olst, J. C. Van, Massingill, M. J., Weissman, J. C., & Brune, D. E. (2002). The controlled eutrophication process: Using microalgae for CO2 utilization and agricultural fertilizer recycling. *Water*, *2*(1), 1433–1438.

Bhakta, J. N., Lahiri, S., Pittman, J. K., & Jana, B. B. (2015). Carbon dioxide sequestration in wastewater by a consortium of elevated carbon dioxide-tolerant microalgae, *10*, 105–112. doi:10.1016/j.jcou.2015.02.001

Bilanovic, D., Andargatchew, A., Kroeger, T., & Shelef, G. (2009). Freshwater and marine microalgae sequestering of CO2 at different C and N concentrations – Response surface methodology analysis. *Energy Conversion and Management*, *50*(2), 262–267. doi:10.1016/j.enconman.2008.09.024

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

Boelee, N. C., Temmink, H., Janssen, M., Buisman, C. J. N., & Wijffels, R. H. (2012). Scenario analysis of nutrient removal from municipal wastewater by microalgal biofilms. *Water*, *4*(2), 460–473. doi:10.3390/w4020460

Bohutskyi, P., Cynamon, D., Byers, N., Khaled, L., Cua, C., Chow, S., … Bouwer, E. J. (2016). Effects of inoculum size, light intensity, and dose of anaerobic digestion centrate on growth and productivity of Chlorella and Scenedesmus microalgae and their poly-culture in primary and secondary wastewater. *Algal Research*, *19*, 278–290. doi:10.1016/j.algal.2016.09.010

Boonchai, R., Seo, G. T., Park, D. R., & Seong, C. Y. (2012). Microalgae photobioreactor for nitrogen and phosphorus removal from wastewater of sewage treatment plant. *International Journal of Bioscience, Biochemistry and Bioinformatics*, *2*(6), 407–410. doi:10.7763/IJBBB.2012.V2.143

Brooker, B. (2011). *Investigation of microalgae growth kinetics using coal-fired flue gas as a carbon source*. California Polytechnic State University, San Luis Obispo, CA, USA.

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

Calicioglu, A. O. (2013). *Integrated nutrient removal and biogas production using microalgal and anaerobic microbial cultures*. Middle East Technical University, Ankara, Turkey.

Cassidy, K. O. (2011). *Evaluating algal growth at different temperatures*. University of Kentucky, Lexington, KY, USA.

Castellanos, C. S. (2013). *Batch and continuous studies of chlorella vulgaris in photo-bioreactors*. The University of Western Ontario, London, Ontario, Canada.

Chae, S. R., Hwang, E. J., & Shin, H. S. (2006). Single cell protein production of Euglena gracilis and carbon dioxide fixation in an innovative photo-bioreactor. *Bioresource Technology*, *97*(2), 322–9. doi:10.1016/j.biortech.2005.02.037

Cheah, W. Y., Ling, T. C., Show, P. L., Juan, J. C., Chang, J. S., & Lee, D. J. (2016). Cultivation in wastewaters for energy: A microalgae platform. *Applied Energy*, *179*, 609–625. doi:10.1016/j.apenergy.2016.07.015

Cheah, W. Y., Show, P. L., Chang, J. S., Ling, T. C., & Juan, J. C. (2014).

Biosequestration of atmospheric CO2 and flue gas-containing CO2 by microalgae. *Bioresource Technology*, *184*, 190–201. doi:10.1016/j.biortech.2014.11.026

Chen, C. Y., & Durbin, E. G. (1994). Effects of pH on the growth and carbon uptake of marine phytoplankton. *Marine Ecology Progress Series*, 109(1), 83–94. doi:10.3354/meps109083

Chen, M. & Graedel, T. E. (2016). A half-century of global phosphorus flows, stocks, production, consumption, recycling, and environmental impacts. *Global Environmental Change*, 36, 139–152. doi:10.1016/j.gloenvcha.2015.12.005

Chinnasamy, S., Bhatnagar, A., Hunt, R. W., & Das, K. C. (2010). Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresource Technology*, *101*(9), 3097–105. doi:10.1016/j.biortech.2009.12.026

Chinnasamy, S., Ramakrishnan, B., Bhatnagar, A., & Das, K. C. (2009). Biomass production potential of a wastewater alga Chlorella vulgaris ARC 1 under elevated levels of CO2 and temperature. *International Journal of Molecular Sciences*, *10*(2), 518–32. doi:10.3390/ijms10020518

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

Chiu, S.-Y., Kao, C.-Y., Chen, C.-H., Kuan, T.-C., Ong, S.-C., & Lin, C.-S. (2008). Reduction of CO2 by a high-density culture of Chlorella sp. in a semicontinuous photobioreactor. *Bioresource Technology*, *99*(9), 3389–96. doi:10.1016/j.biortech.2007.08.013

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

Chiu, S. Y., Tsai, M. T., Kao, C. Y., Ong, S. C., & Lin, C. S. (2009). The air-lift photobioreactors with flow patterning for high-density cultures of microalgae and carbon dioxide removal. *Engineering in Life Sciences*, *9*(3), 254–260. doi:10.1002/elsc.200800113

Choi, O., Das, A., Yu, C.-P., & Hu, Z. (2010). Nitrifying bacterial growth inhibition in the presence of algae and cyanobacteria. *Biotechnology and Bioengineering*, *107*(6), 1004–11. doi:10.1002/bit.22860

Condie, K. C. (2015). *Earth as an Evolving Planetary System*. London: Elsevier Academic Press. doi:10.1016/B978-0-12-803689-1.00008-0

Cordell, D., Rosemarin, A., Schröder, J. J., & Smit, A. L. (2011). Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. *Chemosphere*, 84(1), 747–758. doi: 10.1016/j.chemosphere.2011.02.032

Costa, J. A. V., & de Morais, M. G. (2011). The role of biochemical engineering in the production of biofuels from microalgae. *Bioresource Technology*, *102*(1), 2–9.

doi:10.1016/j.biortech.2010.06.014

Dash, R. R., Gaur, A., & Balomajumder, C. (2009). Cyanide in industrial wastewaters and its removal: a review on biotreatment. *Journal of Hazardous Materials*, *163*(1), 1–11. doi:10.1016/j.jhazmat.2008.06.051

de Godos, I., Arbib, Z., Lara, E., & Rogalla, F. (2016). Evaluation of High Rate Algae Ponds for treatment of anaerobically digested wastewater: Effect of CO2 addition and modification of dilution rate. *Bioresource Technology*, *220*, 253–261. doi:10.1016/j.biortech.2016.08.056

de la Noue, J., & de Pauw, N. (1988). The potential of microalgal biotechnology: A review of production and uses of microalgae. *Biotechnology Advances*, *6*(4), 725–770. doi:10.1016/0734-9750(88)91921-0

de Morais, M. G., & Costa, J. A. V. (2007). Biofixation of carbon dioxide by Spirulina sp. and Scenedesmus obliquus cultivated in a three-stage serial tubular photobioreactor. *Journal of Biotechnology*, *129*(3), 439–445. doi:10.1016/j.jbiotec.2007.01.009

de Vree, J. H., Bosma, R., Janssen, M., Barbosa, M. J., & Wijffels, R. H. (2015). Comparison of four outdoor pilot-scale photobioreactors. *Biotechnology for Biofuels*, *8*(1), 215. doi:10.1186/s13068-015-0400-2

Delgadillo-Mirquez, L., Lopes, F., Taidi, B., & Pareau, D. (2016). Nitrogen and phosphate removal from wastewater with a mixed microalgae and bacteria culture. *Biotechnology Reports*, *11*, 18–26. doi:10.1016/j.btre.2016.04.003

Ding, J., Zhao, F., Cao, Y., Xing, L., Liu, W., Mei, S., & Li, S. (2015). Cultivation of microalgae in dairy farm wastewater without sterilization. *International Journal of Phytoremediation*, 17(3), 222-227. doi:10.1080/15226514.2013.876970

Doucha, J., Straka, F., & Lívanský, K. (2005). Utilization of flue gas for cultivation of microalgae Chlorella sp. in an outdoor open thin-layer photobioreactor. *Journal of Applied Phycology*, *17*(5), 403–412. doi:10.1007/s10811-005-8701-7

Douskova, I., Doucha, J., Livansky, K., Machat, J., Novak, P., Umysova, D., Zachleder, V., Vitova, M. (2009). Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. *Applied Microbiology and Biotechnology*, *82*(1), 179–85. doi:10.1007/s00253-008-1811-9

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

El-behlil, M. A., Sead, E., & Adma, S. A. (2012). *Volatile organic chemicals removal from contaminated water using air stripping low profile sieve tray towers*. Proceedings of the 16th International Water Technology Conference, Istanbul, 1–16.

Fernández, F. G. A., González-López, C. V, Fernández Sevilla, J. M., & Molina Grima, E. (2012). Conversion of CO2 into biomass by microalgae: How realistic a contribution may it be to significant CO2 removal? *Applied Microbiology and*

Biotechnology, *96*(3), 577–86. doi:10.1007/s00253-012-4362-z

Filali, R., Tebbani, S., & Dumur, D. (2011). *Growth modeling of the green microalga Chlorella vulgaris in an air-lift photobioreactor*. Proceedings of the 18th International Federation of Automatic Control World Congress, Milano, 10603–10608.

Fontes, A. G., Angeles Vargas, M., Moreno, J., Guerrero, M. G., & Losada, M. (1987). Factors affecting the production of biomass by a nitrogen-fixing blue-green alga in outdoor culture. *Biomass*, *13*(1), 33–43. doi:10.1016/0144-4565(87)90070-9

Garcia, J., Mujeriego, R., & Hernandez-Marine, M. (2000). High rate algal pond operating strategies for urban wastewater nitrogen removal. *Applied Phycology*, *12*, 331–339.

Gielen, D. (2003). CO2 removal in the iron and steel industry. *Energy Conversion and Management*, *44*(7), 1027–1037. doi:10.1016/S0196-8904(02)00111-5

Goldman, J. C., Azov, Y., Riley, C. B., & Dennett, M. R. (1982). The effect of pH in intensive microalgal cultures. *Journal of Experimental Marine Biology and Ecology*, *57*, 1–13.

Gómez-Villa, H., Voltolina, D., Nieves, M., & Piña, P. (2005). Biomass production and nutrient budget in outdoor cultures of Scenedesmus obliquus (Chlorophyceae) in artificial wastewater, under the winter and summer conditions of Mazatlan, Sinaloa, Mexico. *Vie et Milieu*, *55*(2), 121–126.

Green, F. B., Lundquist, T. J., & Oswald, W. J. (1995). Energetics of advanced integrated wastewater pond systems. *Water Science and Technology*, *31*(12), 9–20. doi: 10.1016/0273-1223(95)00488-9

Grima, E. M., Acie, F. G., & Chisti, Y. (1999). Photobioreactors : light regime, mass transfer, and scaleup. *Journal of Biotechnology*, *70*, 231–247.

Gupta, D., & Singh, S. K. (2012). Greenhouse gas emissions from wastewater treatment plants: A case study of Noida. *Journal of Water Sustainability*, *1*(2), 131–140.

Haiduc, A. G., Brandenberger, M., Suquet, S., Vogel, F., Bernier-Latmani, R., & Ludwig, C. (2009). SunCHem: an integrated process for the hydrothermal production of methane from microalgae and CO2 mitigation. *Journal of Applied Phycology*, *21*(5), 529–541. doi:10.1007/s10811-009-9403-3

Hammitt, J. K., & Adams, J. L. (1996). The value of international cooperation for abating global climate change, *Resource and Energy Economics*, 18(3), 219–241. doi:10.1016/S0928-7655(96)00008-5

Hammouda, O., Gaber, A., & Abdelraouf, N. (1995). Microalgae and wastewater treatment. *Ecotoxicology and Environmental Safety*, *31*(3), 205–210. doi:10.1016/j.sjbs.2012.04.005

Hanagata, N., Takeuchi, T., Fukuju, Y., Barnes, D. J., & Karube, I. (1992). Tolerance of microalgae to high CO2 and high temperature. *Phytochemistry*, *31*(10), 3345–3348. doi:10.1016/0031-9422(92)83682-O

Harrington, L., & Foster, R. (1999). *Australian residential building sector greenhouse gas emissions 1990–2010*, Australian Greenhouse Office, Canberra.

