ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

GROWTH OF MICROALGAE IN PRE-TREATED LANDFILL LEACHATE

M.Sc. THESIS

Umut METİN

Department of Enviromental Engineering

Enviromental Biotechnology Programme

AUGUST 2015

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

ÖN ARITILMIŞ DEPONİ SIZINTI SUYUNDA MİKROALG BÜYÜTÜLMESİ

YÜKSEK LİSANS TEZİ

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Çevre Mühendisliği Anabilim Dalı

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AĞUSTOS 2015

Umut Metin, a **M.Sc.** student of ITU **Institute of Science and Technology** student ID 501121810, successfully defended the **thesis** entitled **GROWTH OF MICROALGAE IN PRE-TREATED LANDFİLL LEACHATE**", which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Date of Submission : 04 May 2015
Date of Defense : 17 August 2015

To my family and friends,

FOREWORD

As a thesis student, I am grateful to my thesis advisor Assistant Professor Dr. Mahmut Altınbaş for the joy root gentle personalities and their valuable support during my graduation project.
In addition, thank you to the academic staff of the ITU Environmental Engineering Department for allow me to all kinds of possibilities during past 3-year education in my life.
I hope that the experience I have gained during the thesis, develop my approach to experimental design.

August 2015

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(Molecular Biologist and Geneticist)

TABLE OF CONTENTS

Page

ABBREVIATIONS

BOD	**:** Biological Oxygen Demand
COD	**:** Chemical Oxygen Demand
FAME	**:** Fatty-acid methyl ester
FFA	**:** Free Fatty Acid
GHG	**:** Greenhouse Gas
OD	**:** Optical Density
PLL	**:** Pre-treated landfill leachate
SS	**:** Suspended Solid
TKN	**:** Total Kjeldahl Nitrogen
VSS	**:** Volatile Suspenden Solids

LIST OF TABLES

Page

LIST OF FIGURES

Page

GROWTH OF MICROALGAE IN PRE-TREATED LANDFILL LEACHATE

SUMMARY

79.7% of world's energy needs is supplied by fossil fuels. Estimates indicate that oil will run out in the next 35 years, natural gas will run out in the next 37 years, and coal will run out in the next 107 years. Renewable and environmentally friendly energy resources should developed to address the world's energy deficit. Biodiesel is a promising energy source that provides this necessary.

Although Biodiesel is a renewable and environmentally friendly energy source, because of the production of biodiesel, which is alternative the petro-diesel usage, is more expensive than production petro-diesel, it does not have widespread use.

Many different methods have been proposed in order to reduce the cost of biodiesel. One of these systems is biorafinery system. When carrying out the production of biodiesel by using biorafinery system, while also valuable products/output work is performed.

On the other hand, to sustain ecological balance to protect our lifestyle and future of our offspings waste reduction and recycling must carried out even more efficiently than before. Lately, In line with this vision, nitrogen and phosphorus, which before given the receiving environment with the effluent water, intended to benefit.

In this study, algal strain *Synechocystis sp. PCC 6803* was growth on pre-treated landfill leachate (PLL) with different dilution rate and determining the biodiesel production potential and wastewater cleaning efficiency of nitrogen and phosphorus were measured. Our goal is to determine that using leachate as medium cost-effective solution for algal biodiesel production and *Synechocystis sp. PCC 6803* is usable for the nitrogen and phosphorus removal from pre-treated landfill leachate.

Synechocystis sp. PCC 6803 strain grown on mediums, of which dilution rate differ from each other, for 500 hours. These dilutions are 20%, 40%, 60%, 80% and 100%. However, *Synechocystis sp. PCC 6803* not grown on medium contains 100% of pre-treated landfill leachate. Thus, data of these mediums were not been taken into account.

Our strain were cultured at 32±2°C with a continuous white led illumination with intensity of 100-300 $\mu E/m^{-2}*s^{-1}$. Our cultures were aerated with air that aeration speed is 1 L/min.

Growths of the culture was determined via optical density measurement. For each different medium, which has different dilution rate, optical density time graphs was prepared.

According to our data, with increased concentration of pre-treated landfill leachate organic N removal efficeny dropped. Especially, medium %80 PLL, removal efficiency dropped significantly. Removal efficiency of %20 PLL, %40 PLL, %60 PLL and %80 PLL are about %90, %86, %83 and %41, respectively.

According to our data, with increased concentration of pre-treated landfill leachate TKN removal efficeny dropped. Especially, medium %80 PLL, removal efficiency dropped significantly. Removal efficeny of %20 PLL, %40 PLL, %60 PLL and %80 PLL are about %51, %44, %40 and %20, respectively.

