ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

MICROALGAL GROWTH IN ANAEROBIC DIGESTATE

M.Sc. THESIS

Mehmet Sadık AKCA

Department of Environmental Engineering

Environmental Science and Engineering Programme

AUGUST 2015

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

ANAEROBİK ÇIKIŞ ATIKSUYUNDA MİKROYOSUNUN ÇOĞALTILMASI

YÜKSEK LİSANS TEZİ

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Mehmet Sadık AKCA, a **M.Sc.** student of **ITU Institute of Science and Technology** student ID **501141754**, successfully defended the **thesis** entitled **"Microalgal Growth in Anaerobic Digestate"**, which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor : **Asst. Prof. Dr. Mahmut ALTINBAŞ**
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[illegible]

Date of Submission : 04 May 2015
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To my family,

FOREWORD

I gratefully express my gratitude to my father Prof. Dr. Lütfi Akça and my whole family for their encouragement and support, my advisor Asst. Prof. Dr. Mahmut Altinbas and my collagues.

August 2015

Mehmet Sadık AKCA
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ABBREVIATIONS

ADE **:** Anaerobic Digestion Effluent
OD **:** Optical Density

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MICROALGAL GROWTH IN ANAEROBIC DIGESTATE

SUMMARY

The reduction of fossil energy resources together with monetary and environmental concerns, drive us to find the new alternative and sustainable energy sources. Therefore, microalgae have received an increasing attention more than ten years. However, the results of the existing research and development projects were not promising to consider the microalgae as an alternative solution. To make this process commercially feasible, new approaches and methodologies are necessary. Coupling microalgae growth for valuable biomass production and wastewater treatment can be one of the sustainable solutions in terms of not only for alternative energy production, but also for the environmental protection. Anaerobic digestion effluent contains high amounts of nitrogen and phosphorus, which are essential for microalgae growth. The aim of this study is to investigate the optimum growth conditions of microalgae in the anaerobic digester effluent. This study consists of two parts. In first part, growth of microalgae in different dilution rates of anaerobic digestion effluent is investigated. All dilutions were made with tap water and reactors were run in continuous illumination with constant light intensity.Cell densities reached 0.625 g/L as dry weight in 20% diluted reactors and lowest dilution rate of considerable microalgal growth is found to be 40% diluted reactors with cell density reaching up to 0.530 g/L as dry weight. In second part, growth in lowest appropriate dilution rate (40%) was investigated with phosphorus addition, due to phosphorus limitation of cells in first part of series. Phosphorus concentrations were adjusted to 0.5, 1, 2 and 4 times of phosphorus concentration of BG-11. Growth of microalgae with phosphorus addition was more efficient than in first part of experiment with cell density reaching up to 0.881 g/L in reactors, which have same concentration of phosphorus with BG-11 medium. No pH adjustment was made and buffer solution was added to cultures, therefore there was a dramatic decline in pH values. In second part of the experiments, pH of reactors dropped to 4-5 at the end of experiments. Lower growth efficiency comparing to cultures grown in BG-11 medium is attributed to decline of pH due to lack of buffer capacity, acclimation problems of BG-11 grown cells to wastewater lower photosynthetic efficiency due to color of wastewater and competition with other microorganisms due to non-sterile conditions.

ANAEROBİK ÇIKIŞ ATIKSUYUNDA MİKROYOSUNUN ÇOĞALTILMASI

ÖZET

En bilinen biyoyakıtlar; biyoetanol, biyodizel, metan gazı gibi hidrokarbonlardır. Bunların biyoetanol dışında hepsi lipitlerden elde edilmektedir. Bitkiler başta olmak üzere algler, mayalar ve diğer mikroorganizmalarda depolama lipitleri mevcuttur.

Biyodizel birinci kaynak olarak bitkilerden elde edilse de, bitki yetiştirmek için gerekli yer ihtiyacı, temiz su ihtiyacı ve uzun bir süreç olması nedeniyle günümüzde bitkiler yerine algler ve mayalar da düşünülmektedir. Algler prokaryot ve ökaryot olmak üzere bir çok türü içinde barındıran, başta deniz ve taze su kaynakları olmak üzere bir çok çevre ortamından izole edilen ve inorganik karbonu enerjiye çeviren organizmalardır. Algler içerdikleri yüksek lipit miktarları, düşük maliyetleri, yüksek büyüme hızları (ikiye katlanma süresi=24 saat) ve bitkilere göre az yer ihtiyacı nedeniyle tercih edilmektedir. Mikroalg yetiştirilmesinde karbon kaynağı (karbondioksit ve bikarbonat gibi), azot kaynağı (üre, nitrit, azot gazı gibi), sülfür kaynağı (sülfatgib), fosforkaynağı (hidrofosfat, fosfatgibi), inorganic tuz (K, Ca, Mg gibi), eserelementler (Fe, Zn, Mn, Pb gibi) ve vitamin (B, C, E gibi) ihtiyacıvardır.

Kısaca organik maddenin oksijensiz ortamda mikroorganizmalar tarafindan metan gazina dönüştürülmesi olarak özetlenebilecek anaerobik çürütme, yenilenebilir kaynaklardan enerji üretmek için kullanılan en yaygın yöntemlerden biridir. Anaerobik çürütücülerle birden fazla çevresel fayda elde edilebilir. Çürütme sonucu oluşan biyogaz, gelişmiş ülkelerde büyük ölçekte ısı ve güç üretmek için kullanılırken, gelişmekte olan ülkelerde mevcut hayvan gübresi yönetimindeki sorunları çözmek ve ısıtma amaçlı kullanılmaktadır. Bu yöntem, sera gazı emisyonlarının özellikle metan gazı salınımının azaltılmasına yardımcı olur. Ayrıca toprak iyileştirme özelliği yüksek olan çürütücü çıkış atığının, tarımda kullanılabilmesi gibi faydaları da vardır. Çürütücü çıkış atığının, bazı istenmeyen koku, viskozite, yüksek nem ve yüksek uçucu yağ asitleri gibi özellikleri, bitkilerde fitotoksik etkiye, icerdiği yüksek nutrient miktarı yüzey sularinda ötrofikasyona, yeraltina sizmasi yeralti sularinda kirlenmeye sebep olabilir. Bu nedenle arıtma yapılmadan tarımsal topraklarda uygulanması riskli görülmektedir ve çürütücü çıkışındaki gübre etkin maddeleri olan azot ve fosforun ekonomik değerini artırıcı faydalı kullanımı gereklidir.

Anaerobik çürüme sırasında, organik azot amonyak azotuna ve toplam fosfor da ortofosfata dönüşür. Bu nedenle, anaerobik çürütücü çıkış atığında, yüksek konsantrasyonda amonyak ve fosfor mevcuttur. Bu değerli kaynakların geri kazanımında algler önemli bir yere sahiptir. Çevre kirliliğinde ana kirleticiler olarak kabul edilen azot ve fosforun alg çoğaltılmasında kullanılması ile hem çevre kirliliği azaltılmış olacak hemde alg çoğaltmak için dışarıdan temin edilen nutrient kaynaklarının kullanımı ve maliyeti ortadan kalkacaktır. Alternatif enerji kaynakları ve değerli biyoürünlerin hammadde sağlayıcısı olarak görülen mikroalgler, ülkemizin enerji açığına kaynak, çevre kirliliğine çözüm, ve ekonomisine katkı sağlayabilecek

potansiyele sahiptir. Üstünlükleri çok fazla olan bu biyokütle kaynağının sürdürülebilir yönetimi oldukça önemlidir.

Bu kapsamda bu çalışmada hem alg çoğaltılması ve hem de atıksu arıtımını öngören bir çalışma yürütülerek sonuçlandırılmıştır. Mikroalg türü olarak hızlı çoğalan Cyanobacteria grubunda olan *Synechocystis* sp. PCC6803 seçilmiş ve türün farklı seyreltme oranları ve nutrient konsantrasyonları ile büyüme verimleri incelenmiştir. Atıksu kaynağı olarak nütrient içeriği açısından zengin ve üretimi hem miktarsal hemde lokasyon olarak çok olan hayvan dışkısı seçilmiştir. Ancak organik maddece zengin olan bu tür atıkların fototrofik çoğalmada kullanımı uygun olmadığı için anaerobik çürütülmüş olan atıklar tercih edilmiştir. Katı maddelerin çürütülmesi sonrası oluşan nütrient içeriği açısından zengin olan bu atıkların askıda katı maddeden de ayrılmış ayrılmış olması gereklidir. Bu nedenle Pamukova'de yer alan biyogaz tesisinde santrifüj dekantör ile katı maddesi kısmen ayrılmış çürütücü çıkış atıksuyu temin edilmiş ve 0.45 µm gözenek çapına sahip filterelerden geçirilerek askıda katı maddeden arındırılmış bir atıksu elde edilmiştir.

Çalışma iki aşamalı olarak yürütülmüştür. Birinci aşamada mikroalgin çoğalacağı anaerobik çürütücü çıkış atıksuyunun en uygun seyreltme oranını bulmak için çalışmalaryürütülmüştür. Seyreltmeoranları %5, 10, 20, 40 ve 60 seçilerek100-300 $\mu E.m^{-2}.s^{-1}$ ışımada ve 32±2°C sıcaklıkta, 1 L/dak hava debisinde ve 500 mL lik sıvı hacminde inkübasyonlar yapılmıştır.Alg çoğalmasını izleyebilmek için çalışma boyunca günlük optik yoğunluk ölçümü yapılmış olup inkübasyonun başında ve sonunda ise pH, alkalinite, KOİ, TKN, amonyak, orto fosfat deneyleri yapılmıştır. Ayrıca çoğalan biyokütleyi karakterize etmek için karbonhidrat, protein ve yağ analizleri de yapılmıştır.

Farklıseyreltme oranları ile yapılan çalışmada en yüksek seyreltme oranı olarak %40'ta büyüme gözlenmiş, %60'ta herhangi bir büyüme gözlenmemiştir, en yüksek biyokütle konsantrasyonuna ise %20 seyreltme oranında ulaşılmıştır (0.625 g/L).

Çalışmanın ilk aşamasında hücrelerin fosfor limitasyonuna girdiği gözlemlenmiş, bu yüzden çalışmanın ikinci aşamada besiyerlerine fosfor eklemesi yapılmıştır. Fosfor konsantrasyonu, sentetikbesiyeri BG-11'in fosforkonsantrasyonun (~5 mg/L) 0.5, 1, 2 ve 4 katı olacak şekilde ayarlanmış ve seyreltme oranı olarak hücrelerin çoğalabildiği en yüksek oran olan %40 seçilmiştir. Fosfor eklemesi yapılmış reaktörlerde calışmanın ilk aşamasına göre daha iyi büyüme gözlemlenmiş ve BG-11'le aynı fosfor konsantrasyonuna sahip reaktörlerde kuru biyokütle ağırlığının 0.881 g/L'ye kadar çıktığı gözlenmiştir. Ancak atıksuya pH ayarlaması yapılmadiğı vetampon çözelti eklenmediği için pH'larda düşüş gözlemiş, deney sonunda reaktörlerin pH'ları mikroalglerin çoğalması için uygun olmayan 4-5 civarına düşmüştür.