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

He, P. J., Mao, B., Shen, C. M., Shao, L. M., Lee, D. J., & Chang, J. S. (2013). Cultivation of Chlorella vulgaris on wastewater containing high levels of ammonia for biodiesel production. *Bioresource Technology*, *129*, 177–81. doi:10.1016/j.biortech.2012.10.162

He, Q., Yang, H., Wu, L., & Hu, C. (2015). Effect of light intensity on physiological changes, carbon allocation and neutral lipid accumulation in oleaginous microalgae. *Bioresource Technology*, *191*, 219–228. doi:10.1016/j.biortech.2015.05.021

Ho, S.-H., Chen, W.-M., & Chang, J.-S. (2010). Scenedesmus obliquus CNW-N as a potential candidate for CO2 mitigation and biodiesel production. *Bioresource Technology*, *101*(22), 8725–30. doi:10.1016/j.biortech.2010.06.112

Hongyang, S., Yalei, Z., Chunmin, Z., Xuefei, Z., & Jinpeng, L. (2011). Cultivation of Chlorella pyrenoidosa in soybean processing wastewater. *Bioresource Technology*, *102*(21), 9884–90. doi:10.1016/j.biortech.2011.08.016

Hsueh, H. T., Chu, H., & Yu, S. T. (2007). A batch study on the bio-fixation of carbon dioxide in the absorbed solution from a chemical wet scrubber by hot spring and marine algae. *Chemosphere*, *66*(5), 878–86. doi:10.1016/j.chemosphere.2006.06.022

Hu, B., Min, M., Zhou, W., Li, Y., Mohr, M., Cheng, Y., Lei, H., Liu, Y., Lin, X., Chen, P., Ruan, R. (2012). Influence of exogenous CO2 on biomass and lipid accumulation of microalgae Auxenochlorella protothecoides cultivated in concentrated municipal wastewater. *Applied Biochemistry and Biotechnology*, *166*(7), 1661–1673. doi:10.1007/s12010-012-9566-2

Hulatt, C. J., & Thomas, D. N. (2011a). Energy efficiency of an outdoor microalgal photobioreactor sited at mid-temperate latitude. *Bioresource Technology*, *102*(12), 6687–95. doi:10.1016/j.biortech.2011.03.098

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

Izhar, M. (2016). *Removal of nitrate from domestic wastewaters using algae*. Hacettepe University, Ankara, Turkey.

Jacob-Lopes, E., Scoparo, C. H. G., Lacerda, L. M. C. F., & Franco, T. T. (2009). Effect of light cycles (night/day) on CO2 fixation and biomass production by microalgae in photobioreactors. *Chemical Engineering and Processing: Process Intensification*, *48*(1), 306–310. doi:10.1016/j.cep.2008.04.007

Jacob, A., Xia, A., & Murphy, J. D. (2015). A perspective on gaseous biofuel production

from micro-algae generated from CO2 from a coal-fired power plant. *Applied Energy*, *148*, 396–402. doi:10.1016/j.apenergy.2015.03.077

Jansson, C., & Northen, T. (2010). Calcifying cyanobacteria-the potential of biomineralization for carbon capture and storage. *Current Opinion in Biotechnology*, *21*(3), 365–71. doi:10.1016/j.copbio.2010.03.017

Jayasingha, P., Pitawala, A. & Dharmagunawardane, H.A. (2012). A review on nutrient pollution of coastal aquifers of Sri Lanka. In V. Subramanian (Ed.) *Coastal Environments: Focus on Asian Regions* (pp 66–77). The Netherlands: Springer. doi:10.1007/978-90-481-3002-3

Ji, F., Liu, Y., Hao, R., Li, G., Zhou, Y., & Dong, R. (2014). Biomass production and nutrients removal by a new microalgae strain Desmodesmus sp. in anaerobic digestion wastewater. *Bioresource Technology*, *161*, 200–207. doi:10.1016/j.biortech.2014.03.034

Jiang, Y., Zhang, W., Wang, J., Chen, Y., Shen, S., & Liu, T. (2013). Utilization of simulated flue gas for cultivation of Scenedesmus dimorphus. *Bioresource Technology*, *128*, 359–364. doi:10.1016/j.biortech.2012.10.119

Johnston, N. A., Blake, D.R., Rowland, F. S., Elliott, S., Lackner, K. S., Ziock, H. J., Dubey, M. K., Hanson H. P., Barr, S. (2003). Chemical transport modeling of potential atmospheric CO2 sinks. *Energy Conversion and Management*, *44*(5), 681–689. doi:10.1016/S0196-8904(02)00078-X

Kadam, K. L. (1997). Power plant flue gas as a source of CO2 for microalgae cultivation: Economic impact of different process options. *Energy Conversion and Management*, *38*, 505–510. doi.org/10.1016/S0196-8904(96)00318-4

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

Keffer, J. E., & Kleinheinz, G. T. (2002). Use of Chlorella vulgaris for CO2 mitigation in a photobioreactor. *Journal of Industrial Microbiology & Biotechnology*, *29*(5), 275–80. doi:10.1038/sj.jim.7000313

Kessler, E. (1985). Upper limits of temperature for growth in Chlorella (Chlorophyceae). *Plant Systematics and Evolution*, *151*(1), 67–71. doi:10.1007/BF02418020

Kiepper, B. H. (2013). Microalgae Utilization in Wastewater Treatment, *University of Georgia Cooperative Extension Bulletin*, 1419, 1-5.

Kim, S.-R., Woo, S.-S., Cheong, E.-H., & Ahn, T.-S. (2003). Nutrient removal from sewage by an artificial food web system composed of phytoplankton and Daphnia magna. *Ecological Engineering*, *21*(4), 249–258. doi:10.1016/j.ecoleng.2003.11.003

Khoeyi, A. Z., Seyfabadi, J., & Ramezanpour, Z. (2012). Effect of light intensity and photoperiod on biomass and fatty acid composition of the microalgae, Chlorella vulgaris. *Aquaculture International*, *20*(1), 41–49. doi:10.1007/s10499-011-9440-1

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

Kumar, A., Ergas, S., Yuan, X., Sahu, A., Zhang, Q., Dewulf, J., … van Langenhove, H. (2010). Enhanced CO2 fixation and biofuel production via microalgae: recent developments and future directions. *Trends in Biotechnology*, *28*(7), 371–80. doi:10.1016/j.tibtech.2010.04.004

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

Laliberté, G., Lessard, P., de la Noüe, J., & Sylvestre, S. (1997). Effect of phosphorus addition on nutrient removal from wastewater with the cyanobacterium Phormidium bohneri. *Bioresource Technology*, *59*(2), 227–233. doi.org/10.1016/S0960-8524(96)00144-7

Larsdotter, K. (2006). Wastewater treatment with microalgae – a literature review. *Solar Energy*, *62*(1), 31–38.

Lau, P. S., Tam, N. F.Y., & Wong, Y.S. (1995). Effect of algal density on nutrient removal from primary settled wastewater. *Environmental Pollution*, *89*(1), 59–66. doi:10.1016/0269-7491(94)00044-E

Lee, E., Jalalizadeh, M., & Zhang, Q. (2015). Growth kinetic models for microalgae cultivation: A review. *Algal Research*, *12*, 497–512. doi:10.1016/j.algal.2015.10.004

Lee, K., & Lee, C.-G. (2001). Effect of light/dark cycles on wastewater treatments by microalgae. *Biotechnology and Bioprocess Engineering*, *6*, 194–199. doi:10.1007/BF02932550

Lee, S.-H., Ahn, C.-Y., Jo, B.-H., Lee, S.-A., Park, J.-Y., An, K.-G., & Oh, H.-M. (2013). Increased microalgae growth and nutrient removal using balanced N:P ratio in wastewater. *Journal of Microbiology and Biotechnology*, *23*(1), 92–8.

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

Li, F.-F., Yang, Z.-H., Zeng, R., Yang, G., Chang, X., Yan, J.-B., & Hou, Y.-L. (2011a). Microalgae capture of CO2 from actual flue gas discharged from a combustion chamber. *Industrial & Engineering Chemistry Research*, *50*(10), 6496–6502. doi:10.1021/ie200040q

Li, Y., Chen, Y.-F., Chen, P., Min, M., Zhou, W., Martinez, B., … Ruan, R. (2011b). Characterization of a microalga Chlorella sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. *Bioresource Technology*, *102*(8), 5138–5144. doi:10.1016/j.biortech.2011.01.091

Liu, G. D., Wu, W. L., & Zhang, J. (2005). Regional differentiation of non-point source pollution of agriculture-derived nitrate nitrogen in groundwater in northern China. *Agriculture, Ecosystems & Environment*, *107*(2–3), 211–220. doi:10.1016/j.agee.2004.11.010

López, C. V. G, Acién Fernández, F. G., Fernández Sevilla, J. M., Sánchez Fernández, J. F., Cerón García, M. C., & Molina Grima, E. (2009). Utilization of the cyanobacteria Anabaena sp. ATCC 33047 in CO2 removal processes. *Bioresource Technology*, *100*(23), 5904–10. doi:10.1016/j.biortech.2009.04.070

Maclean, H. L., Bagley, D. M., Monteith, H. D., Sahely, H. R., & Bagley, D. M. (2005). A rational procedure for estimation of greenhouse-gas emissions from municipal wastewater treatment plants. *Water Environ Res.*, *77*(4), 390–403. doi:10.2175/106143005X51978

Martinez, M. E., Sanhez, S., Jimenez, J. M., Yousfi, F. E., & Munoz, L. (2000). Nitrogen and phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus. *Biosource Technology*, *73*, 263–272.

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

Mennaa, F. Z., Arbib, Z., & Perales, J. A. (2015). Urban wastewater treatment by seven species of microalgae and an algal bloom: Biomass production, N and P removal kinetics and harvestability. *Water Research*, *83*, 42–51. doi:10.1016/j.watres.2015.06.007

Metz, B., Davidson, O., Coninck, H. de, Loos, M. L., & Meyer, L. (2005). *IPCC special report on carbon dioxide capture and storage*. United States: Cambridge University Press.

Mirón, A. S., Cerón García, M.-C., García Camacho, F., Molina Grima, E., & Chisti, Y. (2002). Growth and biochemical characterization of microalgal biomass produced in bubble column and airlift photobioreactors: studies in fed-batch culture. *Enzyme and Microbial Technology*, *31*(7), 1015–1023. doi.10.1016/S0141-0229(02)00229-6

Moroney, J. V and Ynalvez, R. A. (2009) Algal Photosynthesis. In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons Ltd., Chichester. doi: 10.1002/9780470015902.a0000322.pub2

Mortezaeikia, V., Yegani, R., & Tavakoli, O. (2016). Membrane-sparger vs. membrane contactor as a photobioreactors for carbon dioxide biofixation of Synechococcus elongatus in batch and semi-continuous mode. *Journal of CO2 Utilization*, *16*, 23–31. doi:10.1016/j.jcou.2016.05.009

Moss, B., Kosten, S., Meerhoff M., Battarbee R.W., Jeppesen E., Mazzeo N., Havens K., Lacerot G., Liu Z., De Meester L., Paerl H., & Scheffer M. (2011). Allied attack: climate change and eutrophication. *Inland Waters*, 1, 101-105. doi:10.5268/IW-1.2.359

Negoro, M., Hamasaki, A., Ikuta Y., Makita T., Hirayama K.,& Suzuki S. (1993). Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. *Applied Biochemistry and Biotechnology*, 39, 643-653. doi:10.1007/BF02919025

Nyenje, P. M., Foppen, J. W., Uhlenbrook, S., Kulabako, R., & Muwanga, A. (2010). Eutrophication and nutrient release in urban areas of sub-Saharan Africa - A review. *Science of the Total Environment*, *408*(3), 447–55. doi:10.1016/j.scitotenv.2009.10.020

Ogbonna, J. C., & Tanaka, H. (1996). Night biomass loss and changes in biochemical composition of cells during light/dark cyclic culture of Chlorella pyrenoidosa. *Journal of Fermentation and Bioengineering*, *82*(6), 558–564. doi:10.1016/S0922-338X(97)81252-4

Ojo, E. O., Auta, H., Baganz, F., & Lye, G. J. (2014). Engineering characterisation of a shaken, single-use photobioreactor for early stage microalgae cultivation using Chlorella sorokiniana. *Bioresource Technology*, *173*, 367–375. doi:10.1016/j.biortech.2014.09.060

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

Olguín, E. J. (2012). Dual purpose microalgae-bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a biorefinery. *Biotechnology Advances*, *30*(5), 1031–46. doi:10.1016/j.biotechadv.2012.05.001

Oswald, W. J., & Gotaas, H. B. (1957). Photosynthesis in sewage treatment. *Transactions of the American Society of Civil Engineers*, 122, 73–105.