According to our data, with increased concentration of pre-treated landfill leachate ammonia-N removal efficeny was drop. However, ther is no quick drop observed from grap. Removal efficeny of %20 PLL, %40 PLL, %60 PLL and %80 PLL are about %28, %19, %14 and %7, respectively.

According to our data, with increased concentration of pre-treated landfill leachate organic N removal efficeny was drop. Especially, medium %80 PLL, removal efficeny drop significanly. Removal efficeny of %20 PLL, %40 PLL, %60 PLL and %80 PLL are about %90, %86, %83 and %41, respectively.

Removal efficeny of orthophosphate of medium %20 PLL, %40 PLL, %60 PLL and %80 PLL are about %94, %96, %89 and %88, respectively according to our work.

Maximum OD_{680} value which was seen is 1,816 for medium with 20% dilution rate. Spesific growth rate of medium with 20% dilution was found 0,00753 h^{-1} and doubling time of medium with 20% dilution was determined 91,96 hours. Maximum OD_{680} value which was seen is 1,089 for medium with 40% dilution rate. Spesific growth rate of medium with 40% dilution was found 0,00887 h^{-1} and doubling time of medium with 40% dilution was determined 78,35 hours. Maximum OD_{680} value which was seen is 0,815 for medium with 60% dilution rate. Spesific growth rate of medium with 60% dilution was found 0,00937 h^{-1} and doubling time of medium with 60% dilution was determined 73,95 hours. Maximum OD_{680} value which was seen is 0,578 for medium with 80% dilution rate. Spesific growth rate of medium with 80% dilution was found 0,01409 h^{-1} and doubling time of medium with 80% dilution was determined 49,20 hours.

According to our results, high orthophosphorus removal rate was observed. This is expected results because, our medium contain very low orthophosphorus concentration. Because of the low growth amount im medium, total kjeldahl nitrogen removal efficieny is not high.It thought that high organic N removal amount caused by contamination of heterotrophic organisms.

ÖN ARITILMIŞ DEPONİ SIZINTI SUYUNDA MİKROALG BÜYÜTÜLMESİ

ÖZET

Günümüzde, modern Dünya'nın yüzleşmek zorunda olduğu en büyük sorunlar atıkların bertaraf edilmesi süreçlerinin sürdürülebilir kalkınma hedefini sağlanaması için gittikçe yetersiz hale gelinmesi vedurmadan artmaya devam eden enerji ihtiyacını karşılayacak uygun bir kaynağa sahip olunamamasıdır.

Günümüzde içinde yaşadığımız çevrenin dengesinin korunarak sürdürülebilirliğin sağlaması, alıştığımız hayat tarzının devam ettirilebilmek için önemlidir. Bu sebeple doğal kaynakların korunması büyük önem teşkil etmektedir. Özellikle sınırlı su kaynaklarının korunması dikkat edilmesi gereken bir konudur. Bu sebeple hali hazırda kirlenmiş suların temiz su kaynaklarına doğrudan verilmemektedir. Kirlenmiş sular, başta içersindeki organik karbondan, bulanıktan, askıdaki katı maddedelerden, renkten, kokudan, azot, fosfordan ve ağır metalden arındırılmaktadır. Fakat her ne kadar organik karbon giderimi belli standartlara oturtulmuş olup neredeyse tamamen giderimi gerçekleştirilmesine rağmen, azot ve fosfor için böyle bir standarttan bahsetmek mümkün değildir. Azot ve fosfor giderimi 3. aşama arıtım olarak adlandırılmakta olup birçok artım tesisi bu gelişmişlikte olmadığı gibi olan tesislerde ise bu arıtım süreçleri karmaşık olup arıtım maliyetlerini yükseltmektedir. Biyoljik azot ve fosfor giderim sistemleri karmaşık olup, kimyasal giderim süreçleri ise daha farklı kirlenmelere sebep olabilmektedir. Bu maddelerin temizlenmeden alıcı ortamlara alınması ise alıcı ortamlarda ötrofikasyona sebep olmaktadır. Bunun sonucunda sınırlı su kaynakları kirlenerek hem içme ya da diğer amaçlar sebebiyle kullanılmasını engellemekte, hem de alıcı ortaın ekosistemini yok ederek bizi doğrudan ya da dolaylı olarak ekonomik kayba sebepolmaktadır. Dolayısyla bu maddelerin kirlenmiş sulardan daha etkili ve eknomik bir yöntem ile gideriminin yönteminin bulunması gerekmektedir.