Deneylerde, anaerobik çürütücü atıksuyunda alglerin çoğalma verimi sentetik besiyeri BG-11'deki çoğalma veriminin altında kalmıştır. Bunun sebepleri; anaerobik çürütücü çıkış atıksuyunun rengi (siyaha yakın kahverengi) dolayısıyla fotosentez veriminde olan düşüş, daha önce sentetik besi ortamında çogalmış türün atıksuya alışması problemi, steril olmayan calışma koşulları dolayısıyla atıksuda yaşaması muhtemel olan diğer mikroorganizmalarla olan rekabet ve özellikle atıksuya pH

ayarlaması ve tampon ilavesi yapılmaması dolayısıyla besiyerinin pH'ında yaşanan dramatik düşüş olarak yorumlanmıştır. Çalışmanın gelecek icin umut vaadeden sonuçlarından birisi, görece düşük seyreltme oranlarındaki amonyak konsantrasyonlarının, hücrelerde amonyak inhibisyonuna sebep olmaması, hücrelerin 300 mg/L'nin üstündeki amonyak konsantrasyonlarında dahi büyümesi olmuştur. Bu sayede daha yüksek miktardaki atıksuyun daha az maliyetle arıtılması ve atıksuda alg biyokütlesi üretilmesi olanağının oldugu görülmüştür. Deneyler sırasında bütün seyreltmeler çeşme suyuyla yapılmış olup, ışık şiddeti sabit tutulmuştur.

1. INTRODUCTION

The reduction of petroleum resources together with monetary and environmental concerns, drive us to find the new alternative and sustainable energy sources. Therefore, microalgae have received an increasing attention more than ten year. However, the results of the projects were not promising to consider the microalgae as an alternative solution. To make this process commercially available, new approaches and methodologies are necessary. Coupling microalgae growth for a valuable biomass and wastewater treatment can be one of the sustainable solution in terms of not only for alternative energy production, but also for the environmental protection.

1.1 Purpose of Thesis

Anaerobic digestion (AD) is extensively used as a bioconversion technology for farm wastes. The recovered biogas from AD can be combusted to provide heat, electricity or both. Alternatively, the biogas can be upgraded to pure methane, to be utilized in transportation or to feed into the natural gas distribution network. However, the effluent obtained from the digester during the wet anaerobic digestion process, which is called “digestate” may bring problem about overall system efficiency. Generally, digestate is used as a fertilizer, which is spread to farmland. However, larger scale biogas plants (> 1 MW electricity capacity) face with the problem of spreading digestate onto the farmlands due to mainly the economical disadvantages. Nitrogenous fertilizers has been limited to about 210 kilogram per hectare and year by the Commission of the European Communities (Karim et al, 2005). Therefore, transportation cost increases concomitantly with the increasing amount of digestate. After dewatering prosess, solid content of liquid phase goes down less than 2%. So, the digestate concentration becomes lower in liquid fraction with less content of nutrients (N, P and K) than conventional commercial fertilizers (Wen et al, 2007). The long-term use of digestate as a substitute fertilizer may also cause secondary microbial pollution even digestate’s long holding time because pathogens are

numerous in cow manures. It means that anaerobic digestate is a strong wastewater and treatment is essential before discharging through either farmlands or water bodies. Thus, the treatment systems are modified in order to reduce carbon amount and save nutrients. In this concept membranes constitute a major technology for the recovery of valuable resources.

1.2 Scope of Thesis

The scope of the thesis is given below:

- In the Second Chapter, the updated literature survey related to anaerobic digestates and microalgaea cultivation was given in detail. The survey was mainly on the literature coupling microalgae culture for biofuel production and wastewater treatment.
- In the Third Chapter, materials and methods used in the experimental set-up and in the theoretical approaches were given in detail.
- In the Forth Chapter, results and discussions were divided into two parts. In the first part, the results of experimental studies were given, in which the experiments were carried out to find out the highest dilution rate of anaerobic digestate where the microalgaea growth observed. In the second part, the experimental study was extended to observe the effect of phosphorous addition into the microalgaea cultivation.
- In the fifth chapter, the conclusions and concluding remarks were given.

2. LITERATURE SURVEY

2.1 Algae

Algae are prokaryotic and eukaryotic photosynthetic organisms that convert inorganic carbon to energy. For growth, they need carbon source (i.e. CO_2, bicarbonate) nitrogen source (i.e. urea, nitrite, nitrogen gas) sulfur source (i.e. sulfate) phosphorus source (i.e. hydrophosphate, phosphate) inorganic salts (i.e. K, Ca, Mg) trace elements (i.e. Fe, Zn, Mn, Pb) and vitamins (B, C, E) Amount of these elements can be seen on Table 1.1.

Table 1.1 : The necessary elements for algae growth

Nutrient	Appropriate amount
Carbon source	1-10 g/L
Nitrogen source	10-2000 mg/L
Phosphorus source	10-500 mg/L
Sulphure source	1-200 mg/L
Inorganic salts	0,1-100 mg/L
Trace elements	0,01-10 mg/L
Vitamins	0,01-1000 mg/L

Algal growth is directly affected by the nutrient availability (El-Nabarawy and Welter, 1984), light (Sorokin and Krauss, 1958), pH stability (Azov and Shelef, 1987) temperature (Talbot and de la Noiie, 1993) and the initial inoculation density (Lau et al, 1995).

Algae can be isolated from sea and freshwater resources. Additionally 1 ton of algae need 1,8 tons of CO_2 for energy, which makes them a major CO_2 absorber. Turkey receives 1200-2000 kWh/m^2 of solar energy annually, and growing photosynthetic microalgae which uses CO_2 as energy source would significantly reduce carbon footprint in the country (Say, 2010). Apart from being a great CO_2 absorber, algae, comparing to terrestrial plants, do not need big cultivation sites and can grow whole year. Doubling time of algae may be short as 3,5 hours, which is highly shorter than

terrestrial plants. Highest microalgal biomass efficiency is indicated as 358 ton/hectar.yrs (Pulz, 2007). Comparing to algae, biomass efficiency of maize, sorghum and sugar cane have been reported as 13-24, 44 and 73-87 ton/hectar.yrs consecutively (Huber et al, 2006). According to Akkoz (2000) research on aquatic algae has increased parallel to raising awareness about environmental issues. Reason of this is indicated about excess algae growth that leads to eutrophication resulting from wastewater discharges near settlements and in particular industry areas.

Although use of natural sources like daylight decreases the cost of commercial algale production (Janssen, 2003) this might become limiting with seasonal changes (Pulz, 1998). Generally growth of microalgae population depends on three abiotic factors; present light, temperature, nutrients. (nitrogen, phosphorus particularly) Among these factors, light, which directly influences photosynthesis mechanism, is very important for determining convenient culture conditions. In the presence of non-limiting nutrients, microalgal efficiency depends on light intensity and temperature. In addition to these, duration of day, photosynthesis, respiration play crucial role on microalgae growth by affecting cell division and growth rate circadian (Bouterfas, et al. 2006). Increasing temperature increases the growth rate of algae, cyanobacteria in particular (Whitehead and Hornberger, 1984). In summer months, decrease in the flow rate of water increases the growth potential of algae. Additionally increase in the sedimentation rate decreases the water column sediment concentration. This decreases the turbidity and with more light penetration algae growth rate increases. According to Moss et al (2011) blue-green algae that cause eutrophication in the lake and river estuaries prefers high nutrient level and temperature. Reason of this is explained as increasing fish population with increasing temperature consume zooplankton that control algae which results in uncontrolled growth of algae. These factors affecting microalgae species and concentration vary with species. According to study of Aneesh (2013) on the lakes, samples taken for 11 months were investigated and no seasonal variety in the dominance of *Microcystis sp.* (cyanobacteria) and *Scenedesmes sp* (green algae) was observed.

Anaerobic digestion effluent contains high amounts of ammonia and phosphorus (Hobson, 1992) which are essential nutrient sources of algal growth. For the management of anaerobic digestion effluent, the most common method is landfilling. Direct landfilling of digestate is considered a cheap method for applying fertilizers to

agricultural areas. Especially high amount of organic content is a desired condition for some agricultural areas (Grigatt et al, 2011; Chadwick, 2007; Schievano et al, 2009). But easily degradable organic compounds may not be fully consumed in the produced anaerobic digestion effluent. This case may result in problems about storage (odor emission, regrowth of pathogens, phytotoxicity) and may have negative impact on soil-plant system by limiting potential fertilizer value. (Abdullahi et al, 2008; Salminen et al, 2001) This may result in greater impacts that must be investigated like delay on germination of seeds on plants, death of plants and suppression of growth. For agriculture, anaerobic digestion effluent is more commonly used at Northern European countries like Denmark, Sweden, Scotland, Germany, at crop cultivation (Moller and Stinner, 2009; Ortenbald 2002; Rodhe et al, 2006; Smith et al, 2007). Generally, for the low energy capacity digestion plants (energy production <1 MW) landfilling digestate is considered economically viable. But in the case of more energy production, there will be more anaerobic digestion effluent, and this will increase landfilling cost. Besides, landfilling digestate will cause pollution in the groundwater because of high nutrient loading. Due to high N and P content of digestate, leaked water from agriculture sites causes eutrophication in the near water bodies. If digestate is not used directly for landfilling, for its effective management, its liquid and solid phases should be separated. For this process, sedimentation, floatation, band filter, centrifuge decanter, drill press, drying/evaporation technics can be applied (Zhang et al, 2013; Waeger-Baumann et al, 2010; Chiumenti et al, 2013).

Liquid portion of anaerobic digestion effluent contains high amount of nutrients (Table 2.2). Digestate consists of 80-90% of liquid and 10-20% of solid fractions. Significant amount of nitrogen in the digestate is present in the liquid fraction (65-75%) Ammonia form of total nitrogen is about 70-80% in the liquid fraction. For phosphorus, 35-45% of total phosphorus is present in liquid fraction. For potassium, 70-80% of potassium is in liquid fraction. (Fuch et al, 2013) Though liquid portion can be directly used as soil fertilizer, its biodegradable organic material and salt content is a limiting factor for this. Membrane separation and membrane bioreactors are among the technics for treatment of liquid fraction. For ammonia removal nitrification-denitrification, for phosphorus removal, biological or chemical precipitation can be applied. Feeding algae is among the methods of nutrient removal from liquid fraction.