Ota, K., Amano, Y., Aikawa, M., & Machida, M. (2013). Removal of nitrate ions from water by activated carbons (ACs) - Influence of surface chemistry of ACs and coexisting chloride and sulfate ions. *Applied Surface Science*, *276*, 838–842. doi:10.1016/j.apsusc.2013.03.053

Packer, M. (2009). Algal capture of carbon dioxide; biomass generation as a tool for greenhouse gas mitigation with reference to New Zealand energy strategy and policy. *Energy Policy*, *37*(9), 3428–3437. doi:10.1016/j.enpol.2008.12.025

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

Pires, J. C. M., Alvim-Ferraz, M. C. M., Martins, F. G., & Simões, M. (2012). Carbon dioxide capture from flue gases using microalgae: Engineering aspects and biorefinery concept. *Renewable and Sustainable Energy Reviews*, *16*(5), 3043–3053. doi:10.1016/j.rser.2012.02.055

Rahmani, A. R., & Mahvi, A. H. (2006). Use of ion exchange for removal of ammonium: A biological regeneration of zeolite. *Global NEST Journal*, *8*(2), 146–150.

doi:10.3923/pjbs.2005.30.33

Ramanan, R., Kannan, K., Deshkar, A., Yadav, R., & Chakrabarti, T. (2010). Enhanced algal CO2 sequestration through calcite deposition by Chlorella sp. and Spirulina platensis in a mini-raceway pond. *Bioresource Technology*, *101*(8), 2616–22. doi:10.1016/j.biortech.2009.10.061

Ramaraj, R., Tsai, D. D. W., & Chen, P. H. (2015). Carbon dioxide fixation of freshwater microalgae growth on natural water medium. *Ecological Engineering*, *75*, 86–92. doi:10.1016/j.ecoleng.2014.11.033

Reddy, M. H. (2002). *Application of Algal Culture Technology for Carbondioxide and Flue Gas Emission Control*. Arizona State University, Tempe, AZ, USA.

Ringsmuth, A. K., Landsberg, M. J., & Hankamer, B. (2016). Can photosynthesis enable a global transition from fossil fuels to solar fuels, to mitigate climate change and fuel-supply limitations? *Renewable and Sustainable Energy Reviews*, *62*, 134–163. doi:10.1016/j.rser.2016.04.016

Rowley, W. M. (2010). *Nitrogen and phosphorus biomass-kinetic model for Chlorella Vulgaris in a biofuel production scheme*. Air Force Institute of Technology, Wright-Patterson Air Force Base, OH, USA.

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

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

Ruiz, J., Álvarez-Díaz, P. D., Arbib, Z., Garrido-Pérez, C., Barragán, J., & Perales, J. A. (2013). Performance of a flat panel reactor in the continuous culture of microalgae in urban wastewater: Prediction from a batch experiment. *Bioresource Technology*, *127*, 456–63. doi:10.1016/j.biortech.2012.09.103

Ruiz, J., Olivieri, G., de Vree, J., Bosma, R., Willems, P., Reith, J. H., … Barbosa, M. J. (2016). Towards industrial products from microalgae. *Energy Environ. Sci.*, *9*, 3036–3043. doi:10.1039/C6EE01493C

Ryu, H. J., Oh, K. K., & Kim, Y. S. (2009). Optimization of the influential factors for the improvement of CO2 utilization efficiency and CO2 mass transfer rate. *Journal of Industrial and Engineering Chemistry*, *15*(4), 471–475. doi:10.1016/j.jiec.2008.12.012

Sakarika, M., & Kornaros, M. (2016). Effect of pH on growth and lipid accumulation kinetics of the microalga Chlorella vulgaris grown heterotrophically under sulfur limitation. *Bioresource Technology*, *219*, 694–701. doi:10.1016/j.biortech.2016.08.033

Samorì, G., Samorì, C., Guerrini, F., & Pistocchi, R. (2013). Growth and nitrogen removal capacity of Desmodesmus communis and of a natural microalgae consortium in a batch culture system in view of urban wastewater treatment. *Water Research*, *47*(2), 791–801. doi:10.1016/j.watres.2012.11.006

Sathasivan, A. (2009). Biological Phosphorus Removal Processes for Wastewater Treatment. In S. Vigneswaran (Ed.), *Waste Water Recyle, Reuse and Reclamation - Volume II* (pp. 1–23). EOLSS (Encyclopaedia of Life Support Systems) Publications, Oxford, UK.

Selvaratnam, T., Pegallapati, A., Montelya, F., Rodriguez, G., Nirmalakhandan, N., Lammers, P. J., & van Voorhies, W. (2014). Feasibility of algal systems for sustainable wastewater treatment. *Renewable Energy*, *82*, 71–76. doi:10.1016/j.renene.2014.07.061

Seviour, R. J., Mino, T., & Onuki, M. (2003). The microbiology of biological phosphorus removal in activated sludge systems. *FEMS Microbiology Reviews*, *27*(1), 99–127. doi:10.1016/S0168-6445(03)00021-4

Shahrom, Z., & Ahmed, E. (2012). Biological aerated filters (BAFs) for carbon and nitrogen removal: A review. *Journal of Engineering, Science and Technology*, *7*(4), 428–446.

Sierra, E., Acién, F. G., Fernández, J. M., García, J. L., González, C., & Molina, E. (2008). Characterization of a flat plate photobioreactor for the production of microalgae. *Chemical Engineering Journal*, *138*(1–3), 136–147. doi:10.1016/j.cej.2007.06.004

Sims, R. C., Bedingfield, S. K., Thompson, R., & Sims, J. L. (2016). Bioenergy from wastewater-based biomass. *AIMS Bioengineering*, *3*(1), 103–124. doi:10.3934/bioeng.2016.1.103

Singh, S. P., & Singh, P. (2014). Effect of CO2 concentration on algal growth: A review. *Renewable and Sustainable Energy Reviews*, *38*, 172–179. doi:10.1016/j.rser.2014.05.043

Skjånes, K., Lindblad, P., & Muller, J. (2007). BioCO2 - A multidisciplinary, biological approach using solar energy to capture CO2 while producing H2 and high value products. *Biomolecular Engineering*, *24*(4), 405–13. doi: 10.1016/j.bioeng.2007.06.002

Spellman F. R. & Drinan J. E. (2014) *Waste Stabilization Ponds*. Boca Raton, FL: CRC Press.

Su, Y., Mennerich, A., & Urban, B. (2012). Coupled nutrient removal and biomass production with mixed algal culture: impact of biotic and abiotic factors. *Bioresource Technology*, *118*, 469–76. doi:10.1016/j.biortech.2012.05.093

Taher, H., Al-Zuhair, S., Al-Marzouqi, A., Haik, Y., & Farid, M. (2015). Growth of microalgae using CO2 enriched air for biodiesel production in supercritical CO2. *Renewable Energy*, 82, 61–70. doi:10.1016/j.renene.2014.08.013

Tam, N. F. Y., & Wong, Y. S. (1990). The Comparison of growth and nutrient removal

efficiency of Chiorella pyrenoidosa in settled and activated sewages. *Environmental Pollution, 65,* 93–108.

Tang, D., Han, W., Li, P., Miao, X., & Zhong, J. (2011). CO2 biofixation and fatty acid composition of Scenedesmus obliquus and Chlorella pyrenoidosa in response to different CO2 levels. *Bioresource Technology*, *102*(3), 3071–6. doi:10.1016/j.biortech.2010.10.047

Tang, H., Chen, M., Ng, K. Y. S., & Salley, S. O. (2012). Continuous Microalgae Cultivation in a Photobioreactor. *Biotechnology and Bioengineering*, *109*(10), 2468–74. doi:/10.1002/bit.24516

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

Tercero, E. A. R, Sforza, E., Morandini, M., & Bertucco, A. (2013). Cultivation of Chlorella protothecoides with urban wastewater in continuous photobioreactor: Biomass productivity and nutrient removal. *Applied Biochemistry and Biotechnology*. doi:10.1007/s12010-013-0629-9

Torzillo, G. and Vonshak, A. (2013). Environmental stress physiology with reference to mass cultures. In A. Richmond and Q. Hu (Eds.), *Handbook of Microalgal Culture: Applied Phycology and Biotechnolog* (pp. 90–113). John Wiley & Sons, Ltd, Oxford, UK. doi: 10.1002/9781118567166.ch6

Travieso, L., Benítez, F., Sánchez, E., Borja, R., & Colmenarejo, M. F. (2006). Production of Biomass (Algae-Bacteria) by Using a Mixture of Settled Swine and Sewage as Substrate, *Journal of Environmental Science and Health*, 41(3), 415-429. doi: 10.1080/10934520500428302

Ugwu, C. U., Aoyagi, H., & Uchiyama, H. (2008). Photobioreactors for mass cultivation of algae. *Bioresource Technology*, *99*(10), 4021–4028. doi:10.1016/j.biortech.2007.01.046

Uncer, M. T., & Alçuk, A. Y. (2012). Environmental master plans for preserving natural and cultural environment: The Zonguldak, Bartın and Karabük planning region. *Gazi University Journal of Science*, *25*(3), 815–821.

U.S. Environmental Protection Agency (U.S. EPA). (2004). *Primer for Municipal Wastewater Treatment Systems*. EPA 832-R-04-001.

U.S. Environmental Protection Agency (U.S. EPA). (2008). *Municipal Nutrient Removal Technologies Reference Document Volume 1, Technical Report*. EPA 832-R-08-006.

Usher, P. K., Ross, A. B., Camargo-Valero, M. A., Tomlin, A. S., & Gale, W. F. (2014). An overview of the potential environmental impacts of large-scale microalgae cultivation. *Biofuels*, *5*(3), 331–349. doi:10.1080/17597269.2014.913925

Van Den Hende, S., Vervaeren, H., & Boon, N. (2012). Flue gas compounds and microalgae: (Bio-)chemical interactions leading to biotechnological opportunities. *Biotechnology Advances*, *30*(6), 1405–1424. doi:10.1016/j.biotechadv.2012.02.015

Von Sperling, M., & Augusto De Lemos Chernicharo, C. (2002). Urban wastewater

treatment technologies and the implementation of discharge standards in developing countries. *Urban Water*, *4*(1), 105–114. doi:10.1016/S1462-0758(01)00066-8

Wang, B., Li, Y., Wu, N., & Lan, C. Q. (2008). CO2 bio-mitigation using microalgae. *Applied Microbiology and Biotechnology*, *79*(5), 707–18. doi:10.1007/s00253-008-1518-y

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

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

Wang, M., Kuo-Dahab, W. C., Dolan, S., & Park, C. (2013). Kinetics of nutrient removal and expression of extracellular polymeric substances of the microalgae, Chlorella sp. and Micractinium sp., in wastewater treatment. *Bioresource Technology*, *154*, 131–137. doi:10.1016/j.biortech.2013.12.047

WEF (Water Environment Federation) and ASCE (American Society of Civil Engineers). (1998). *Design of Municipal Wastewater Treatment Plants.* WEF Manual of Practice No. 8, Volume II, 4th ed. pp 15-1 through 15-114. American Society of Civil Engineers, Reston, VA, USA.

Weisse, T., & Stadler, P. (2006). Effect of pH on growth, cell volume, and production of freshwater ciliates, and implications for their distribution. *Limnology and Oceanography*, *51*(4), 1708–1715. doi:10.4319/lo.2006.51.4.1708

White, D., Silkina, A., Skill, S., Oatley-Radcliffe, D., Van Den Hende, S., Ernst, A., De Viser, C., Van Dijk, W., Davey, M., Day, J. (2015). *Best Practices for the Pilot-Scale Cultivation of Microalgae*. Public Output report of the EnAlgae project, Swansea, UK, 34 pp. Available online at www.enalgae.eu.

WHO (World Health Organization). (2011). *Nitrate and Nitrite in Drinking-Water*. Background Document for Development of WHO Guidelines for Drinking-Water Quality, Geneva, Switzerland.

Woertz, I. (2007). *Lipid Productivity of Algae Grown on Dairy Wastewater as a Possible Feedstock for Biodiesel*. California Polytechnic University, San Luis Obispo, CA, USA.