2010 yılı verilerine göre Dünya enerji ihtiyacının %79,7'si fosil yakıtlardan sağlanmaktadır. 2011 yılı verilerine göre, tahminler eğer yeni bir kaynak bulunmadığı takdirde petrolün 35 yıl, doğalgazın 37 yıl, kömürün ise önümüzdeki 107 yıl içinde tükeneceğini göstermektedir. Tükenmeye yüz tutmuş bu kaynakların yerine yenisi konunması şarttır. Bu enerji açığının giderilebilmesi için yenilenebilir ve çevre dostu enerji kaynakları geliştirilmesi gerekmektedir. Ayrıca, fosil yakıt kullanımı atmosferde CO2 birikmesine, ve dolayısyla sera etkisine sebep olması ve bu etkinin iklim değişikline yol açması büyük bir sorun teşkil etmektedir. Yenilenebilir birçok farklı enerji kaynağı olmasına rağmen biyoyakıtlar üretilmesi için yüksek teknoloji gerekmemesi ve hali hazırda kullanımda olan sistemler değişikliğe gidilmeden ya da çok az değişiklik gerekterimesi sebebiyle diğer kaynaklardan 1 adım öne çıkmaktadır.

Birçok farklı biyoyakıt tipi vardır. Bunlara örnek olarak biyohidrojen, bioyogaz, singaz, biyoethanol ya da diğer biyoalkoller, biyodizel veya yeşil dizel örnek verilebilir. Bu farklı kaynaklar 3 farklı grupta yoğunluklarına göre gaz biyoyakıtlar, düşük yoğunluklu (ve düşük moleküler ağırlıklı) sıvı biyoyakıtlar ve yüksek yoğunluklu (ve yüksek moleküler ağırlıklı) sıvı biyoyakıtlar olarak sınıflandırılabilir. Gaz biyoyakıtlar birim kütle başına en fazla enerji içeriğini sahip olmasına rağmen, depolamanın ve taşımanın sorunlu olması bunları biyoyakıtlar arasında ilk tercih olmasına engel olur. Düşük yoğunluklu sıvı biyoyakıtlar birim kütle başına üç tip biyoyakıt arasından en az enerji içeriğine sahip olması sebebiyle tercih edilebilirliğini

azaltır. Yüksek yoğunluklu biyoyakıtlar hem birim kütle başına düşük yoğunluklu sıvı biyoyakıtlardan daha yüksek enerji sahip olması ve gaz biyoyakıtların doğasında olan sorunlara sahip olmaması sebebiyle, biyoyakıt çeşitleri arasından en tercih edilebilcek olandır. Yüksek yoğunluklu sıvı biyoyakıtlar arasından biyodizel en geniş kullanım alanı olan ve gelecek vaad eden bir yakıt türüdür.

Biyodizel, petrol kaynaklı dizele eşdeğer olarak kullanılabilir bir yakıttır. Günümüzde, biyodizel günümüzde büyük çoğunlukla yağ içeriği yüksek kültür bitkilerinden elde edilir. Ayrıca mikroalgal ve selülozik bir biyokütleden de biyodizel üretimi vardır. Fakat bunlardan yapılan üretim daha çok pilot ölçekli olup, toplam biyodizel üretimi içindeki hacmi %1'den azdır. Yağ içeriği yüksek kültür bitkilerinin biyodizel üretiminde kullanılması yukarıda bahsedilen sorunlara çözüm olmasına rağmen yeni bir sorun ortaya çıkarmaktadır. Bu sorun, gıda kaynağı olabilecek olan kaynaklar yakıt olarak kulanılması sorunudur. Dahası Dünya genelinde sadece taşımacıklıkta kullanılan yakıta eşdeğer biyodüzel üretmek için Dünya'daki bütün tarım alanları bile yetersiz gelmektedir. Dolayısıyla yağ içeriği yüksek kültür bitkilerinin biyodizel üretiminde kullanılması karşılaştığımız sorunu çözmekte yetersizdir. Fakat, eğer biyodizel üretiminde mikroalgal biyokütlenin kullanılması durumunda bu sorun ortadan kaldırabilmekte, ayrıca mikroalglerin kültür bitkilerinden çok daha hızlı büyüme yeterliliğine sahip olması, yetişitirilmesinde verimli tarım alanlarına ihtiyaç duyulmaması, atık ve kirlenmiş sularda büyüyebilme yetenekleri gibi karakteristik sahip olduğu birçok faklı özellikten dolayı mikroalgal biyokütledenbiyodizel üretimini çok daha verimli hale getirmektedir.