There is a number of study on algal growth in anaerobic digestion effluent in the literature (Table 2.3). Golueke and Oswald (1959) have been successful to grow Scenedesmus sp. in digestate. Olguin et al. have observed the growth rate of *spiriluna maxima* in the swine manure digestate diluted with seawater, observed increase in the growth rate with CO_2 supply and have concluded that merging anaerobic digestion and algae growth systems would be applicable for nutrient reuse and wastewater treatment. Blier et al (1995) have observed *phormidium bohneri* (cyanobacteria) and *micractinium pusillum* (microalgae) growth and 100% inorganic nutrient removal in anaerobic digestion effluent from cheese factory. Bjornsson et al (2013) have investigated growth of *Scenedesmus* sp. in the cow and swine manure digestate diluted with distilled and lake water. Results indicated that maximum growth rate is in digestate diluted with lake water. In the following experiments it has been seen that growth rates and biomass efficiencies are higher in the species grown in swine manure digestate and supported with Mg^{2+}. In this study, it has been observed that Mg^{2+} enters the system with lake water and pointed out that Mg^{2+} could be the key element for high biomass efficiency. Supporting this data, in the study made by Park et al (2010) increase in the growth rate of *Scenedesmus acuminatus* with Mg^{2+} addition grown semi-continously in swine manure digestate. Franchino et al (2013) observed growth of 3 different algae species (*Neochloris oleoabundans, Chlorella vulgaris* and *Scenedesmus obliquus*) in the digestate of cow manure, sewage sludge and raw whey. Wastewater was diluted and used for growth of species. Results indicated the maximum growth rate of all species in 1:10 dilution. In another study made by Prajapati et al (2014) diluted anaerobic digestate has been used for growth of *Chroococcus* sp. Results showed that anaerobic digestate diluted with 30% BG-11 medium is the most suitable for this species.

Table 2.1 : Composition of liquid portion of anaerobic digestion effluent.

Waste Type	Parameters															References
	S, %	VS, %	SS, g/L	VSS, g/L	pH	TN, g/L	NH_4-N, g/L	NO_3-N mg/L	TP, g/L	K, g/L	Mg, g/L	Ca, g/L	Na, g/L	COD, g/L	BOD_5, g/L	
Crop waste	1.9	-	-	-	8.3	3.8	-	-	0.22	2.00	0.18	0.50	524	-	-	Alburquerqe et al, 2012
Swine manure	11±1	-	-	-	7.5±0.6	6.8±0.4	5.5±0.3	-	2.4± 1.1	3.8± 0.8	-	2.4± 0.2	2.8± 0.5	-	-	Vaneeckhaute et al, 2013
Cow manure	4.8±0.2	3.7± 0.3	-	-	7.5±0.1	2.5±0.1	0.87± 0.04	0	0.51	3.38	-	-	-	10±0.1 (Soluble)	-	Seppala et al, 2013
Animal manure	0.15-1.27	-	-	-	5.6-8.2	0.6-4.9	0.4-3.5	-	0.1-1.2	0.8-3.1	0.1-0.7	-	-	-	1.2-62.5	Magri et al, 2013
Calf manure	-	-	29.6± 3.4	38.5± 2.8	8.3±0.3	1.55±0.2	1.06± 0.2	-	0.46± 0.08	-	0.15± 0.02	0.57± 0.02	-	-	-	Siciliano et al, 2013
Cow manure	3.14	-	-	-	7.42	1.7	0.9	-	0.3	1.4	0.29	1.29	0.53	-	8.3	Alburquerqe et al, 2012

Table 2.2 : Algal growth in anaerobic digestion effluent.

Digestate	Mikroalgae Species	Valuable Product	Maximum Algae Concentration (g/L)	References
Cow and swine manure, algae	*Scenedesmus* sp. AMDD	Biomass	0.55	Bjornsson et al. (2013)
Sewage sludge	*Scenedesmus* sp. dominant mixed microalgae consort	Biomass	2.6	Uggetti et al. (2014)
Cattle manure and swey	*Neochloris oleoabundans, Chlorella vulgaris and Scenedesmus obliquus*	Biomass	1.13	Franchino et al. (2013)
Cow manure	*Chlorella* sp.	Lipid (max. 19%)	1.6	Wang et al. (2010b)
Swine manure + domestic wastewater	*Scenedesmus* sp.	Lipid (<10%) Chlorophyll a 2.5 mg/g Chlorophyll b 1.2 mg/g Lutein 0.3 mg/g β-karoten 0.06 mg/g	0.96	Dickinson et al. (2015)
Cow manure	*Neochloris oleoabundans*	Lipid (10%)	~1.0	Levine et al. (2011)
Swine manure	*Scenedesmus accuminatus*	Biomass	~1.1	Park et al. (2010)
-	*Chlorella* sp.	Biomass	~0.6	Yan and Zheng (2013)
-	*Synechocystis* sp.	Lipid (13.5%)	~0.5	Cai et al. (2013)
Farm waste (manure)	*Scenedesmus* sp. and *Chlorella* sp.	Biomass	0.85	Marcilhac et al. (2015)
Sewage sludge	*Chlorella* sp.	Lipid (12.7%)	0.22	Smith (2012)
Swine manure	*Desmodesmus* sp.	Biomass	0.4	Ji et al. (2014)
Swine manure	*C. sorokiniana*	Biomass	-	Hernandez et al. (2013)
-	*Tetraselmis* sp.	Lipid (49%)	-	Erkelens et al. (2014)
Algae	*Chroococcus* sp.	Biomass	0.8	Prajapati et al. (2014)

Bjornsson et al (2013) investigated the growth of *Scenedesmus* sp. AMDD and recycle of nutrients on effluents from anaerobic digestion of cow manure, swine manure and co-digestion of swine manure and algal biomass with and without additional CO_2 supply. They have observed maximum biomass yield and nutrient assimilation when swine manure digestate was mixed with algal biomass digestate and diluted with lake water or supplemented with magnesium. They have concluded that magnesium is limiting for algal growth on swine manure digestate and supplying magnesium is essential for recycling nutrients of swine manure digestate for algal growth. In flask cultures, for all growth conditions (swine manure, cow manure, swine manure + algal biomass) growth rate of algae was greatest between inoculation and day 1. There was a retardation between days 1-3 and growth stopped by the third day, before start of N and P limitation. At the end of day 6, relatively high amounts of N and P remained in the culture. According to these results it has been concluded that cultures had excess macronutrients for growth and some other factor was limiting. In 1 L experiments, similar to flask cultures, exponential growth ceased by day 3 but N and P continued to be drawn down near depletion. In the experiments run with algal biomass digestate, complete N drawdown was observed, the cultures grown in swine manure digestate, amended with Mg^{2+} assimilated virtually all N in the cultures. In the cultures, swine manure digestate diluted with lake water, highest biomass yield was achieved (0.55 g dw/L) and nearly all of the N and P was removed.

According to these results, in cultures with a fraction of algal biomass digestate, satisfactory nutrient removal and biomass yields were achieved. In cultures with animal manure only digestates with DI water, complete drawdown of NH_3-N was not achieved, however with the Mg^{2+} addition, increase has been observed on algal growth and nutrient drawdown. Differences in the results of animal manure only digestates and algae-animal manure co-digestates suggested there was at least one factor required for optimum growth, present in algal digestate but missing in animal manure digestate. Further experiments showed that biomass yields and growth rates of *Scenedesmus* sp. AMDD approached optimum values with the addition of Mg^{2+}, suggesting that this element was the key nutrient, which was present in algae digestate.

Uggetti et al (2014) investigated the growth of mixed microalgae culture dominated by *Scenedesmus* sp. using 500 ml flasks in the anaerobic digestion effluent coming from a wastewater treatment plant, while some samples diluted with tap water. The initial growth rates of the cultures which are calculated at the end of the exponential growth varied between 0.04 to 0.9 d^{-1} and are inversely proportional to the absorbance. They have observed decrease in the growth rate in varied concentrations of digestate with the increase of algal concentration. This suggests effect of mutual shading; limitation of light penetration causes improved O_2 concentration due to dark respiration of algal cells. Initial ammonia concentration is another factor affecting the growth rate. High ammonia concentrations (about 2.3 um) can inhibit microalgal growth and parallel to this, a decrease in the growth rate is observed with the increase of initial ammonia concentration.

Unlike the initial growth rate, biomass production is found to be directly proportional to the algae concentration. With the highest initial digestate concentration, (260 mg NH_4-N/L) highest biomass concentration (2.6 gTSS/L) was achieved, which means the more initial microalgal concentration means more biomass production. It is explained as O_2's stimulating effect of aerobic bacteria present in the culture. By the enhanced production of O_2 from photosynthesis with the higher initial algal concentration, aerobic bacteria converted more organic matter to carbon dioxide and ammonia needed by algae, thus improved the biomass production.

Franchino et al. cultivated three microalgae strains, *Neochloris oleoabundans, Chlorella vulgaris and Scenedesmus obliquus* on an agro-zootechnical digestate coming from a pilot plant anaerobic digester of several mixes of cattle slurry and raw cheese whey. Microalgae strains almost completely removed different forms of N and P, chlorella vulgaris with the highest elimination of 96% ammonia removal in 1:10 diluted digested sample.

In the experiments with lower dilutions (1:1 and 1:2, diluted with tap water) c. vulgaris did not survive due to high turbidity, while in 1:5 dilution survived with 87% biomass lost. All three strains survived in higher dilution ratios. (1:10, 1:15, 1:20, 1:25). In all dilution ratios no significant change of mean productivity was observed for any strain, so it is concluded that initial substrate concentration did not affect algal growth. For differences of growth were not significant, 1:10 dilution ratio is seen not likely to limit algal growth and is thought to be suitable for full scale

application to treat larger amount of digestate with higher initial substrate concentration.

Most of the N in the digestate was in the ammonia form and *vulgaris* and *oleobundas* removed of ammonia in all dilution ratios with more than 99% efficiency while for *obliquus* this was between 83%-93%. Due to high NH_4^+/NO_3^- ratio, clear nitrate removal was not observed; NH_4^+ is preferred form of N for microalgae. Phosphorus removal was also efficient, for all strains more than 94% phosphate removal efficiency was achieved. For elimination capacity, EC of phosphate and ammonium were higher in 1:10 mediums, indicating that with higher initial nutrient concentrations, more nutrient is removed.

With the same dilution ratios (10, 15, 20, 25) Wang et al. grew oil-rich green microalgae *Chlorella* sp. in dairy manure digestate. A slow growth rate was observed in the less diluted samples with the reverse linear relationship between specific growth rates (first 7 days) and initial turbidity. With algal biomass, removal of 100%, 75.7-82.5%, 62.5-74.7% and 27.4-38.4% of removal was achieved respectively for ammonia, total nitrogen, total phosphorus and COD, in differently diluted samples. It has also been proven that mixotrophic *Chlorella* uses COD in the digestate as alternative carbon source, for experiment was performed in axenic condition. Incomplete removal of total nitrogen suggests that there was some organic compounds not converted to ammonium nitrogen. For the first 7 days in the 1:20 and 1:25 diluted samples, faster growth was observed, though it started to level off due to exhaustion of nutrients. For the four dilutions, growth rates in first 7 days were 0.282, 0.350, 0.407 and 0.409 d^{-1} respectively.

Dickinson et al (2015) grew *Scenedesmus* sp. AMDD in continuous chemostats of 1.8 l in municipal wastewater and municipal wastewater blended with swine manure and algal biomass anaerobic co-digestate to observe nutrient remediation and effects of wastewater on algal growth. With anaerobic digestate, 1.6 times and 2.4 times more nitrogen (N) was supplied. Though not directly proportional, an increase was observed in biomass concentration and productivity with higher nutrient amounts, however light became limiting with increasing cell densities. Also, increase in the cellular quotas of carbon, nitrogen and phosphorus was observed, with the increase of these elements in the growth medium. Protein was directly proportional to cell density while total carbohydrate was inversely proportional and fatty acid contents

remained relatively constant. In all experiments, complete ammonium and phosphate removal was achieved, with highest removal rates 41.2 mg N $l^{-1}d^{-1}$ for and 6.7 mg P $l^{-1}d^{-1}$ in wastewater with 2.4 times higher N level. Highest specific growth rates were observed 2nd and 3rd days of initial batch growth. Cell numbers increased for 7 days of cultivation and after that point wastewater or wastewater blended with digestate was supplied and chemostat cultivation continued for further 37 days. In this period it has been seen that soluble N and P were completely assimilated by algae.