Woertz, I., Feffer, A., Lundquist, T., & Nelson, Y. (2009). Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. *Journal of Environmental Engineering*, *135*(11), 1115–1122. doi:10.1061/(ASCE)EE.1943-7870.0000129

WNA (Word Nuclear Association). (2013). Comparison of lifecycle greenhouse gas emissions of various electricity generation sources. London, UK.

Wu, L. F., Chen, P. C., Huang, A. P., & Lee, C. M. (2012). The feasibility of biodiesel

production by microalgae using industrial wastewater. *Bioresource Technology*, *113*, 14–8. doi:10.1016/j.biortech.2011.12.128

Xin, L., Hu, H., Ke, G., & Sun, Y. (2010). Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga Scenedesmus sp. *Bioresource Technology*, *101*(14), 5494–500. doi:10.1016/j.biortech.2010.02.016

Yadav, K., Sarma, V. V. S. S., Rao, D. B., & Kumar, M. D. (2016). Influence of atmospheric dry deposition of inorganic nutrients on phytoplankton biomass in the coastal Bay of Bengal. *Marine Chemistry*, *187*, 25–34. doi:10.1016/j.marchem.2016.10.004

Yao, L., Shi, J., & Miao, X. (2015). Mixed wastewater coupled with CO2 for microalgae culturing and nutrient removal. *PLoS One*, *10*(9), 1–17. doi: 10.1371/journal.pone.0139117

Yuan, Y., Liu, J., Ma, B., Liu, Y., Wang, B., & Peng, Y. (2016). Improving municipal wastewater nitrogen and phosphorous removal by feeding sludge fermentation products to sequencing batch reactor (SBR). Bioresource Technology, 222, 326–334. doi:10.1016/j.biortech.2016.09.103

Yun, Y.-S., Lee, S. B., Park, J. M., Lee, C.-I., & Yang, J.-W. (1997). Carbondioxide fixation by algal cultivation using wastewater nutrients. *Journal of Chemical Technology & Biotechnology*, 69(4), 451–455. doi:10.1002/(SICI)1097-4660(199708)69:4<451::AID-JCTB733>3.0.CO;2-M

Zhang, K., Kurano, N., & Miyachi, S. (2002). Optimized aeration by carbon dioxide gas for microalgal production and mass transfer characterization in a vertical flat-plate photobioreactor. Bioprocess and Biosystems Engineering, 25(2), 97–101. doi:10.1007/s00449-002-0284-y

Zhang, X. (2015). *Microalgae removal of CO2 from flue gas*. IEA Clean Coal Center, London, UK.

Zhao, B., & Su, Y. (2014). Process effect of microalgal-carbon dioxide fixation and biomass production: A review. Renewable and Sustainable Energy Reviews, 31, 121–132. doi:10.1016/j.rser.2013.11.054

Zhao, C. H., Peng, Y. Z., Wang, S. Y., & Takigawa, A. (2008). Effects of influent C/N ratio, C/P ratio and volumetric exchange ratio on biological phosphorus removal in UniFed SBR process. *Journal of Chemical Technology and Biotechnology*, 83(12), 1587–1595. doi:10.1002/jctb.1943

Zhu, J. (2010). *Algal Greenhouse Gas Mitigation for Coal-Fired Flue Gas*. Duke University, Durham, NC, USA.

Zhu, L. (2015). Microalgal culture strategies for biofuel production: A review. *Biofuels, Bioproducts & Biorefining*, *9*(6), 801–814. https://doi.org/0.1002/bbb.1576

Znad, H., Naderi, G., Ang, H. M., & Tade, M. O. (2012). CO2 biomitigation and biofuel production using microalgae: Photobioreactors developments and future directions.

In Z. Nawaz and S. Naveed (Eds.), *Advances in Chemical Engineering* (pp. 229–244). doi:10.13140/2.1.4712.0328

APPENDICES

APPENDIX A: Calculation of Specific Growth Rate for B3-50, B3-100, B4-50 and B4-100 Photobioreactors

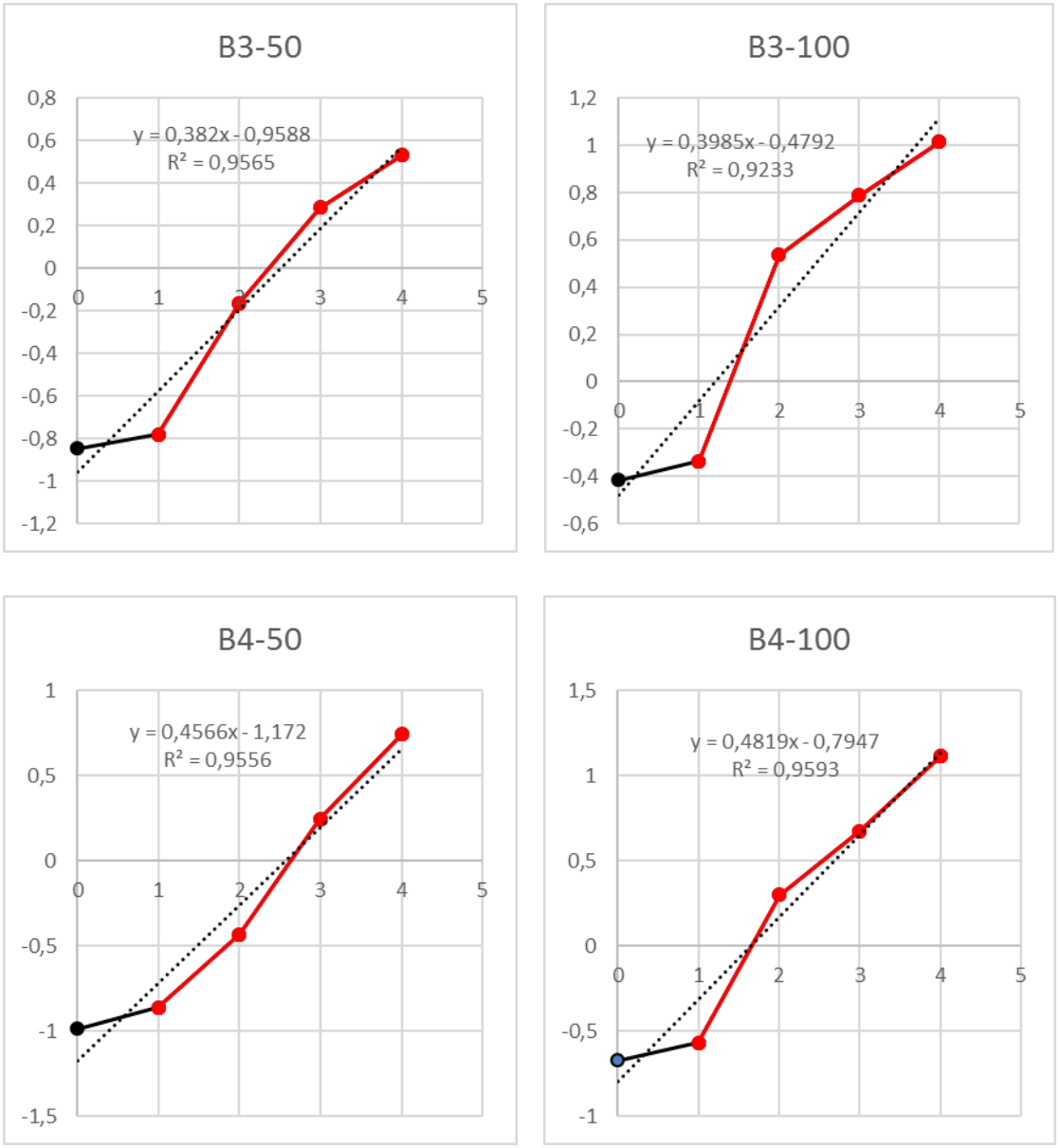


Figure 32 Natural log O.D. versus time plots for B3-50, B3-100, B4-50 and B4-100 Reactors

APPENDIX B-1: Calculation of Specific Growth Rate for DB6, DB8 and DB10 Photobioreactors

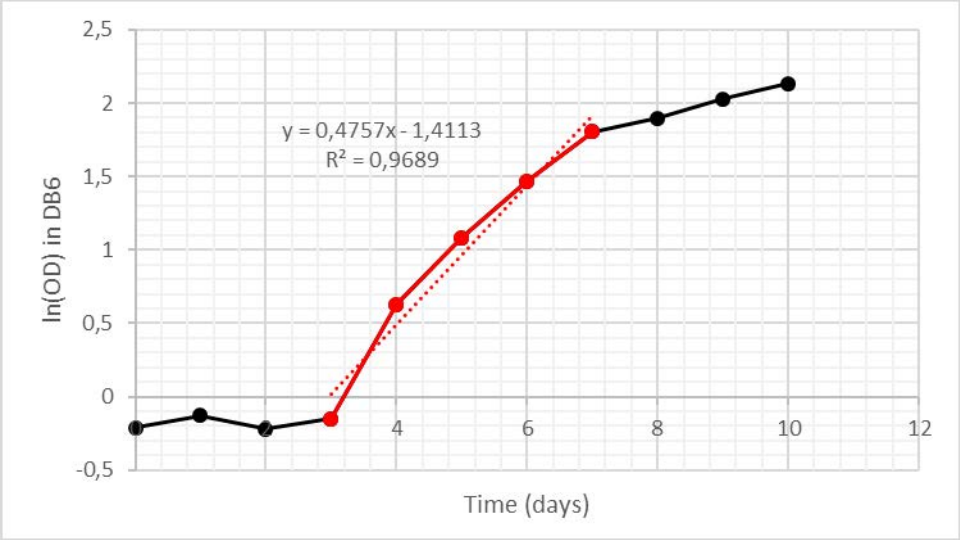


Figure 33 Ln(OD) versus Time for DB6

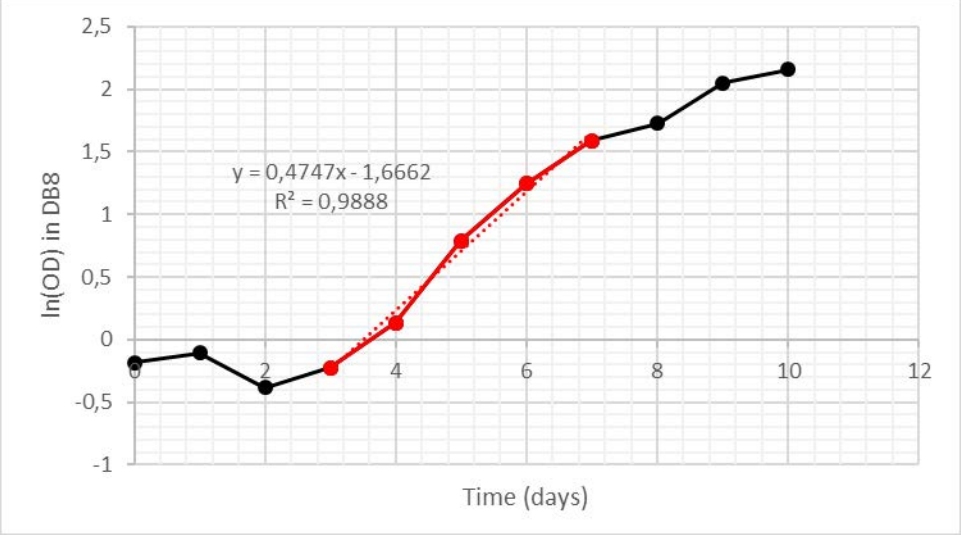


Figure 34 Ln(OD) versus Time for DB8

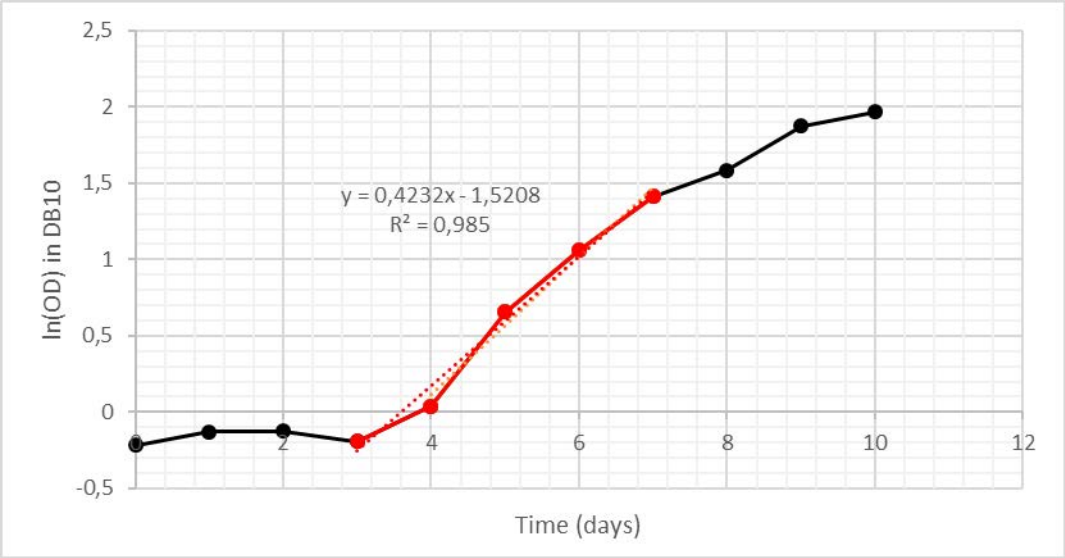


Figure 35 Ln(OD) versus Time for DB10

APPENDIX B-2: Calculation of Microalgal Biomass Productivity for DB6, DB8 and DB10 Photobioreactors