Bütün bu sahip olduğu avantajlara ve 1960'lardan beri süre gelen iyileştirme çalışmalarına rağmen, mikroalgal kütleden ekonomik olarak petrol kaynaklı dizelden ile rekabet edebilir biyodizel üretimi gerçekleştirilememiştir. Bunun başarılamasının en asıl sebebi ise teknik olmaktan ziyade mikroalgal biyokütleden biyodizel üretim maliyetlerinin yüksek olmasıdır.

1960 yılından beri yapılan çalışmalar göstermiştir ki hali hazırda birçok farklı etmenden kaynaklanan yüksek maliyet sorunu, sorunların sadece biri ya da bir kısmının çözülerek aşılamıyacağını göstermiştir. Bu maliyet sorununun çözülebilmesi çözülmesi gereken sorunlar şöyle sıralanabilir. İlk olarak mikroalg üretim sürecinde besi yerinde kullanılcak besin maddelerinin daha ucuza temini ve yine mikroalg üretimin içinde gerçekleştiği fotobiyoreaktörin yüksek büyüme verimini yüksek olmayan maliyetler gerçekleştirilebilcek şekilde tasarımlanması sağlanmalıdır. İkinci olarak biyodizel üretiminde kullanılacak mikroalgal biyokütlenin hasat ve yağ çıkarılması işlemlerinin, dahası bu yağın biyodizele dönüşümünü sağlayan transesterifikasyon maliyetlerinin düşürülmesi sağlanmalıdır. Üçüncü olarak, biyorafineri üretim modeli uygunlmalı yani düşük değerlikli bir ürün olan biyodizel üretimi yapılırken, mikroalglerin sentezlediği bilindiği birçok farklı yüksek değerlikli madde yağ hasadı yapıldıktan sonra kalan biyokütleden katma değer üretme amacıyla bu yüksek değerlikli maddelerin saflaştırması gerçekleştirilmedilir. Dördüncü olarak ise tümleşik tesis yaklaşımın gerçekleştirimesi gerekmektedir. Yani, örneğin, mikroalg üretimi yapılırken, mikroalglerin büyümesi olan CO2 kaynağı tümleşik tesiste bulunan termik santralin baca çıkış gazından karşılanmalı, böylelikle besi yerinde kullanılcak besin maddedis olan CO2 elde edilmiş olacak iken, aynı zamanda tesis baca filtreleme masrafların kurtulacak ve dolayı bu duruda biyodizel maliyetlerinin düşmesine katkıda bulunacaktır. Son olarak ise, mikroalglerin kendi doğalarından kaynaklanan sınırlandırmalar genetik mühendisliği yöntemleri aşılarak çok daha verimli suş elde edilmesi gerekmektedir.

Yukarıda değilen sorunla çözüm üretebilme amacıyla geniş kapsamlı bir çalışmanın ilk aşaması olarak *Synechocystis sp. PCC 6803* alg türü değişik oranda seyreltilmiş ön arıtımı gerçekleştirilmiş sızıntı suyunda büyümesi gözlemlenmiş, kültürleme işleminin bitiminden sonra azot ve fosfor giderim oranları tespit edilmiştir. Burda besi ortamı olarak ön arıtılmış sızıntı suyu seçimiyle üretim sürecinde besi yerinde kullanılcak besin maddelerinin masrafsız temini sağlanmış, ayrıca bu kirlenmiş suda bulunan azot ve fosforun giderilmesi için yeni bir yöntem önerilmiş olmaktadır. Ayrıca, tümleşik tesis yaklaşımı çerçevesine uygun olarak hem biyodizel üretimi hem de atık giderimin gerçekleştirilip gerçekleştirilemiyeceği gözlenmesi amaçlanmıştır. Yüksek büyüme yeteneğine sahip, genetik haritası hali hazırda çıkartılmış, uyum sağlama yeteneği yüksek olan ve genetik müdahele için kullanılması gereken araçları iyi tanımlanmış *Synechocystis sp. PCC 6803* türü suş iyileştirilmesi amacının daha kolay gerçektirilebilesine olanak sağladığı için seçilmiştir.