Levine et al (2011) investigated the batch growth of oleaginous green algae *Neochloris oleabundans* in diluted (1:50, 1:100, 1:200, with distilled water) dairy manure digestate and synthetic media and achieved 90-95% initial nitrate and ammonium assimilation in digestate after 6 days. Algae yielded 10-30% fatty acid methyl esters on dry weight basis. Lipid contents and N concentrations in the growth medium were inversely proportional. The highest biomass productivity and cell density was observed in MBBN-NO_3^- with N concentration of 100 mg L^{-1} for 7 days while in the lower NO_3^- concentrations, exponential growth lasted for 4 days. High NH_4^+-N concentrations (100 mg/L) seemed to have toxic effect on *Neochloris oleabundans*, cell density never exceeded 0.1 g/L. For NH_4^+ treatments, highest cell growth was observed in mid-level NH_4^+-N, (25 mg/L) a maximum of 0.4 g/L. In the lowest NH_4^+-N concentration (10 mg/L) cell growth was supported over a 10 days period and final biomass density was similar with the culture grown with same level of NO_3^-.

With N. *oleabundans* grew on synthetic medium with the NH_4^+ as sole N source, it was hypothesized that anaerobic digestion effluent which is rich by NH_4^+ would support algal growth. In the experiments, it has been seen that the highest biomass productivity is in the most concentrated digestate, while it decreased when digestate became more dilute. In the digestate with 1:50 dilution, cells grew more quickly and maintained higher biomass productivity than all levels of MBBM-NH_4^+. Maximum biomass densities were observed in the digestate dilutions 1:50 and 1:200 similar to MBBM-NO_3^- with N concentrations of >50 mg/L and 10 mg/L respectively.

In all treatments, 90-95% removal of N was observed after 6 days. For algae grown in MBBM-NH_4^+, N removal rates were higher in the higher initial NH_4^+ concentrations. It declined significantly after day 2, likely to be because of declining biomass productivity. In cultures grown in digestate, N removal rates were equal to

those in MBBM-NH_4^+ in first 2 days and overall removal rates in digestate were higher than any other group in first 4 days.

Park et al (2010) investigated growth and nitrogen removal of *Scenedesmus* in livestock digestate (swine manure) effluent containing high amounts of ammonium and alkalinity. No ammonium inhibition was observed up to 100 ppm NH_4^+-N but cell density decreased by 70% in concentrations of 200-500 ppm. Algae consumed bicarbonate quickly and this resulted in attenuation of cell growth. Aeration is thought to be beneficial due to stripping of ammonium to ammonia gas and stripping of oxygen which may have inhibitory effect on photosynthesis. Magnesium consumption was very fast so supplying additional Mg^{2+} is essential. Cells preferred both NO_3^- and NH_4 in the same manner. 100 ppm NH_4-N cultures demonstrated the best cell growth, slightly better than that of 100 ppm NO_3^- - N. Growth rates of cultures with 200-500 ppm NH_4-N were similar to that of 100 ppm but decreased after 7-8 days, showing that inhibition levels of 200 ppm and 500 ppm were similar. Inhibition became severe after 800 ppm. Net growth rate of cells were higher in higher seeding cell densities, while higher specific growth rates were observed in lower seeding concentration. Different seeding concentrations affected ammonia removal during the cultivation. In first 4 days of cultivation, lowest seeding concentration (0.5 g/L) resulted in the highest ammonia removal rate. After 4 days, its removal rate decreased and overall removal rate became the lowest. The overall ammonium removal rates were directly proportional to net growth rates and initial seeding concentrations. Near whole inorganic carbon, -mostly bicarbonate- that was 80-90 mg/L as C at the beginning, was consumed rapidly within 2 days. With the consumption of inorganic carbon the cell growth was attenuated. Ammonia removal also decreased and only 13% was removed in 10 days. Effect of aeration was positive in terms of ammonium removal and increased algal biomass.

Yan and Zheng (2013) investigated biogas upgrade by algal growth in anaerobic digester effluent. The main purpose was to use CO_2 in the biogas and nutrients in the biogas reactor effluent for algal growth and increase the methane percent in the biogas, that is thought as the most cost effective way to sequester CO_2 from biogas. Highest dry weight value of microalgal growth was achieved in moderate light intensity with middle photoperiod. For best biogas upgrading, low light intensity and long photoperiod and moderate light intensity with middle photoperiod is found to be

most suitable. With moderate light intensity and middle photoperiod, nutrient reduction was also highest, thus the optimal parameters are decided to be moderate light intensity with 350 umol m^{-2} s^{-1} and middle photoperiod of 14 hours light and 10 hours dark.

Cai et al (2013) investigated the growth of two strains; cyanobacteria *Synechocystis* sp. and seawater algae *Nanochloropsis Salina* in artificial seawater supplemented with anaerobic digester effluent. Potential use of *Synechocystis* sp. was compared to *Nanochloropsis Salina.* Growth rates of cyanobacteria was higher than that of marine microalgae by 83% and 20% in 3% and 6% digestate loading ratios respectively. Cyanobacteria achieved highest biomass activity of 212 mg l^{-1} d^{-1}. However its lipid productivities were lower than marine microalgae by 7-28%. Decreasing the harvesting interval in the semi-continuous growth of cyanobacteria may be useful to improve its lipid productivity. Lipids extracted from cyanobacteria also had higher palmitic acid, linoleic acid, cetane number and oxidative stability than those of marine microalgae. Compared to marine microalgae, cyanobacteria favored lower digestate loading ratios and achieved higher growth rates and biomass productivities.

Marcilhac et al (2015) investigated the effect of N:P ratio and phosphorus limitation in nutrient removal from anaerobic digestion effluent by algae. They have observed no growth limitation over 14 days, even in the depletion of P, in the N:P ratios between 3 and 76. For the trials with N:P ratios 26 and 76, phosphorus concentration dropped below 0.1 mg L^{-1}; as microalgae required phosphorus to grow, cellular phosphorus storage was clearly observed. Highest phosphorus removal rate was observed in the trial with N:P ratio is 3, while nitrogen removal rate was same for all experiments. N:P ratios affected phosphorus removal but did not affect algal growth. Nitrogen removal was not affected by N:P ratios and phosphorus concentration, however nitrification was highly affected by N:P ratio and phosphorus concentration. Biomass N:P ratio was found to be a function of influent N:P ratio, thus cellular phosphorus storage was proven. Experiments were made in 4 conditions in terms of N:P ratio (N:P ratios; 3, 9, 26, 76) and all demonstrated same growth kinetics, with the final maximum microalgae concentration reaching 1 x 10^7 cells mL^{-1}.

Smith (2012) investigated the growth of *Chlorella* sp. under mixotrophic conditions in unsterilized digestate. It has been seen that *Chlorella* sp had stronger growth in whole digestate rather than lower turbidity centrifuged samples, which may be due to

higher nutrient levels and mixotrophic growth. *Chlorella* sp. also demonstrated faster growth rate in mixotrophic conditions rather than autotrophic and heterotrophic growth combined, indicating a synergistic boost due to increase in local CO_2 concentration. In mixotrophic conditions, also higher lipid productivities were achieved.

Ji et al (2014) investigated the growth and nutrient removal of a newly isolated algae strain which have a close relationship with *Desmodesmus* sp. in anaerobic digestion effluent. The strain was capable remove 100% of NH4–N (68.691 mg/L), TP (4.565 mg/L) and PO4–P (4.053 mg/L), and 75.50% TN (84.236 mg/L) at 1:10 digestate, with removal rates of 5.284, 0.326, 0.290, 4.542 mg/L/d respectively. Highest biomass production was achieved after 14 days of cultivation as 0.412 g/L.

Prajapati et al (2014) studied a "closed loop system" for algae to biomethane production. *Chroococcus* sp. was anaerobically digested and its liquid effluent was used for further algal biomass production. Digested algal biomass has a biomethane potential of 317.31 +- CH_4 g/VS_{fed}. 30% diluted liquid digestate was found to be the optimum growth medium for algal biomass with 0.79 g dry biomass/L biomass production and 69.99–89.31% nutrient and sCOD removal by algae was achieved. Higher growth was observed when digestate was diluted with rural sector wastewater and BG11 broth.

3. MATERIALS AND METHODS

3.1 Algal Culturing Technique

Algal strain used in this study is cyanobacteria *PCC 6803* is supplied from The Pasteur Culture Collection of Cyanobacteria (PCC) (France). This strain isolated from freshwater in 1968 (Waterbury & Stanier, 1981). Cells were grown in synthetic BG-11 growth medium (Table 3.1) in 1 L flasks for 2 months before the cultivation with liquid anaerobic digestate. This strain were cultured at 32±2°C with a continuous white led illumination with intensity of 100-300 $\mu E/m^{-2}*s^{-1}$. Aeration is carried out with a rate of 1 L/min by air diffusers located at the bottom of erlenmayer.

Table 3.1 : The components of BG-11 medium.

Component	Amount	Stock Solution Concentration	Final Concentration
$NaNO_3$	10 mL/L	30 g/200 mL dH_2O	17.6 mM
KH_2PO_4	10 mL/L	0.8 g/200 mL dH_2O	0.23 mM
$MgSO_4.7H_2O$	10 mL/L	1.5 g/200 mL dH_2O	0.3 mM
$CaCl_2.2H_2O$	10 mL/L	0.72 g/200 mL dH_2O	0.24 mM
Citric acid.H_2O	10 mL/L	0.12 g/200 mL dH_2O	0.031 mM
Ferric ammonium citrate	10 mL/L	0.12 g/200 mL dH_2O	0.021 mM
$Na_2EDTA.2H_2O$	10 mL/L	0.02 g/200 mL dH_2O	0.0027 mM
Na_2CO_3	10 mL/L	0.4 g/200 mL dH_2O	0.19 mM
BG-11 Trace metals solution	1 mL/L		
Sodium thiosulfate pentahydrate	1 mL/L	49.8 g/200 mL dH_2O	1 mM

3.2 Anaerobic Digestate

The liquid digestate used in this study as a growth medium was provided from Biosun Pamukova Katı Atık İşleme Enerji ve Çevre Sanayi Ticaret A.Ş. located at Pamukova (Sakarya). This plant mainly digest the mixture of organic fraction of municipal solid wastes, waste activated sludge, chicken and cattle manures. Digestate was first centrifuged for 30 minutes at 9000 rpm and then filtered through 0.45 μm

filters to obtain suspended solid free liquid. Characteristics of ADE after centrifugation and filtration were shown in Table 3.2.

Table 3.2 : The composition of liquid anaerobic digestate.