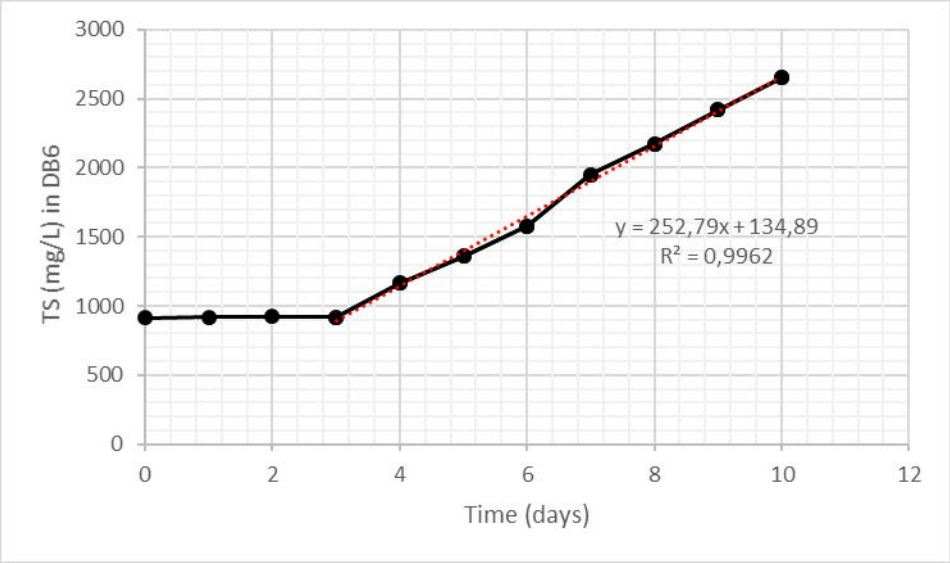


Figure 36 Productivity determination for DB6

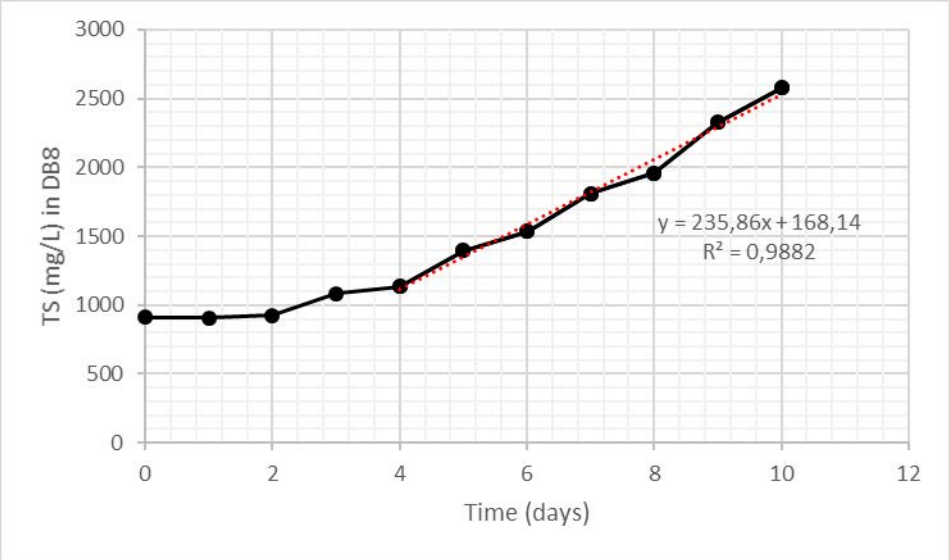


Figure 37 Productivity determination for DB8

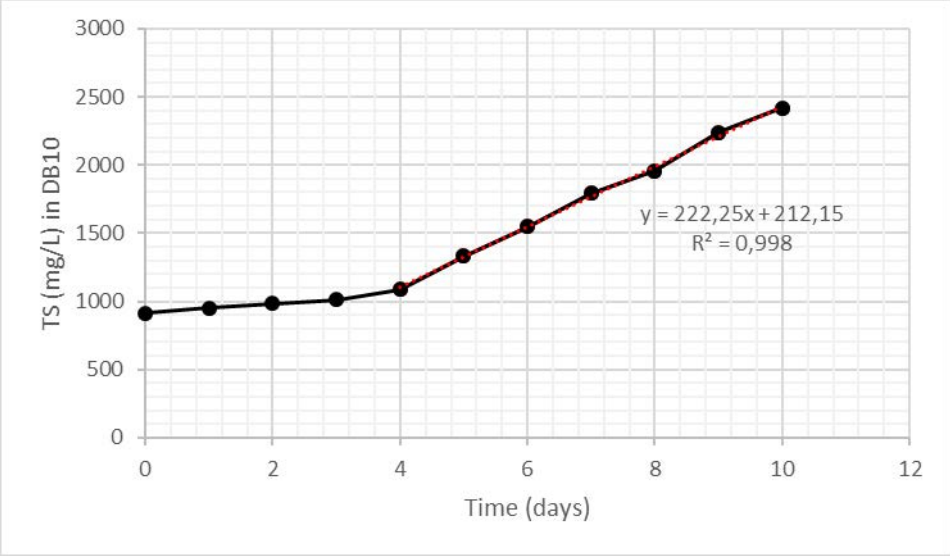


Figure 38 Productivity determination for DB10

APPENDIX C: Calibration Curve for the GC Analysis

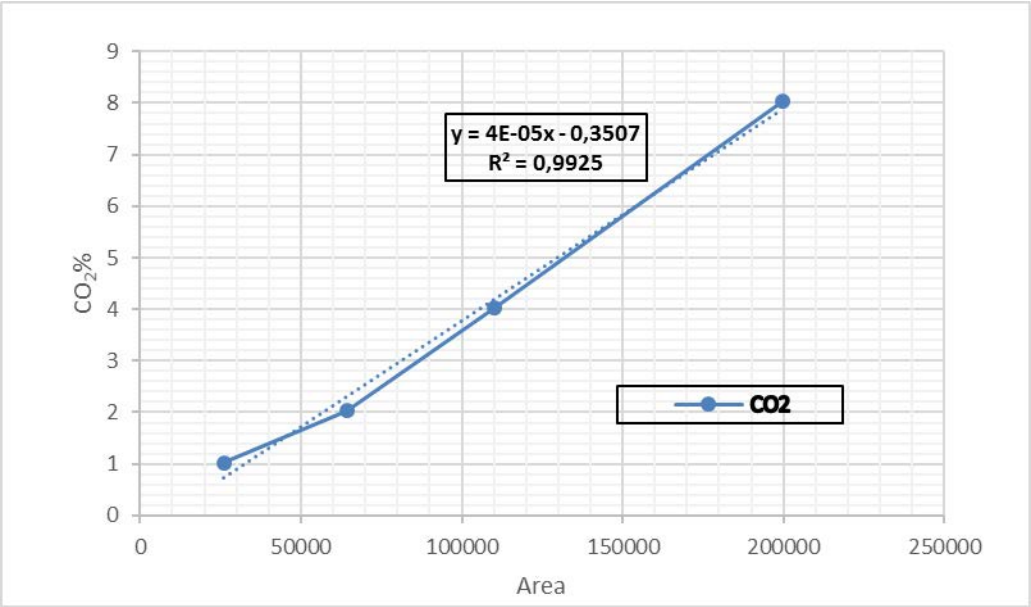


Figure 39 Calibration curve used for the calculation of inlet and outlet CO_2 concentrations