Synechocystis sp. PCC 6803 türü %20, %40, %60, %80 ve %100 oranın derişimlerde ön arıtılmış sızıntı suyu içeren besi ortamlarında büyütülmüştür. Kültürlerimiz 32±2°C sıcklığında, içerisine 1 L/dk miktarında hava verilerek, 100-300 değerleri arasında değişen soğuk beyaz led ışık altında 24 saat kesintisiz aydınlatma altında 500 saat süreyle büyütülmüştür. Karıştırma, kültür içersine verilen havalandırmanın şiddetiyle sağlanılmıştır. Kültürler 1'lik erlenler içersinde büyütülmüş olup başlangıç kültür hacmi ise 500 ml'dir. Her farklı derişim 4'lü tekrar yapılarak çalışılmıştır.

Büyüme 680 nm'de optik yoğunluk ölçülerek yapılmıştır. Her farklı derişim için optik yoğunluk-zaman grafiği çıkartılmıştır. Ayrıca, her farklı derişim için özgül büyüme hızı ve ikiye katlanma süresi hesaplanmıştır. Ayrıca "optik yoğunluk-toplam askıda katı madde (TAKM)" grafiğinden elde edilen değerler ile her bir besi yerinde üretien teorik TAKM değeri hesaplanmıştır.

Ön arıtımı yapılmış sızıntı suyunun ve besi yerlerinin büyütmenin başlangıcında ve bitişinde olmak üzere karakterizasyonu yapılmıştır. Total kjeldahl azotu, amonyok azotu, ortofosfat, toplam askıda katı madde, uçucu askıda katı madde, pH ve alkanite değerleri hesaplanmıştır. Total kjeldahl azotu, amonyok azotu ve ortofosfat tayini standart yöntemler kullanılarak gerçekletirildi.

Yapılan çalışmaların sonuçlarına göre artan sızıntı suyu derişimi ile birlikte kültürlerde gözlmelenen büyümenin azaldığı gözlemlenmiştir. Seyreltilmemiş sızıntı suyunda (%100 derişim) ise büyümenin nerdeyse tamamen baskılandığı gözlemlenmiştir. Dolayısıyla bu derişimdeki ölçümler sonuçlara katılmamıştır. %20 derişimde en yüksek gözlemlenen OD_{680} değeri 1,816, %40 derşimde en yüksek gözlemlenen OD_{680} değeri 1,0893, %60 derşimde en yüksek gözlemlenen OD_{680} değeri 0,8146 ve %80 derşimde en yüksek gözlemlenen OD_{680} değeri 0,578'dir. %20 derişimde bulunan özgül büyüme hızı 0,00753 h^{-1} ve ikiye katlanma süresi ise 91,95 saat, %40 derişimde bulunan özgül büyüme hızı 0,008868 h^{-1} ve ikiye katlanma süresi ise 78,35 saat, %60 derişimde bulunan özgül büyüme hızı 0,00937 ve ikiye katlanma süresi ise 73,95 saat, ve %80 derişimde bulunan özgül büyüme hızı 0,014088 ve ikiye katlanma süresi ise 49,19 saattir.

%20 derişimde gözlemlenen TKN giderim oranı %51,819, amonyak ozanı giderim oranı %28,855 ve organik azot giderim oranı ise 90,33'dir. %40 derişimde gözlemlenen TKN giderim oranı %44,57, amonyak ozanı giderim oranı %19,36 ve organik azot giderim oranı ise 86,85'dir. %60 derişimde gözlemlenen TKN giderim oranı %40,603, amonyak ozanı giderim oranı %14,88 ve organik azot giderim oranı

ise 83,72'dir. %80 derişimde gözlemlenen TKN giderim oranı %20,196, amonyak ozanı giderim oranı %7,473 ve organik azot giderim oranı ise 41,53'dir.

%20 derişimde gözlemlenen ortofosfat giderim oranı %94,88, %40 derişimde gözlemlenen ortofosfat giderim oranı %96,69, %60 derişimde gözlemlenen ortofosfat giderim oranı %89,22, ve %80 derişimde gözlemlenen ortofosfat giderim oranı %88,08'dir.

Yapılan çalışmalrın sonuçlarına göre sonuçlara göre ön arıtıma tabi tutulmuş sızıntı suyunda gözlemlenen büyüme oranlı istenilen seviyede olmadığı gözlemlenmiştir. Yüksek fosfat giderimi gözlemlenmiştir. Fakat başlangıç fosfor derişim düşük olduğun gözlemlenen değerlerin yüksek olması beklenen bir durumdur. Yeterince büyüme olmadığı için tam olarak azot giderimi gerçekleştirilememiştir. Organik azot giderimin yüksek olmasının sebebi olarak ise heterotrofik büyümenin varlığı olduğu düşünülmektedir.