Parameter	Unit	Value
TKN (Total Kjeldahl Nitrogen)	mg/L	956
NH_3-N	mg/L	803
Total Phosphorus	mg/L	5.51
Ortophosphate	mg/L	4
pH	-	8.66
Conductivity	mS/cm	19.36
Alkalinity	mg/L $CaCO_3$	1604

3.3 Cultivation

Experiments were performed in duplicate with 0%, 5%, 10%, 20%, %40 and 60% dilution rates for 21 days. All dilutions were made with tap water. With 1 L erlen flasks 28.75 mL inoculum grown in BG-11 was added to 500 mL media to obtain around 0.1 absorbance in the working flask at 680 nm. The cultivation was carried out continuous white led light with illumination intensity of 100-300 $\mu E.m^{-2}.s^{-1}$ at 32±2°C by using incubation chamber (Figure 3.1).

Optical density measurements were made on daily basis with spectrophotometer at 680 nm (Jenway 6300, Staffordshire, UK). For the loss of media volume due to evaporation, tap water was added to flasks in every 2-3 days to keep culture volume constant. For suspension and inorganic C demand of cells air was supplied into reactors with aquarium motor (Quik SB-988, Istanbul, Turkey). The flowrate of the feeding each flask was kept constant as 1 L/min. Experiments were conducted for 21 days. 4 ml sample was taken throughout the entire growth phase of the culture for the optical density measurements. By this way the viability of the culture was controlled and growth charts were prepared.

Figure 3.1 : Cultivation chamber of *Synechocystis* sp. PCC6803.

After first series, second cultivations were made with phosphorus addition at 40% dilution rate, with concentrations set to 0.5, 1, 2 and 4 times of phosphorus concentration of BG-11, which has TP of 4.96 mg/L. K_2HPO_4 was used as phosphorus source as in BG-11. Dilution was again made with tap water. Optical densities were measured on daily basis with spectrophotometer at 680 nm wavelength, same as in first series.

After growth experiments, algal cultures were centrifuged at 9000 rpm for 15 minutes (Hettich, Universal 320, Germany). Supernatant is taken for COD, TKN, NH_3-N, TP, orthophosphate analysis. Suspended biomass was kept in freeze drier (Thermo Savant, ModulyoD, USA) for 48 hours for lipid, protein and carbohydrate analysis.

3.4 Analytical Methods

For lipid analysis modified Bligh and Dyer (1959) methods was used (Li et al, 2014). Pre weighted the algal powder was eluted by 5 ml of chloroform and methanol (1:2, v/v; $CHCl_3$/MeOH) in a 15 ml falcon tube. Tube placed in an Ultrasonic Cleaner at room temperature (25 derece) for 90 minute. Then, sample kept at this condition one day (approx. 20 hrs). After that, 2 ml $CHCl_3$ and 3.6 ml water added, vigorously vortexed and centrifuged at 1,000 × g for 5 min. The organic phase was pipetted into new tube for lipid analysis (GC analysis and weighing). The remaining phase was pleaced into the freeze dryer again to determine the pure lipid amount.

The lipid composition of algal biomass was determined by a gas chromatograph (Shimadzu GC-2010) equipped with a flame-ionisation detector and a 100 m × 0.25 mm inner diameter and film thickness 0.2 μm, TR-CN100 capillary column (Teknokroma, Barcelona, Spain). The temperature of the injection port and detector were 260°C and 260°C, respectively. The oven temperature reached 140°C in first 6 min and then 140°C to 240°C (4°C/min) and fixed at 240 °C in 10 min. Helium was the carrier gas at 30 ml/min. In addition, hydrogen gas was used at 40 ml/min flow rate and air flow was used at 400 ml/min. 1.0 μL injection at 100:1 split ratio was was use to transfer the sample to gas chromatography.

Carbohydrate analysis was carried our according to Gerdhart et al (1994).

Protein by Folin Reaction (Lowry et al, 1951) was used to estimate the amount of proteins (already in solutions or easily-soluble in dilute alkali) in biological samples.

Suspended solid and volatile suspended solid experiments, alkalinity analysis and pH measurements (Thermo, Orion 720A+) were performed before centrifuge.

Suspended Solids (SS), Volatile Solids (VS), pH, alkalinity, COD, COD, TKN, NH_3-N, TP, orthophosphate analysis were all analyzed according to ‘‘Standard Methods” (2005).

4. RESULTS AND DISCUSSION

The study was divided into two parts. In the first part of the study, cultivations of *Synechocystis* sp. PCC6803 was carried out with different liquid anaerobic digestate ratios by diluting with tap water. The aim was to determine the maximum ratio of liquid anaerobic digestate where the growth ratio of *Synechocystis* sp. PCC6803 was observed. In the second part of the study, the cultivations were carried out with different phosphorous concentrations. Since, phosphorous concentration in liquid anaerobic digestate was low, phosphorous was added externally to provide the need in comparison with the BG11 medium.

4.1 Maximum ratio of liquid anaerobic digestate

Cultivations of microalgaea on liquid fraction of anaerobic digestate were carried out up to 60% dilution with water to find out the maximum ratio where the microalgaea could grow. The dilution of liquid fraction of anaerobic digestate was managed with tap water to adjust the concentrations of disgestates as 5, 10, 20, 40, and 60%. Optical Density results of microalgaea cultivation on liquid digestate demonstrated that how the dilution rate affect the growth efficiency. While the growth of *Synechocystis* sp. PCC6803 was observed at 40% dilution, at the 60% dilution, not any growth was observed up to around 30 days. The optical density changes during cultivation of *Synechocystis* sp. PCC6803 with 5, 10, 20, 40% ADE were given in Figure 4.1-4.4, respectively. Overall optical density change was given in Figure 4.5.

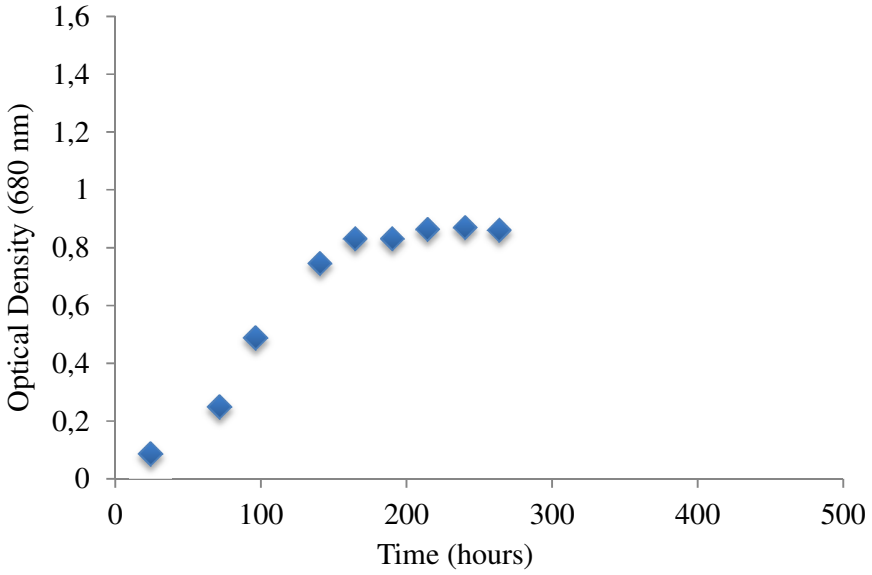

Figure 4.1 : Average optical density change during cultivation of *Synechocystis* sp. PCC6803 with 5% ADE.

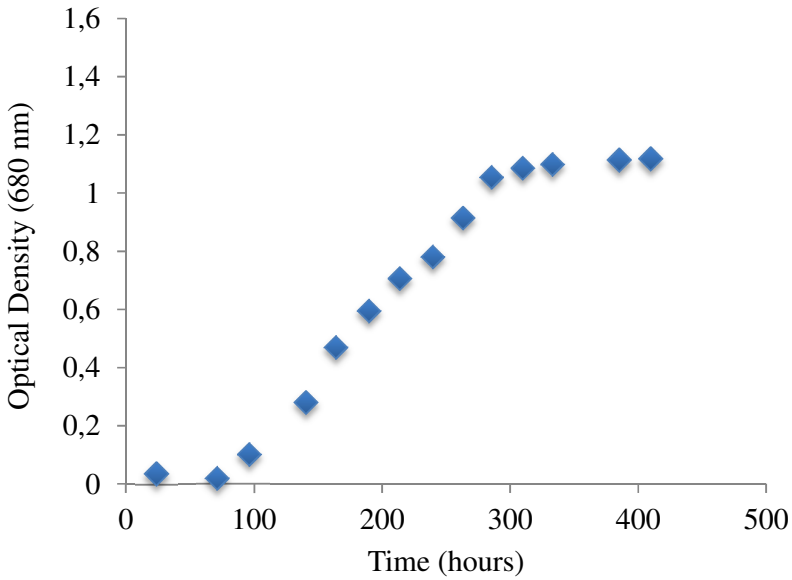

Figure 4.2 : Average optical density change during cultivation of *Synechocystis s*p. PCC6803 with 10% ADE.

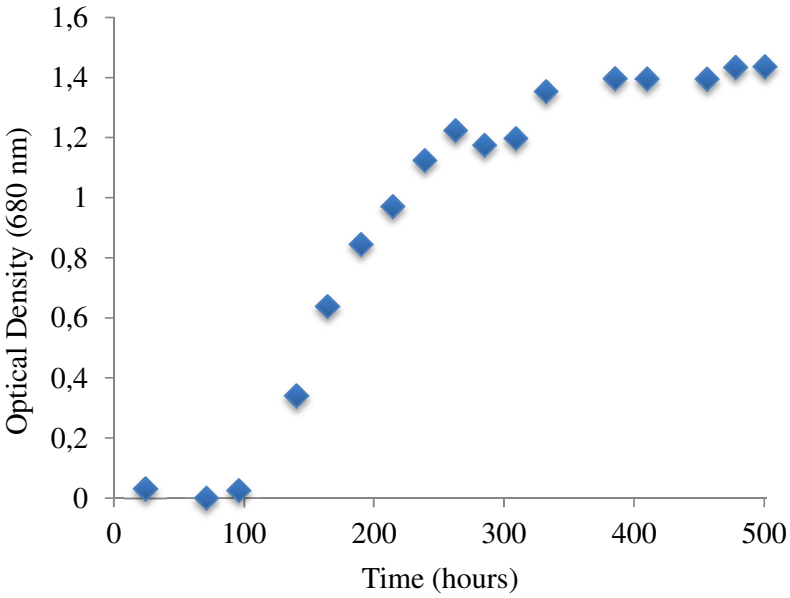

Figure 4.3 : Average optical density change during cultivation of *Synechocystis* sp. PCC6803 with 10% ADE.