APPENDIX D: Photographs of Microscopic Analyses

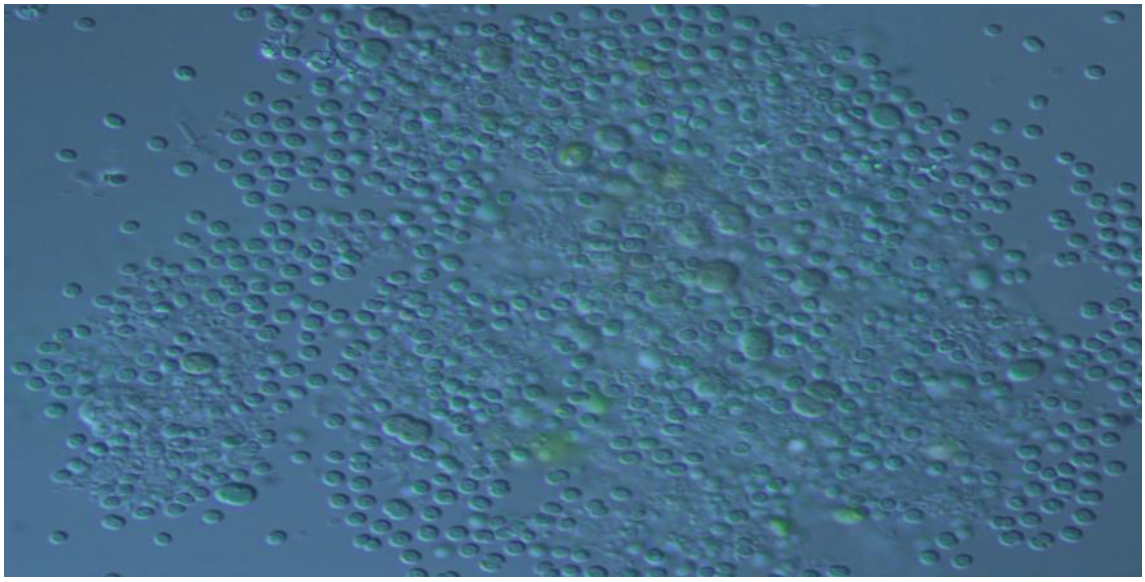


Figure 40 *Chroccoccus Turgidis*-I

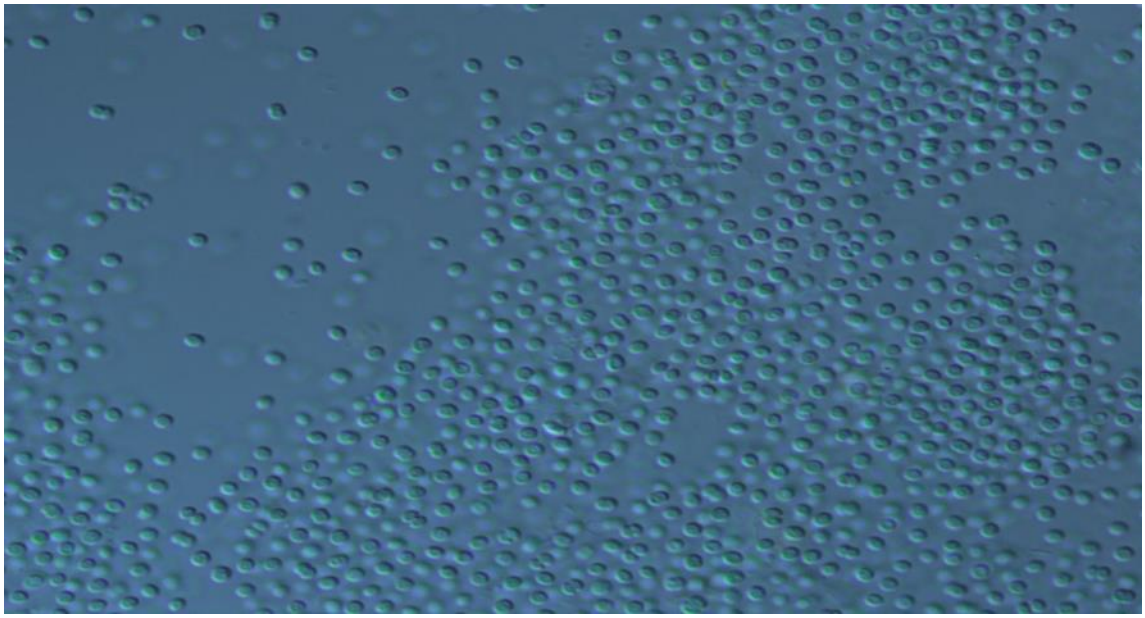


Figure 41 *Chroccoccus Turgidis*-II

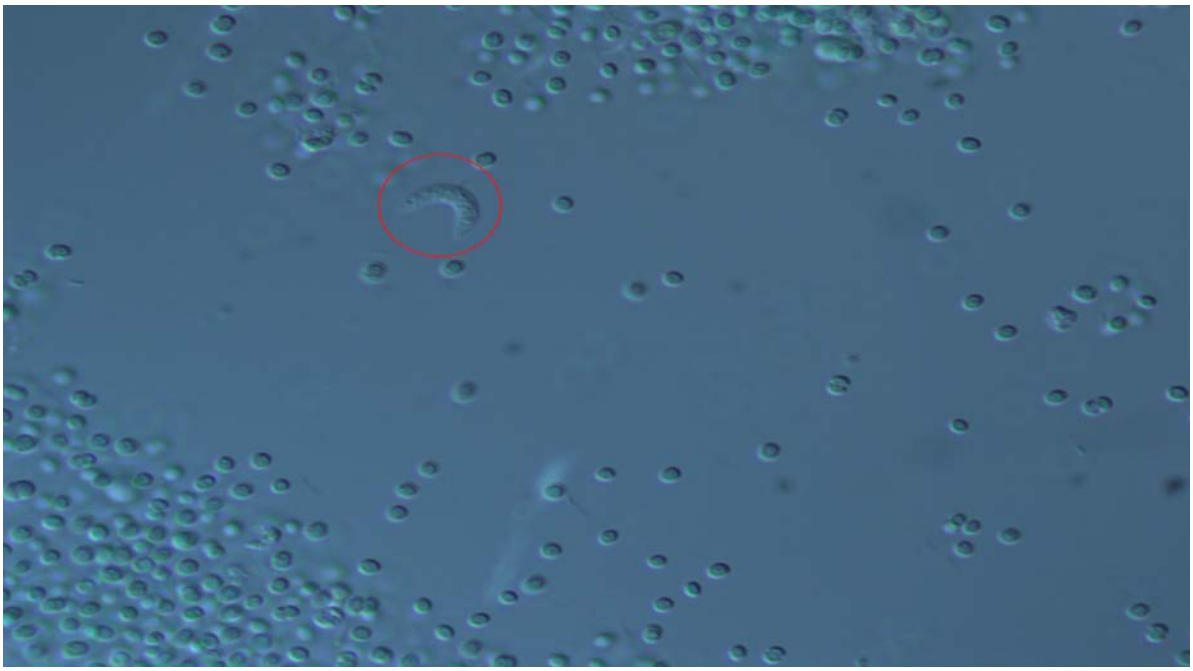


Figure 42 *Chroccoccus Turgidis* and *Cryptomanas sp*. (in the red circle)

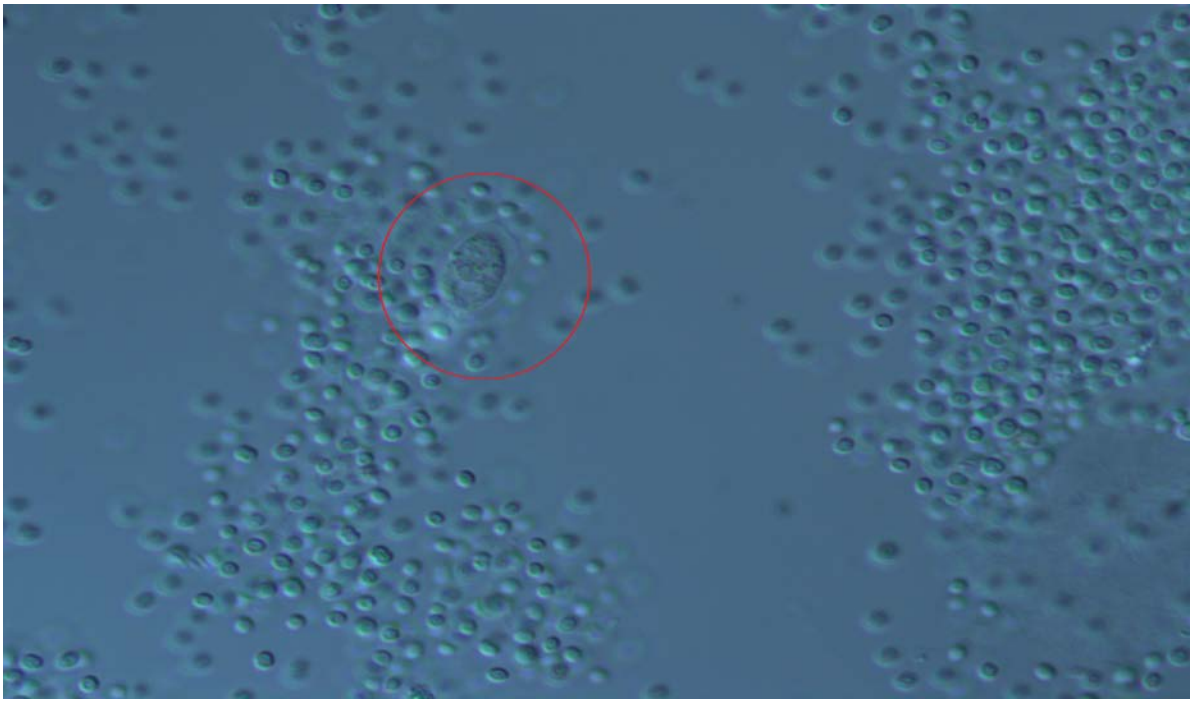
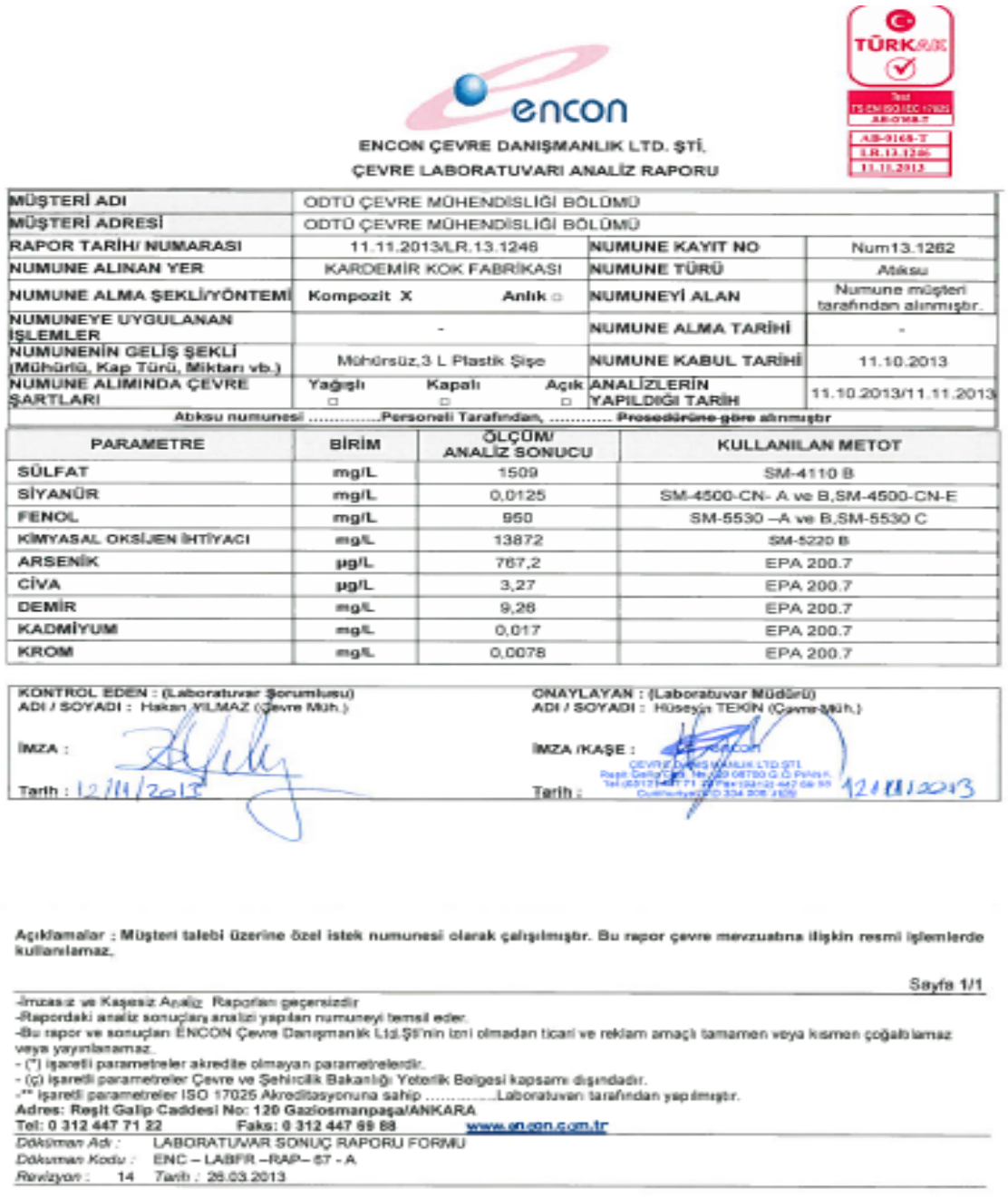


Figure 43 *Chroccoccus Turgidis* and *Kirchneriella sp*. (in the red circle)

APPENDIX E: Industrial Wastewater Analysis Results



ENCON ÇEVRE DANIŞMANLIK LTD. ŞTİ.
ÇEVRE LABORATUVARI ANALİZ RAPORU

MÜŞTERİ ADI	ODTÜ ÇEVRE MÜHENDİSLİĞİ BÖLÜMÜ		
MÜŞTERİ ADRESİ	ODTÜ ÇEVRE MÜHENDİSLİĞİ BÖLÜMÜ		
RAPOR TARİH/ NUMARASI	11.11.2013/LR.13.1248	NUMUNE KAYIT NO	Num13.1262
NUMUNE ALINAN YER	KARDEMİR KOK FABRİKASI	NUMUNE TÜRÜ	Atıksu
NUMUNE ALMA ŞEKLİ/YÖNTEMİ	Kompozit X Anlık □	NUMUNEYİ ALAN	Numune müşteri tarafından alınmıştır.
NUMUNEYE UYGULANAN İŞLEMLER	-	NUMUNE ALMA TARİHİ	-
NUMUNENİN GELİŞ ŞEKLİ (Mühürlü, Kap Türü, Miktarı vb.)	Mühürsüz,3 L Plastik Şişe	NUMUNE KABUL TARİHİ	11.10.2013
NUMUNE ALIMINDA ÇEVRE ŞARTLARI	Yağışlı □ Kapalı □ Açık □	ANALİZLERİN YAPILDIĞI TARİH	11.10.2013/11.11.2013
Atıksu numunesi .....Personeli Tarafından, ... Prosedürüne göre alınmıştır			

PARAMETRE	BİRİM	ÖLÇÜM/ ANALİZ SONUCU	KULLANILAN METOT
SÜLFAT	mg/L	1509	SM-4110 B
SİYANÜR	mg/L	0,0125	SM-4500-CN- A ve B,SM-4500-CN-E
FENOL	mg/L	950	SM-5530 –A ve B,SM-5530 C
KİMYASAL OKSİJEN İHTİYACI	mg/L	13872	SM-5220 B
ARSENİK	µg/L	767,2	EPA 200.7
CİVA	µg/L	3,27	EPA 200.7
DEMİR	mg/L	9,28	EPA 200.7
KADMİYUM	mg/L	0,017	EPA 200.7
KROM	mg/L	0,0078	EPA 200.7

KONTROL EDEN : (Laboratuvar Sorumlusu)
ADI / SOYADI : Hakan YILMAZ (Çevre Müh.)
İMZA :
Tarih : 12/11/2013

ONAYLAYAN : (Laboratuvar Müdürü)
ADI / SOYADI : Hüseyin TEKİN (Çevre Müh.)
İMZA /KAŞE :
Tarih : 12/11/2013

Açıklamalar : Müşteri talebi üzerine özel istek numunesi olarak çalışılmıştır. Bu rapor çevre mevzuatına ilişkin resmi işlemlerde kullanılamaz.