1. INTRODUCTION

1.1 The need for sustainable liquid fuels

The use of fossil fuels is widely recognized as unsustainable due to the expected depletion of fossil fuel and the increasing emission of the greenhouse gas (GHG) CO_2. However, for their energy requirements, people worldwide and the global economy largely depend on the use of these fossil fuels. 79.7% of world's energy needs is supplied by fossil fuels (Persson, 2010). Therefore, today one of greatest challenges for our society is to develop renewable and GHG emissions free fuels (Stern, 2006). Although there are many options, which has recently been explored for sustainable energy production such as biomass, hydroelectric, photovoltaic, wind, tidal & geothermal, however, most of these technologies are aimed at electricity production and because of that, these technologies are not ideally suitable to replace oil consumption caused by sectors such as transportation and the petrochemical industry. According to data of year 2012, oil covers over 31,4% of the world's energy production (EIA, 2014). Roughly 63,7% of all oil supply is used as liquid fuel in the transportation sector (EIA, 2014). In 2012 this amounted to 20,0018% (20.882 TWh) of global energy consumption. Moreover, it is predicted that, this amount increase with 10 EJ each 5 years (EIA, 2008). This is a considerable amount and therefore sustainable alternatives for liquid fuels must be investigated.

It can be argued that developing technologies such as electric engines and hydrogen based engines could overcome this reliance on liquid fuels. Although it can be achivable from a technological point of view, there are some problems about these technologies that will make realization difficult. As fuel source, hydrogen has highest energy production capacity per weight. However, a hydrogen-economy requires not only large scale infrastructural changes for hydrogen storage and distribution, but most importantly it will require large scale methods for cost efficient renewable hydrogen production. This scenario seems unlikely because of the current prices of photovoltaic technology and limited availability of semiconductor materials, (Feltrin and

Freundlich 2008). Relying on electric engine technologies faces a similar problem with regards to large scale sustainable electricity production. Moreover, dependence on batteries may have some disadvantages such as a limited life span and storage capacity of the batteries. Moreover reducing overall energy efficiency will decrease when the additional energy conversion step is introduced. In addition to these, it can be argued that large scale replacement of combustion engines with engines which usa battery would deplete world lithium resources rapidly because of the production batteries require lithium (Taniguchi, 2008). Besides these problems, it can be expected that various sectors such as aviation, shipping and road freight, but also the petrochemical industry and remote energy consumption, are most likely to remain largely dependent on carbon based liquid fuels.

Because of these reason, the production of liquid biofuels, such as ethanol and biodiesel, which are mainly produced from agricultural crops, has strongly increased over the past few years.

1.2 Purpose of Thesis

Purpose is this thesis is to define capacity of pre-treated landfill leachate to sustain appropriate medium for *Synechocystis sp. PCC 6803* to produce economically biodiesel production. Moreover, while producing *Synechocystis sp. PCC 6803* for biodised production, we try to determine the capacity *Synechocystis sp. PCC 6803* to clean pre-treated landfill leachate from nitrogen and phosphorus. We try to define that the suitability of *Synechocystis sp. PCC 6803* for tertiary wastewater treatment. For this aim, *Synechocystis sp. PCC 6803* strain grown on mediums, of which dilution rate differ from each other, for 500 hours. These dilutions are 20%, 40%, 60%, 80% and 100%. After the finising the cultivation, total khejdahl nitrogen and orthophosphate removal efficeny was determined.

1.3 Scope of Thesis

In this thesis, chapter 2 is about literature review. In this chapter, first of all The definition of biofuels made and source of biofuels was defined. Then, brief information about microalgae was given and then was started to talk about biodiesel. After that, the advantages and disadvantages of producing biofuels from microalgae are

discussed. Following, comprehensive information on cynobacteria are given. Finally, properties of *Synechocystis sp. PCC 6803* was explained.

In chapter 3, chemcicals, laboratory equipments and methods, which used during our thesis, was described.

In chapter 4, results of our work was given.

Finally, in chapter 5, briefly what have to be done in future according to interpretation of our results, was explained.

2. LITERATURE REVIEW

2.1 Biofuel

Biofuels is a type of fuels, which are generated from biological material (Lee & Lavoie, 2013). Variety of biomasses from different sources, including forestry, agricultural, and aquatic sources have been used as the feedstock to produce biofuel so far (Li et al., 2008). There are many different type of biofuels exist such as bioethanol and other bioalcohols, biodiesel, green diesel, biogasoline, bioether, biogas and syngas. Each one has quite different production method and did not mentioned here. Aim of this work is biodiesel and it explain in detail.