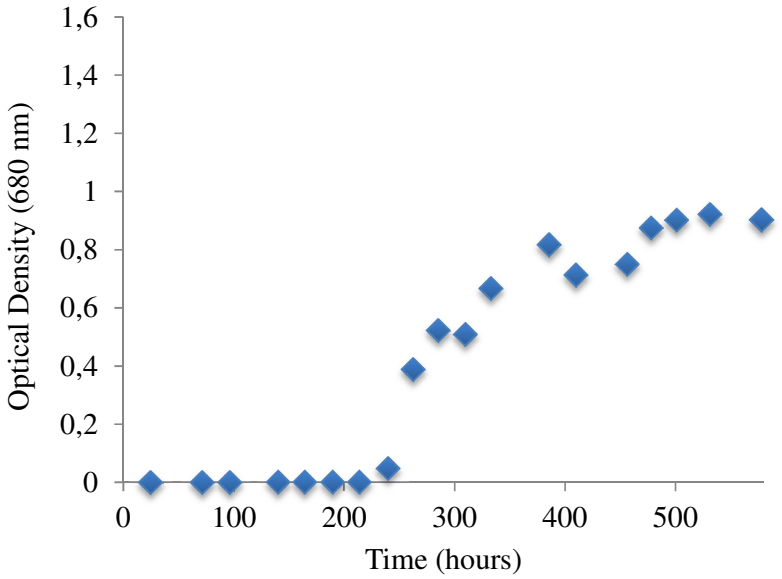

Figure 4.4 : Average optical density change during cultivation of *Synechocystis* sp. PCC6803 with 40% ADE.

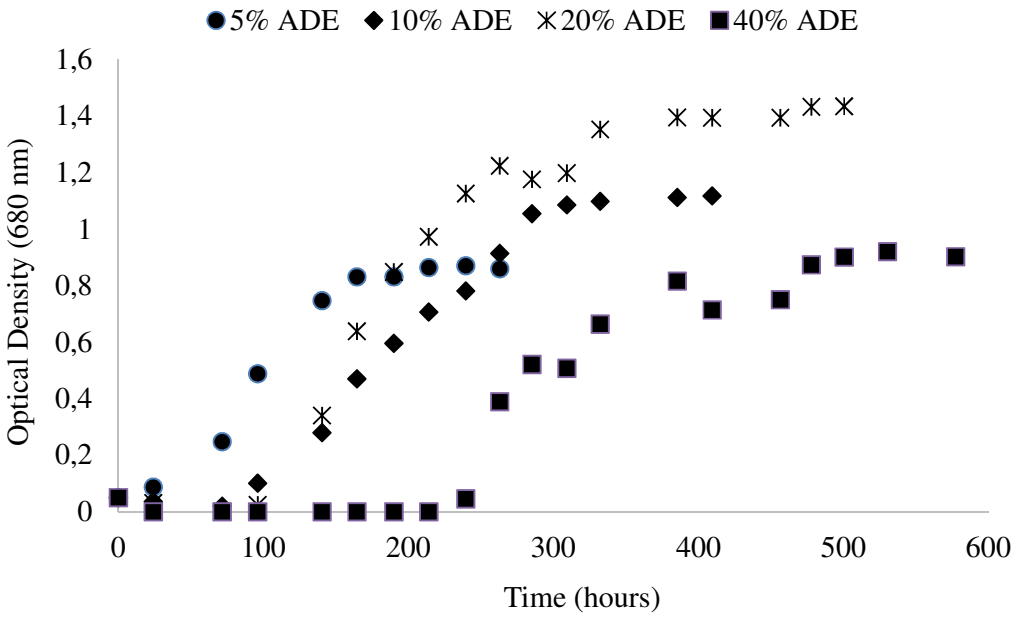

Figure 4.5 : Average optical density change during cultivation of *Synechocystis* sp. PCC6803.

The dry weight changes during cultivation of *Synechocystis* sp. PCC6803 with 5, 10, 20, 40% ADE were given in Figure 4.6.

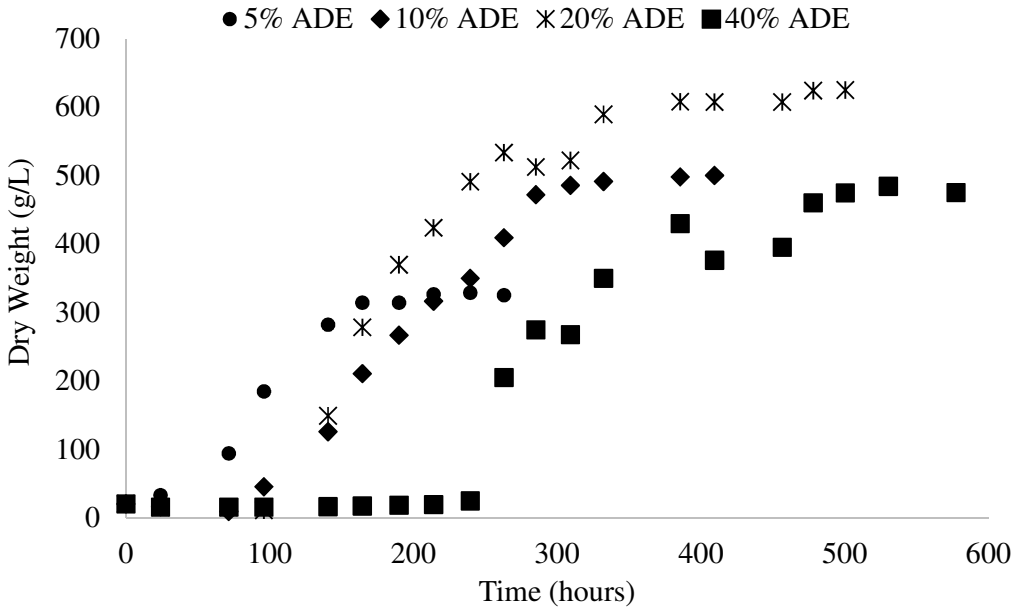

Figure 4.6 : Average dry weight change during cultivation of *Synechocystis* sp. PCC6803.

Average TKN change during cultivation of *Synechocystis* sp. PCC6803 was given in Figure 4.7.

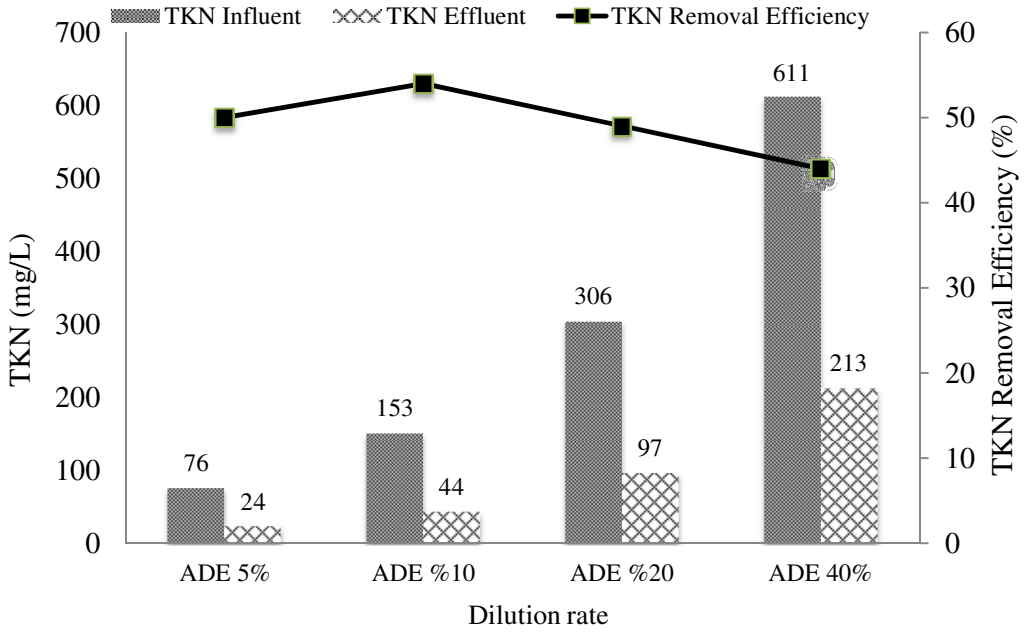

Figure 4.7 : Average TKN change during cultivation of *Synechocystis* sp. PCC6803.

Average ammonia change during cultivation of *Synechocystis* sp. PCC6803 was given in Figure 4.8.

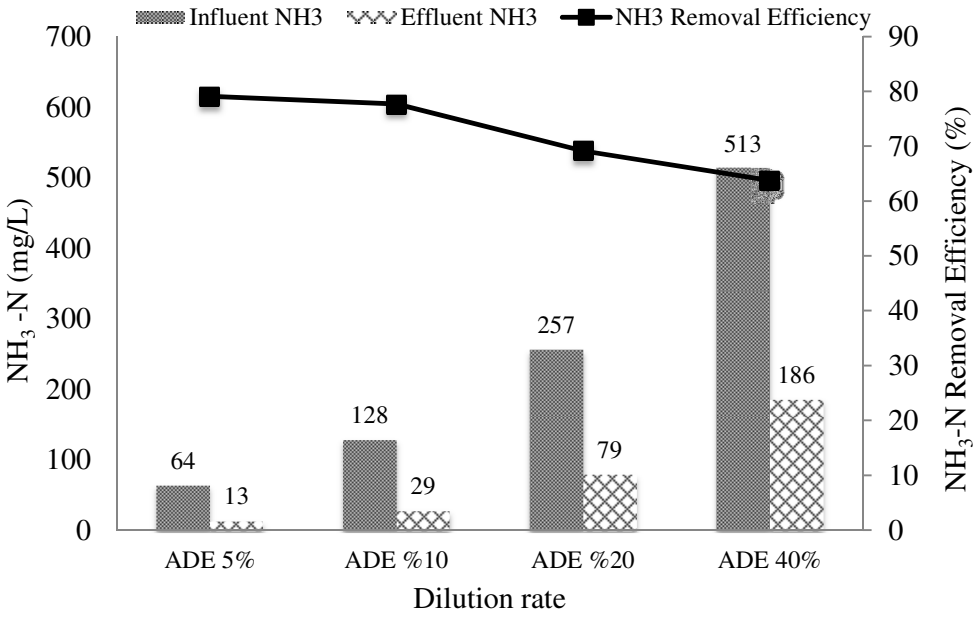

Figure 4.8 : Average ammonia change during cultivation of *Synechocystis* sp. PCC6803.

Average ortophosphate change during cultivation of *Synechocystis* sp. PCC6803 was presented in Figure 4.9.