Sayfa 1/1

-İmzasız ve Kaşesiz Analiz Raporları geçersizdir
-Rapordaki analiz sonuçları analizi yapılan numuneyi temsil eder.
-Bu rapor ve sonuçları ENCON Çevre Danışmanlık Ltd.Şti'nin izni olmadan ticari ve reklam amaçlı tamamen veya kısmen çoğaltılamaz veya yayınlanamaz.
- (*) işaretli parametreler akredite olmayan parametrelerdir.
- (ç) işaretli parametreler Çevre ve Şehircilik Bakanlığı Yeterlik Belgesi kapsamı dışındadır.
-** işaretli parametreler ISO 17025 Akreditasyonuna sahip .....Laboratuvarı tarafından yapılmıştır.
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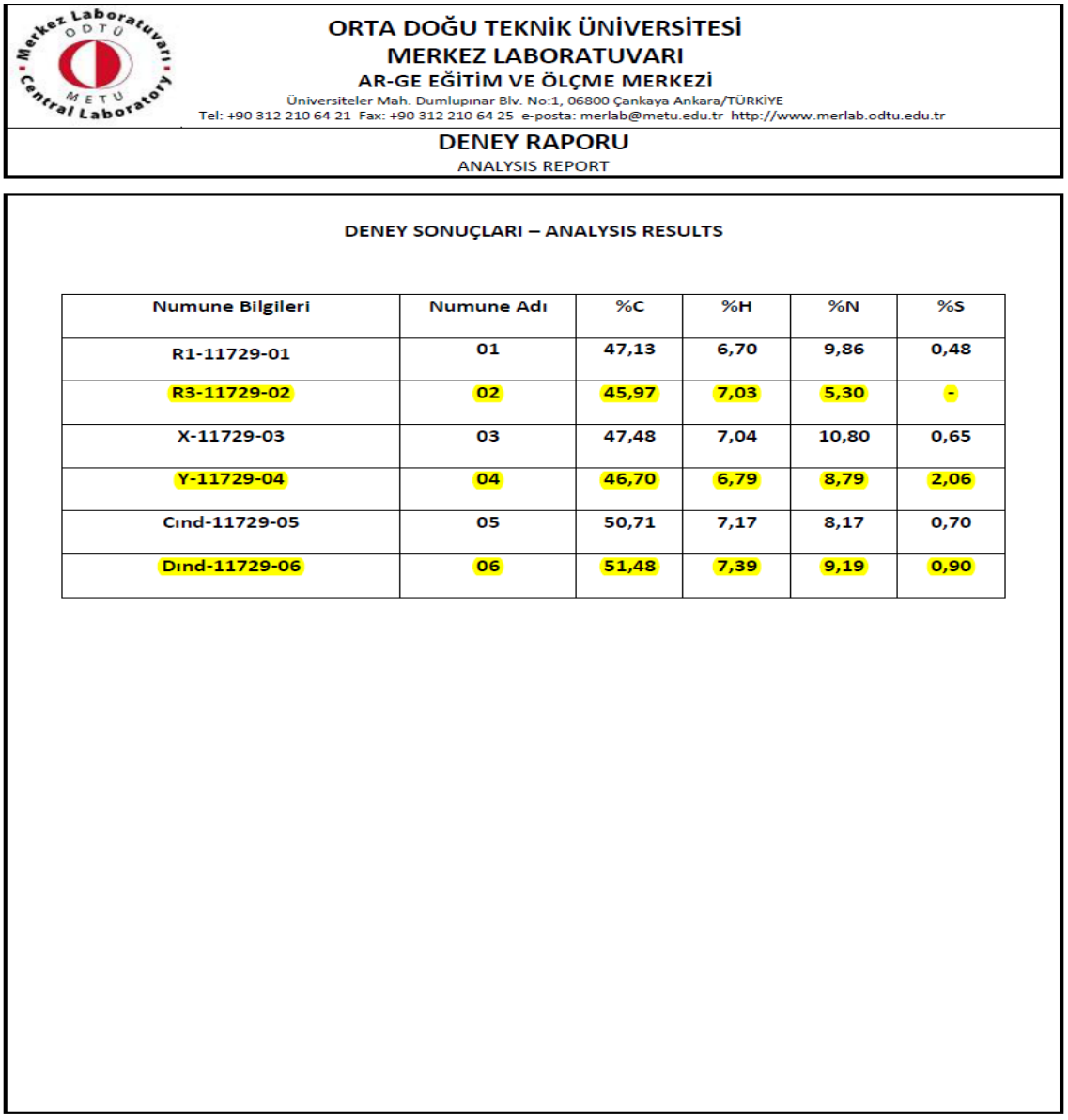
Dökümanı Kodu : ENC – LABFR –RAP– 57 - A
Revizyon : 14 Tarih : 26.03.2013

Figure 44 Results of industrial wastewater characterization study conducted by ENCON

APPENDIX F: CHN Analysis Results



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Üniversiteler Mah. Dumlupınar Blv. No:1, 06800 Çankaya Ankara/TÜRKİYE
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DENEY RAPORU
ANALYSIS REPORT

DENEY SONUÇLARI – ANALYSIS RESULTS

Numune Bilgileri	Numune Adı	%C	%H	%N	%S
R1-11729-01	01	47,13	6,70	9,86	0,48
R3-11729-02	02	45,97	7,03	5,30	-
X-11729-03	03	47,48	7,04	10,80	0,65
Y-11729-04	04	46,70	6,79	8,79	2,06
Cınd-11729-05	05	50,71	7,17	8,17	0,70
Dınd-11729-06	06	51,48	7,39	9,19	0,90

Figure 45 Results of CHN Analysis conducted by Central Laboratory at METU for the microalgal biomass samples taken from R3 cultivation reactor, Y1-B reactor and D12 reacto

APPENDIX G: Correlation Between Chlorophyll-a and TVS Concentrations

The correlation between Chlorophyll-a and TVS data obtained during the operation of semi-continuous Y1 PBR with 2-day HRT is given in Figure 46.

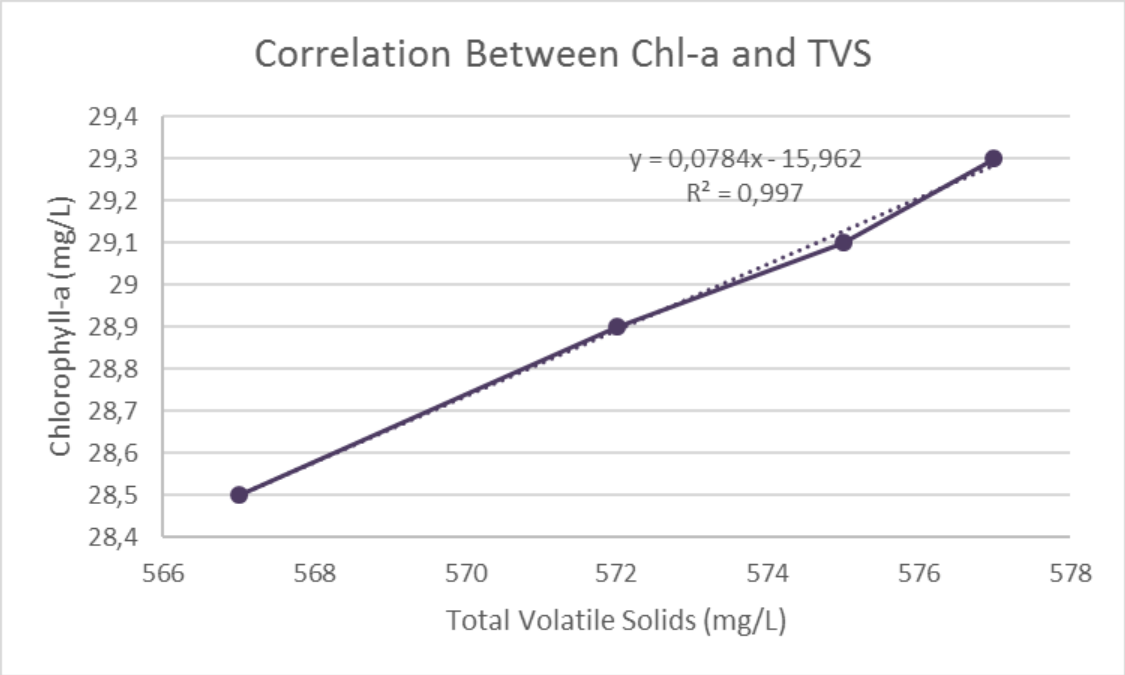

Figure 46 Correlation Between Chlorophyll-a and TVS

APPENDIX H: Simple Regression for Chl-a in Microalgae vs. TAN in Microalgae

Dependent variable: Chl-a in Microalgae

Independent variable: TAN in Microalgae

Linear model: Y = a + b*X

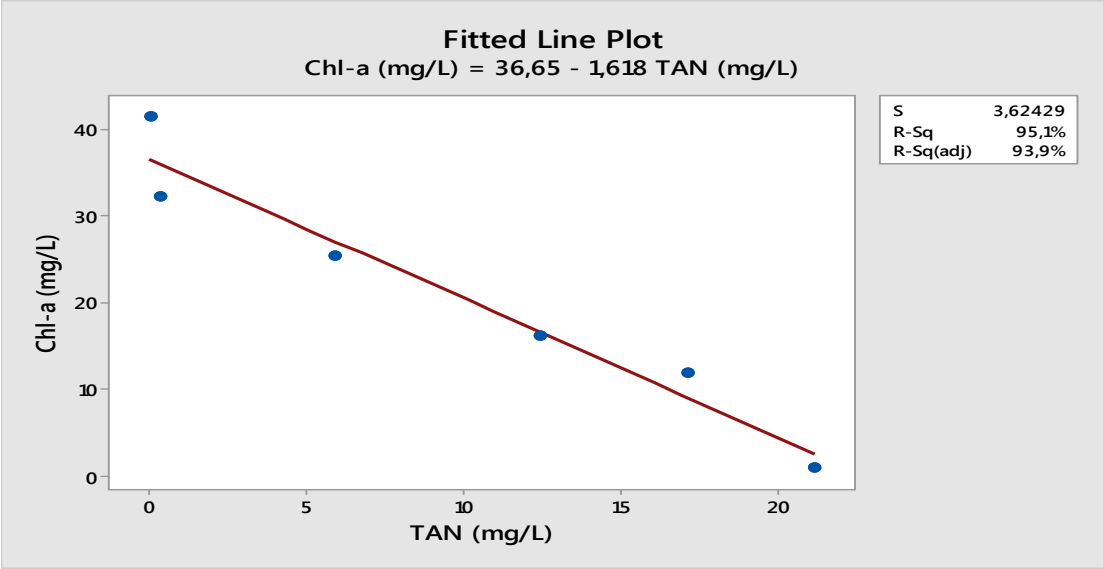

Figure 47 Fitted line Plot for Chl-a (mg/L) versus TAN (mg/L)

The regression equation is:

Chl-a in Microalgae (mg/L) = 33.65 - 1.618 (TAN in Microalgae (mg/L))

Analysis of Variance

	Sum of Squares	D_f	*Mean Square*	*F-Ratio*	*P-Value*
Model	1020,24	1	1020,24	77.67	0.001
Residual	52,54	4	13,14		
Total (Corr.)	1072,78	5			

Correlation coefficients = - 0.951

R-squared = 93.9%

Based on the assessment, there is a statistically significant relationship between Chlorophyll-a in microalgae and TAN in microalgae at the 95.0% confidence level since the P-value in the ANOVA table is less than 0.05.

The R-Squared statistic indicates that the model as fitted explains 93.9% of the variability in Chlorophyll-a in Microalgae. The correlation coefficient equals to -0.951 which indicates that there is a relatively strong relationship between the variables.