Biofuels are classified into four classes, which are called as generations depending on source biomass, and these are first generation biofuels, second-generation biofuels, third-generation biofuels, and fourth-generation biofuels (Smith, 2010). Figure 1 represents generations of biofuels.

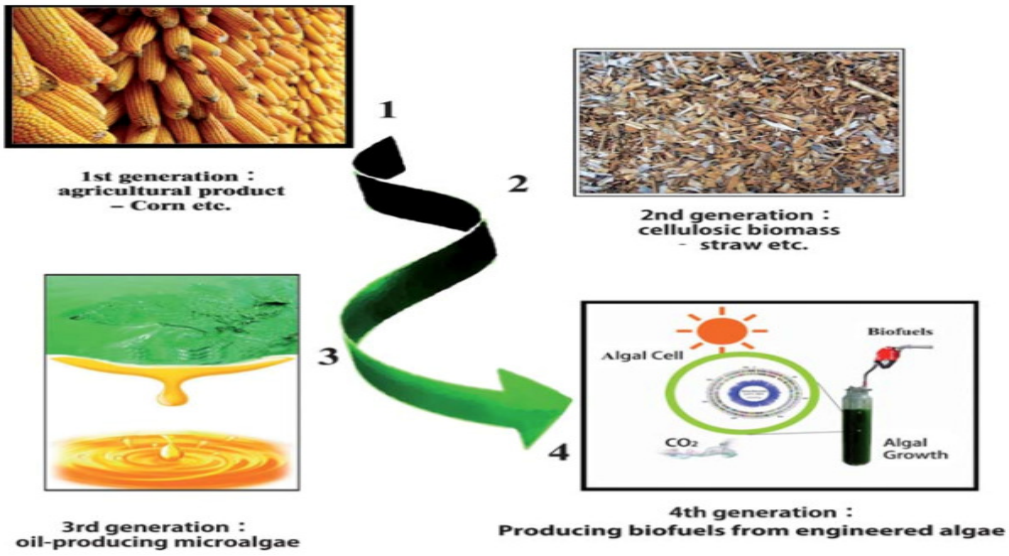


Figure 2.1: Four generations of biofuel production: from agricultural products to algae (Sims et. al., 2010).

First generation biofuels are based on fuel production from a mostly edible cultivated and harvested biomass such as rapeseed oil, sugarcane, sugar beet, and maize as well as vegetable oils and animal fats using conventional technology. (Brennan & Owende, 2010; Collet et al., 2013). Production of biofuel from first-generation biomass is rather simple process which it differentiate for ethonol and biodiesel. However, increases in the sugar price are a problem for the bioethanol business (Lee & Lavoie, 2013). In addition to that, competition with food production for arable land usage, lack of developed agricultural practices, requirement for high water and fertilizer make it impossible to meet overall energy demand (Brennan & Owende, 2010).

Second generation biofuels are fuels which are produced from a wide array of different feedstocks, ranging from non-edible lignocellulosic feedstocks to forest residues. The cost of second-generation biofuel feedstock is less than the cost of first-generation biofuels suc as vegetable oil, corn, and sugarcane (Lee & Lavoie, 2013). However, conversion of these biomasses to final product is more complex than using other feedstocks and mostly conversion of these biomasses requires sophisticated and expensive technologies for the pre-treatment with special enzymes, which make it less profitable for commercial production (Brennan & Owende, 2010; Alam et al., 2012).

Third generation biofuels are fuels which are produced from algal biomass Lam & Lee, 2012). However, other feedstock such as yeast and fungus can be used for production of third generation biofuels. (Nigam & Singh, 2011). A technically and economically viable biofuel resource should be able to compete with petroleum fuels in terms of price and usability in standart engines; should use small land; should be GHG emissions free (reduce CO_2 emmision); and should require less water usage. Microalgae meet most of these conditions and therefore make it suitable biomass for biofuel production (Khosla, 2008). However, in terms of price there is huge different betweem petrolum derived fuel and third generation biofuels (Chisti, 2007). For example, according to calculation made by Chisti (2007), in USA, while tax free on-highway petro-diesel price is $0.49/L, tax free algal biodiesel price is $2.80/L.