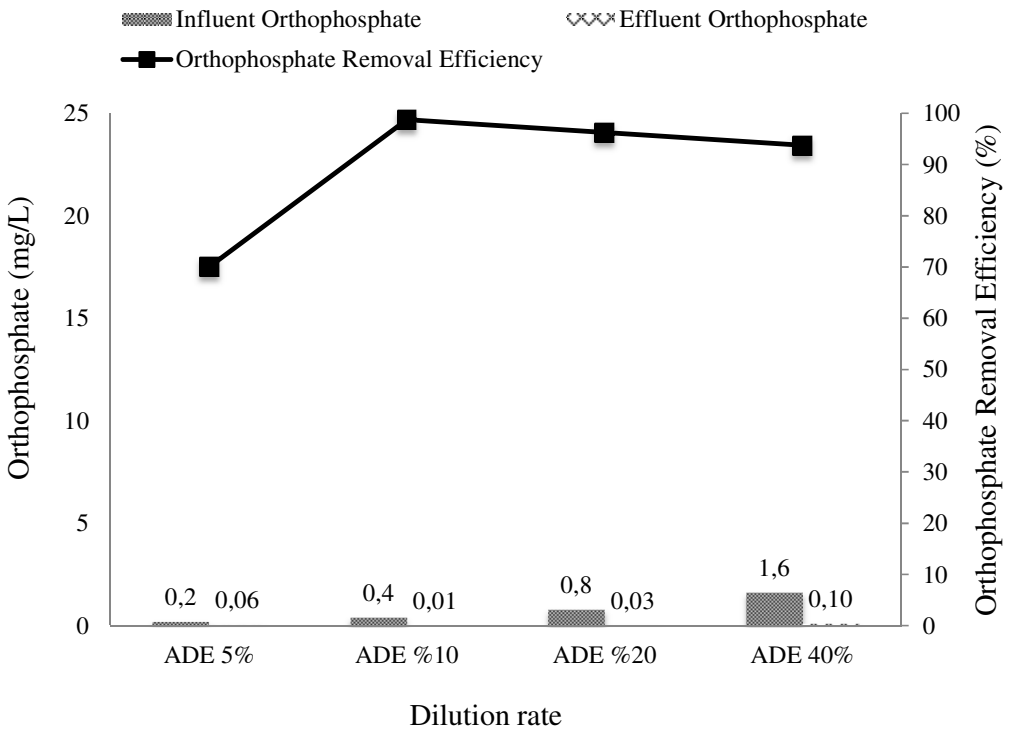

Figure 4.9 : Average ortophosphate change during cultivation of *Synechocystis* sp. PCC6803.

For 5% dilution rates, lag phases were relatively short (0-2 days) comparing to other dilutions, due to brighter color of medium and related photosynthetic efficiency. After 2 days, cells grew rapidly until 5th day. In the end of day 5, culture reached 310 mg/L concentration as dry weight. In day 6 they entered stationary phase. At the end of day 9 dry weight of cells were 325 mg/L, which was only slightly higher than day 5, end of exponential phase. In 9 days, cells consumed 79% of NH_3 and 70% of orthophosphate.

Similar to 5%, 10% diluted samples also took 2 days lag phase and started to grow exponentially in 3rd day. Between days 3 to 10 cells grew exponentially, reaching 470 mg/L biomass concentration as dry weight at the end of 10th day. After 10 days, stationary phase began, dry weight concentration reaching only 0.5 g/L at the end of 14th day. In 14 days cells consumed nearly all of orthophosphate (99%) and 78% of NH_3 present in the culture.

In 20% dilutions lag phase lasted a bit longer, cells started to grow after 4 days. Exponential growth phase lasted for 8 days, at the end of day 12, culture reached 590 mg/L dry weight concentration. After 12th day, stationary phase began, biomass

concentration reached 625 mg/L as dry weight at the end of 17th day. In 17 days, cells consumed 69% of NH_3 and 96% of orthophosphate.

Lag phase of 40% dilutions were higher than any other sample, lasting for 7 days. This long durations is mainly attributed to darker color which causes limited light penetration into the reactor. Exponential growth began at 8th day lasting until 13th day. After 13 days, biomass concentration reached 429 mg/L as dry weight. Stationary phase began at day 14, at the end of 19 days dry weight of cells was 475 mg/L. In 19 days cells consumed 64% of NH3 and 94% of orthophosphate present.

Biomass concentrations at the end of experiments were lower than cultures grown in synthetic medium. This is mainly attributed to several factors. Experiments were not conducted in sterile condition, impurities in the anaerobic digestion effluent affected the growth of algae. Inoculum used in this study was isolated from freshwater in 1968 and has grown in synthetic medium since then. Acclimation of cells to wastewater is thought to affect the growth. Higher turbidities of wastewater caused stress conditions, limiting light penetration and thus directly affecting to photosynthetic efficiency.

Table 4.1 : pH and Alkalinity at different dilutions of ADE.

Dilution rate	pH	Alkalinity (mg/L $CaCO_3$)
%5	6.85	75.6
%10	6.77	70.2
%20	6.61	63.3
%40	6.53	73.7

4.1.1 Macromolecule structure of *Synechocystis* sp. PCC6803

Macromolecule structure regarding carbohydrate, protein and lipid was given in Table 4.2.

Table 4.2 : Composition of *Synechocystis* sp. PCC6803.

Dilution ratio	Carbohydrate (%)	Protein (%)	Lipid (%)
5% ADE	12,94±2,17	31,20±8,3	29,4±1,2
10% ADE	5,42±1,29	24,14±1,56	32,7±0,02
20% ADE	3,00±0,31	37,63±0,92	37,63±0,23
40% ADE	5,13±0,71	27,11±5,02	29,6±0,58

4.2 External Phosphorus Supply

Average optical density change during cultivation of *Synechocystis* sp. PCC6803 with the addition of phosphorous was demostrated in Figure 4.10.

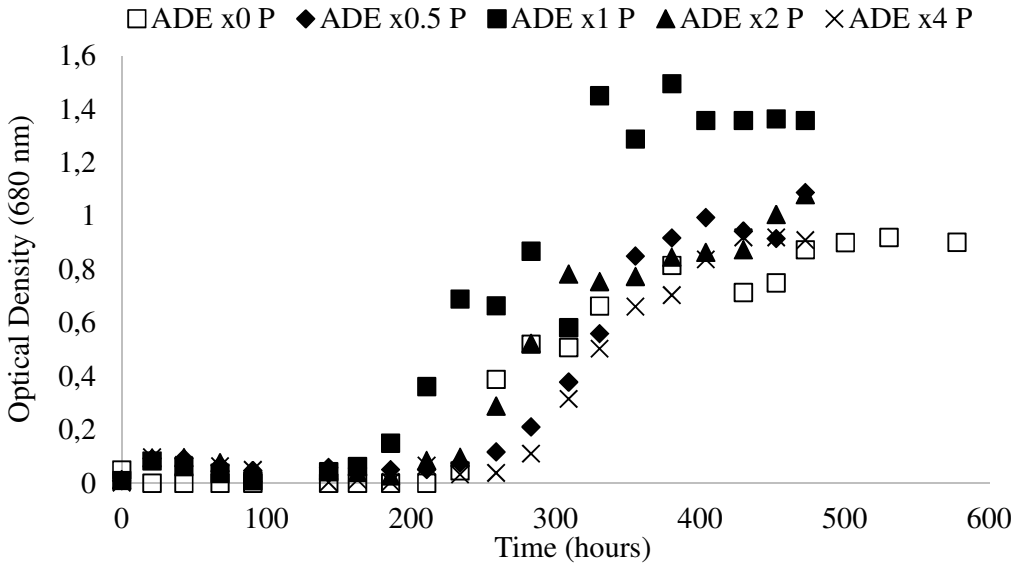

Figure 4.10 : Average optical density change during cultivation of *Synechocystis* sp. PCC6803 with the addition of phosphorous.

Average dry weight change during cultivation of *Synechocystis s*p. PCC6803 with the addition of phosphorous was presented in Figure 4.11.

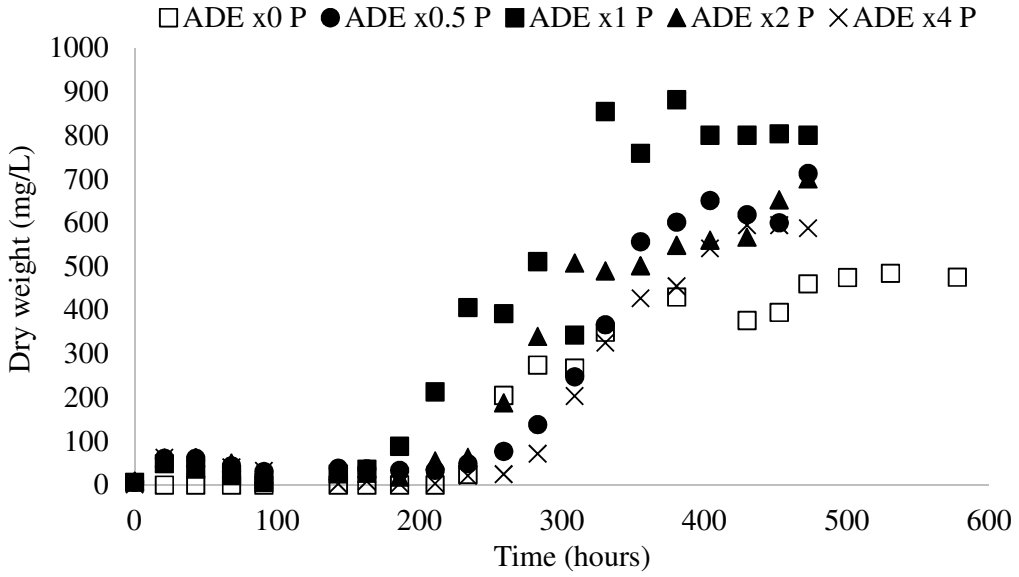

Figure 4.11 : Average dry weight change during cultivation of Synechocystis sp. PCC6803 with the addition of phosphorous.

Average TKN and ammonia change during cultivation of Synechocystis sp. PCC6803 with the addition of phosphorous was showed in Figure 4.12 and 4.13, respectively.

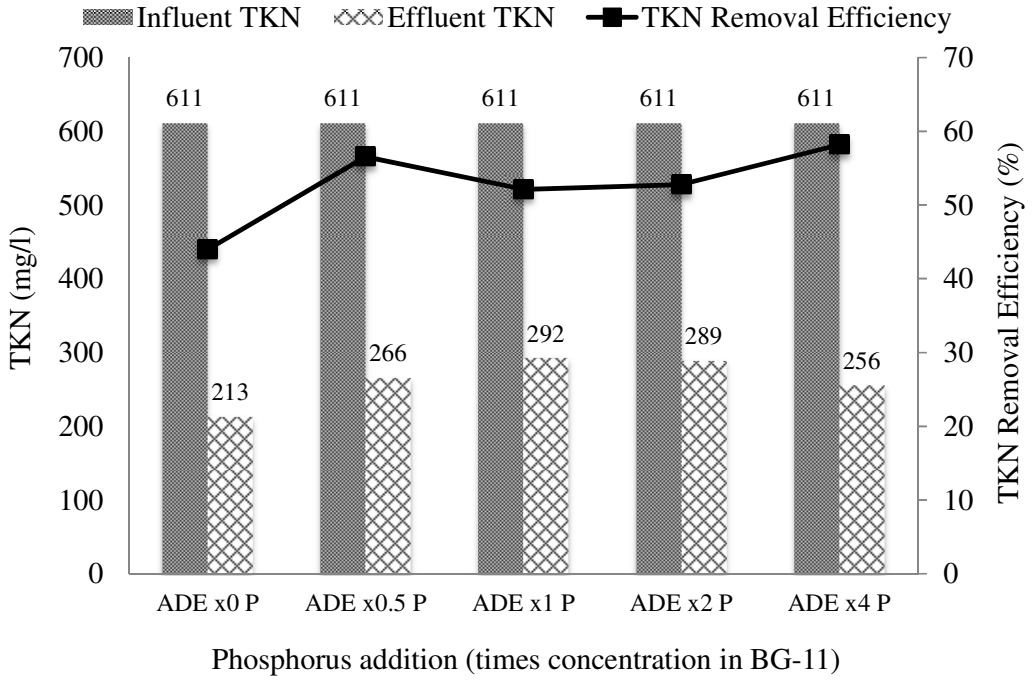

Figure 4.12 : Average TKN change during cultivation of Synechocystis sp. PCC6803 with the addition of phosphorous.