APPENDIX I: Simple Regression for Chl-a in Microalgae vs. PO_4^{3-}-P in Microalgae

Dependent variable: Chl-a in Microalgae

Independent variable: PO_4^{3-}-P in Microalgae

Linear model: Y = a + b*X

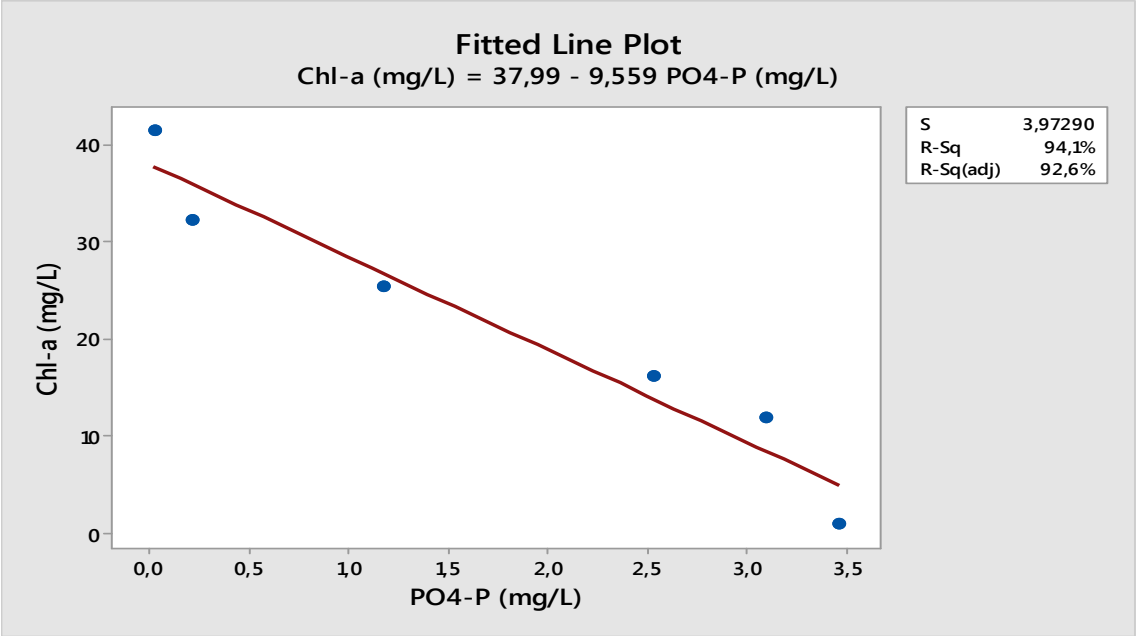

Figure 48 Fitted Line Plot for Chl-a (mg/L) versus PO_4^{3-}-P (mg/L)

The regression equation is:

Chl-a in Microalgae (mg/L) = 37.99 – 9.559 (PO_4^{3-}-P in Microalgae (mg/L))

Analysis of Variance

	Sum of Squares	D_f	*Mean Square*	*F-Ratio*	*P-Value*
Model	1009,65	1	1009,65	63,97	0,001
Residual	63,14	4	15,78		
Total (Corr.)	1072,78	5			

Correlation coefficients = - 0.941

R-squared = 92.6%

Based on the assessment, there is a statistically significant relationship between Chlorophyll-a in microalgae and PO_4^{3-}-P in microalgae at the 95.0% confidence level since the P-value in the ANOVA table is less than 0.05.

The R-Squared statistic indicates that the model as fitted explains 92.6% of the variability in Chlorophyll-a in Microalgae. The correlation coefficient equals to -0.941 which indicates that there is a relatively strong relationship between the variables.

APPENDIX J: Research Performed Using Microalgae

Table 31 Summary of research performed using microalgae

Species	Nutrient Source	PBR Design	Temperature (ºC)	Light	CO_2 Supply	Mode	HRT	Biomass Productivity	Biomass Concentration	Growth Rate (d^{-1})	Nutrient Removal Efficiency	CO_2 Fixation	Reference
Spirulina platensis	Zarrouk's medium	2-L Erlenmeyer flasks	30	12 h:12 h light:dark cycle; 2500 lux (35 µmol $m^{-2}s^{-1}$)	Ambient air	Semi-Cont.	2-4 days	Between 29.2 ± 3.9 and 42.3 ± 6.0 mg/L/day	750 mg/L	Between 0.050 and 0.111	-	-	(Reichert et al., 2006)
Euglena gracilis	Cramer-Myers medium	100-L PBR	27±0.5	480 ± 10 µmol $m^{-2}s^{-1}$	CO_2-enriched air (10%)	Semi-Cont.	Between 3 and 10 days	113.8 mg/d	510 mg/L at 5 day-HRT and 910 mg/L at 8-day HRT	-	-	7.4 g/L.d or 19% at 8-day HRT	(Chae et al., 2006)
Chlorella sp.	Modified f/2 medium	1.15-L Cylindrical glass reactor	26±1	Continuous light at 300 µmol $m^{-2}s^{-1}$	CO_2-enriched air (2%, 5%, 10%, and 15%)	Semi-Cont.	2 days	527, 457, 178 and 155 mg/L/day for 2%, 5%, 10%, and 15% CO_2 enriched-air, respectively	-	0.492 at 2% CO_2	-	58%, 27%, 20% and 16% removal of the inlet CO_2 for 2%, 5%, 10%, and 15% CO_2 enriched air, respectively	(Chiu et al., 2008a)
Chlorella sp. NCTU-2	Artificial seawater	4-L Air-lift PBRs	26±1	Continuous light at 300 µmol $m^{-2}s^{-1}$	CO_2-enriched air (5% and 10 %)	Semi-Cont.	2, 3 and 8 days	510-610 mg/L.d	5000 mg/L	0.106-0.132	-	Maximum CO_2 reduction of 63% under 10% CO_2	(Chiu et al., 2009)
Mixed culture	Municipal wastewater (primary clarifier effluent)	1-L Pyrex Roux bottles	23-25	16 h:8 h light:dark cycle; 4300 lux (60 µmol $m^{-2}s^{-}1$)	CO_2-enriched air to stabilize pH	Semi-Cont.	2-4 days	271 mg/L/day for 3-day HRT	700–800 mgVSS/L at steady state	-	Over 99% removal of ammonium and orthophosphate	-	(Woertz et al., 2009)

Table 31 (continued)

Mixed culture	Effluent from the anaerobic digester (10% and 25% diluted dairy wastewater with tap water)	40-L rectangular glass aquarium tanks	30.6 (average)	Outdoor culture	CO_2-enriched air to stabilize pH	Batch	-	-	500 mgVSS/L for 10% dilution; 900 mgVSS/L for 25% dilution	-	96% ammonium and 99% orthophosphate removal	-	(Woertz et al., 2009)
Scenedesmus accuminatus	anaerobic digestion effluent	1-L cylindrical PBR	-	12 h:12 h light: dark cycle at 200 µmol $m^{-2}s^{-1}$	-	Semi-Cont.	10 days	213 mg/L.d	2400 mg/L (maximum)	-	-	-	(Park et al., 2010)
C.vulgaris and *S.obliquus*	Urban wastewater	3-L PBRs	25±1	Continuous light at 135 µmol $m^{-2}s^{-1}$	Ambient air	Batch	-	-	-	0.186 and 0.285 for *C.vulgaris* and *S.obliquus*, respectively	60.1-80% and 96.6-100% ammonium removal and 53.3-80.3% and 55.2-83.3 orthophosphate removal by by *C.vulgaris* and *S.obliquus*, respectively	-	(Ruiz-Marin et al., 2010)
S.obliquus	Urban wastewater	3-L PBRs	25±1	Continuous light at 200 µmol $m^{-2}s^{-1}$	Ambient air	Semi-Cont.	35 hours	-	-	-	88% ammonia and 63% orthophosphate removal	-	(Ruiz-Marin et al., 2010)
Chlorella vulgaris (UTEX 2714)	20x diluted undigested and digested dairy manure	4-L flasks	25±2	Continuous light at 120 µmol $m^{-2}s^{-1}$	CO_2-enriched air (2%) to stabilize pH	Semi-Cont.	5 days for undigested dairy manure, 10-20 days for digested dairy manure	-	1000 and 1380 mg/L at steady state operation with undigested dairy manure	-	For undigested dairy manure; 99.7% ammonium, 89.5% total nitrogen and 92.0% total phosphorus removal.	3724 mg CO_2 eq. for undigested manure; 2276 mg CO_2 eq. for digested dairy manure	(Wang et al., 2010)

Table 31 (continued)

Scenedesmus obliquus	Jaworski Medium	500-L horizontal tubular	10-24	Outdoor	Pure CO_2-to stabilize pH	Semi-Cont.	Few days to maintain nitrate at a desired level	11.31 $g/m^2.d$	Between 1.5 g/L and 3.5 g/L	-	-	-	(Hulatt et al., 2011a)
Chlorella minutissima	Modified Bold 3N medium	3-L glass PBR vessel	25	Continuous light at 50 µmol $m^{-2}s^{-1}$	CO_2-enriched air (4%)	Cont.	1.56-13.15 days	137 mg/L/day at 3-day HRT	61-726 mg TS/L at steady state	0.076-0.64 (equals to dilution rate)	-	-	(Tang et al., 2012)
Mixed culture	Submerged anaerobic membrane bioreactor effluent	10-L cylindrical PBR	28-32	Continuous light at 209 µmol $m^{-2}s^{-1}$ (at the middle section)	Pure CO_2-to stabilize pH	Semi-Cont.	2 days	-	595 mg/L (maximum)	0.66	67.2% ammonium and 97.8% phosphate	-	(Ruiz-Martinez et al., 2012)
Mixed culture	2nd Lagoon Effluent	24-L plastic baskets	23.8-25.8	-	Ambient and CO_2-enriched air (2%)	Semi-Cont.	3 and 6 days	-	-	-	80% nitrogen and 90% phosphorus removal when N:P ratio is balanced	-	(Lee et al., 2013)
Chlorella vulgaris (C9-JN 2010)	Sterilized municipal wastewater (primary clarifier effluent)	7.5-L PBR	25	16 h:8 h light:dark cycle: 40–60 µmol $m^{-2}s^{-1}$	CO_2-enriched air (2%)	Semi Cont.	2-4 days	-	425-550 mg/L	0.259	Over 98% ammonium, 93% orthophosphate removal	-	(Li et al., 2013)
Chlorella prototheocoides	Non-sterilized municipal wastewaters	0.25-L flat plate vertical reactor	23	Continuous light at 100 µmol $m^{-2}s^{-1}$	CO_2-enriched air (5%)	Cont.	1.26 days	500 mg/L/day	470 mg TS/L at steady state	0.97-1.03	90% ammonia and 70% orthophosphate removal from the primary treated wastewater	-	(Ramos et al, 2013)
Desmodesmus sp.	Anaerobic digestion wastewater	100 mL Flask	24±1	15 h:9 h light:dark cycle: 120 µmol $m^{-2}s^{-1}$	-	Batch	-	190-290 mg/L.day	max. conc. of 412 mg TSS/L	-	75.5% TN and 100% TP removal	-	(Ji et al., 2014)

Table 31 (continued)

Chlorella sorokiniana and *Desmodesmus communis*	Mixed swine and municipal wastewaters (at 1:3 ratio)	1-L	28±2	12 h:12 h light:dark cycle; 126 µmol $m^{-2}s^{-1}$	CO_2-enriched air (5%)	Batch	-	193 mg/L.day for *Chlorella*	Max biomass concentrations of 1220 mg/L and 840 mg/L for *Chlorella*	-	-	-	(Yao et al., 2015)
Mixed culture	Natural fresh water	4-L CSTR	29.5	Continuous light at 27.11± 2.67 µmol $m^{-2}s^{-1}$	Ambient air	Cont.	10 days	13 mg/L.day	Average biomass conc. 130 mg/L	-	63.4% TN and 81.9% TP removal	137 mg/L.d	(Ramaraj, et al. 2015)
Chlorella vulgaris, Chlorella Sorokiniana, Scenedesmus acutus f. alternans and Scenedesmus dimorphus	Primary and secondary treated urban wastewater	0.44-L cylindrical PBR	Room Temperature	Continuous light at 140-200 µmol $m^{-2}s^{-1}$	-	Batch	-	40-60 mg/L.day	Dry weights between 100-380 mg/L	03-0.9	-	-	(Bohutskyi et al., 2016)