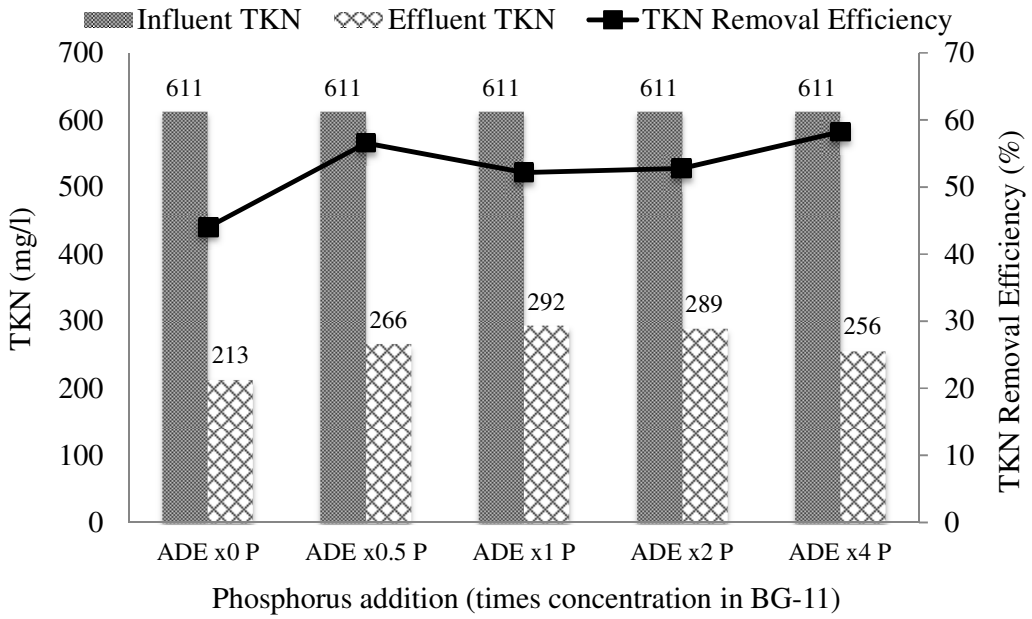

Figure 4.13 : Average ammonia change during cultivation of Synechocystis sp. PCC6803 with the addition of phosphorous.

Average ortophosphate change during cultivation of Synechocystis sp. PCC6803 with the addition of phosphorous was given in Figure 4.14.

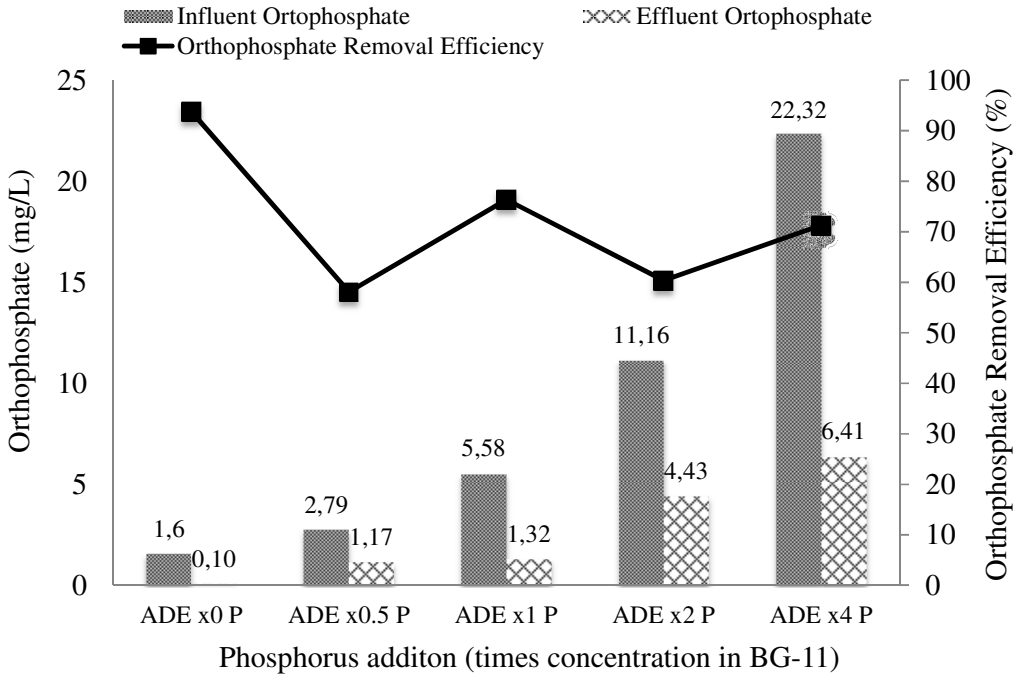

Figure 4.14 : Average ortophosphate change during cultivation of Synechocystis sp. PCC6803 with the addition of phosphorous.

Other factor contributing to limited growth is thought as phosphorus limitation. BG-11 has 5,51 mg/L of phosphorus and our undiluted anaerobic digestion effluent contained 5.58 mg/L total phosphorus which makes initial phosphorus concentrations of cultures considerably lower than ideal conditions. For that reason, second part of experiments was run with phosphorus addition. KH_2PO_4, which is the phosphorus source of BG-11 was added to %40 diluted cultures. Phosphorus concentrations were adjusted to 0.5, 1, 2 and 4 times of BG-11 in order to observe the effect of phosphorus to growth. In the exponential phase, cells turned out to yellow color which is a sign of nutrient limitation.

Phosphate added cultures growth better than the first series. In 0.5 times phosphorus added reactor, cells took 9 days for lag phase. After 9 days, exponential phase began and lasted until the end of day 16. At the end of exponential growth cell density reached 650.9 mg/L as dry weight and entered stationary period. After 21 days dry weight reached 712.5 mg/L. Cells consumed 53% of NH_3 and 58% of orthophosphate.

For cultures that have same phosphorus concentrations as BG-11, lag phase of cells lasted for 5 days. After 6 days cells grew exponentially for 12 days reaching biomass concentration of 881 mg/L as dry weight, higher than any other sample, and entered stationary phase. At the end of 21 days biomass concentration was 800 mg/L as dry weight. Cells consumed 46% of NH_3 and 76% of orthophosphate.

In the reactors containing phosphorus 2 times of BG-11 lag phase lasted for 8 days and until the end of experiment at 21st day. Cell density reached concentration of 700 mg/L as dry weight and cells consumed 51% of NH_3 and 60% of orthophosphate.

Cultures with 4 times phosphorus concentration of BG-11 had nearly 10 days of lag phase which is higher than any other experiment in both series. After 10 days, cells grew exponentially until the end of 19th day, reaching a biomass concentration of 594 mg/L as dry weight. Biomass concentration after 21 days was 587.5 mg/L. Cells consumed 55% of NH_3 and 71% of orthophosphate.

Table 4.3 : pH effect of phosphorous addition.

Dilution rate	Phosphorus concentration	pH
%40	½ x BG-11	4.54
%40	1 x BG-11	4.40
%40	2 x BG-11	4.31
%40	4 x BG-11	4.16

Buffer solution was not added to reactors at the beginning of the experiments and especially for second series pH of the reactors was very low. Though cells grow better in second series (with phosphorus addition) there was still a limited growth. Significant decrease in the pH is the main factor of limited growth with acclimation problem of cells to wastewater and darker color in the 40% diluted reactors. There was no nutrient limitation for the second series.

5. CONCLUSIONS AND RECOMMENDATIONS

The study was divided into two parts. In the first part of the study, cultivations of *Synechocystis* sp. PCC6803 was carried out with different liquid anaerobic digestate ratios by diluting with tap water. The aim was to determine the maximum ratio of liquid anaerobic digestate where the growth ratio of *Synechocystis* sp. PCC6803 was observed. In the second part of the study, the cultivations were carried out with different phosphorous concentrations. Since, phosphorous concentration in liquid anaerobic digestate was low and nutrient starvation was observed in first part, phosphorous was added externally to provide the need in comparison with the BG11 medium.

At the first part of the study, lag phases of the cultivations increased up to 9 days concamitantly with the increasing ratio of liquid anaerobic digestate and considerable growth was observed in 40% dilutions while cells did not survive in 60% dilution.

In general, biomass concentrations at the end of experiments were lower than cultures grown in synthetic medium. This is mainly attributed to several factors. Experiments were not conducted in sterile condition, impurities in the anaerobic digestion effluent affected the growth of algae. Inoculum used in this study was isolated from freshwater in 1968 and has grown in synthetic medium since then. Acclimation of cells to wastewater is thought to affect the growth. Higher turbidities of wastewater caused stress conditions, limiting light penetration and thus directly affecting to photosynthetic efficiency.

The phosphate amendment triggered somehow the growth of *Synechocystis* sp. PCC6803, although it was not alike the growth in BG11. The highest productivity, 881 mg/L obtained by providing the same amount of phosphorous present in BG11.

Buffer solution was not added to reactors at the beginning of the experiments and especially for second series pH of the reactors was very low. Though cells grow better in second series (with phosphorus addition) there was still a limited growth. Significant decrease in the pH is the main factor of limited growth with acclimation

problem of cells to wastewater and darker color in the 40% diluted reactors. There was no nutrient limitation for the second series.

One of the significant finding of this research was to detect the amonia concentration as high as 513 mg N/L did not have a toxic effect on *Synechocystis* sp. PCC6803. This will be promising to grow the microalgal species in the wastewater having high ammonia concentrations.

For sustainable and commercial microalgal growth, modelling of photosynthetic efficiency is one of the main concerns for algal growth in full scale bioprocessing plants. Providing contionous light is not economically feasible and using solar energy is essential for photosynthesis. Turkiye receives sufficient amount of sunlight throughout the year essential for microalgal growth. Modelling and designing the most effective photobioreactors is one of the most significant aspects in sustainable biomass production. Today in 2015, in full scale plants, raceway ponds, tubular photobioreactors and flat panel reactors are widely used. For dilutions of wastewater using sea water comes up as a somewhat viable option since many of algae species can be tolerant to saline conditions. With the production of high biomass amounts, valuable biomass production is also an important concern. Lipids and proteins in structure of microalgae may not be in sufficient amount for economical feasibility of production and therefore increasing the proportion of lipids, proteins or other macromolecules may be essential regarding to purpose of production.

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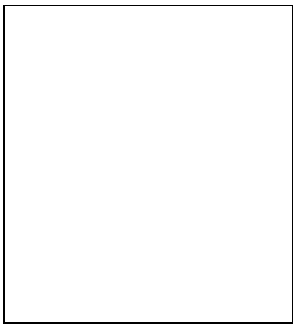

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