Fourth generation biofuels type of fuels which is produced using by genetically modifed photosynthetic microorganisms. To create or increase biofuel production, bioengineering techniques is used to change cellular metabolism algae via introduction of new genetic metarial, deletion, and/or modification of algal genetic system. (Sims et al., 2010). In fourth-generation biofuels organisms engineered in a way that they

become efficient to procude biofuels or biofuel precursor. To aim of develop genetically modifed photosynthetic microorganisms is to overcome thr problems of which facing in third generation biofuels.

2.2 Microalgae

Microalgae, which also called as Microphytes, are a diverse group of prokaryotic and eukaryotic photosynthetic microorganisms that they able to convert carbon dioxide to potential biofuels, foods, feeds and high-value bioactives (Chisti, 2007, Li et al, 2008, Thurman, 1997). Because of their simple structure, microalgae can adapt to changed environmental conditions quickly (Brennan & Owende, 2010). The term “algae” is covers a large group contains more than thousands of species, which are distributed over Protista, Chromista, and Plantae (Woese et al., 1990). Micro part of microalgae define the part of unicellular organism in the group of algea. Microalgae can be eukaryotic or prokaryotic (Aguirre et al., 2013). Microalgae produce oxygen by process called photosynthesis. Most of the microalgae autotrophs and they use atmospheric CO_2 as carbon source while some microalgae able to use an organic source of carbon in addition to CO2. In addition, there are obligate heterotrophic algea, which cannot perform photosynthesis (Buesseler, 2012). Microalgea can be found in any environment. They can live in hyper-saline to fresh water environments, low pH to high pH. In addition, they found in dry environments such as soil and rocks. Because of their high physiological diversity microalgae have been used as living “cell factories” for production of variety of high value compounds such as fatty acids, carotenoids, antioxidants, enzymes, polymers, peptides, toxins and sterols and for biotechnologic applications. Microalgae have also been used for biofuel production since they sustain many potential advantages (Tabatabaei et al., 2011).

Microalgae contain 7–70% lipids, 5–23% carbohydrates, 6–52% proteins and other components such as nücleic acid or metals and this chemical composition of microalgea are strongly dependent on the species and environmental conditions (Chisti, 2007, Diltz adnd Pullammanappallil, 2013). Mainly because of the high lipid and carbohydrates content, microalgae accepted as an important biofuel feedstock to produce of variety of biofuels such as biodiesel, bioethanol, biogas, bio-hydrogen and many other fuel types.

Because of the possible high carbohydrate content of microalgae, they are ideal feedstocks for ethanol production. Carbohydrate extraction from algal cells easy due to the lack of structural biopolymers such as hemicellulose and lignin and this simplifies the algal bioethanol production process. Ethanol from microalgae is produced through extraction of starch or cellulose from the algal biomass, hydrolysis of the starch/cellulose to sugars, and fermentation of the sugars to produce ethanol by ethanol producing organisms (Zhu et al., 2014; Amin, 2009).

Biogas is produced by anaerobic digestion and produced methane and carbon dioxide can be used as a heat source or for electricity generation. Biomass of microalgae or algal residues after lipid extraction process use as feedstock for anaerobic digestion process since cells still contain starch and protein content without lignin. However, because of the tough cell wall of algae, digestion process is difficult and high protein content of residual algal biomass imbalance anaerobic digestion process (Zhu et al., 2014; Amin, 2009).

Microalgea are capble to produce biohydrogen by using sunlight and water in a closed culture system and the absence of oxygen. During photolysis reaction in photosynthesis, water molecules are divided into their molecules and in this manner, oxygen molecule and hydrogen ions are produced (Pushkar et al., 2008). Then, hydrogen ions are converted by hydrogenase enzyme into hydrogen molecules. However, nowadays biohydrogen production by using microalgae in large scale is not applicable due to lack of efficient microalgae to yield biohydrogen. (Amin, 2009).

By using microalgae, bio-oils or syngas fuels can be produced. Bio-oils or syngas fuels can be produced through thermochemical conversion algal biomass (Zhu et al., 2014). By changing temperature, oxygen availability and thermochemical process produced product can be altered. End products can be gas, liquid, or solid fuel. Synthetic gas, (which is mixture of CO_2, CO, CH_4, and H) are produced by gasification of carbonaceous materials in the algal biomass (Zhu et al., 2014).

Microalgea is cabaple the produce oil, which can be used as a precursor of biodiesel. Due to this thesis associated with the production of biodiesel, biodisel produvtion from microalgae is needed to explain in deatil.rebum.

2.3 Biodiesel

Biodiesel is a renewable, biodegradable fuel that can be manufactured domestically from vegetable oils, animal fats, microbial oils or recycled restaurant grease. Biodiesel is the clean alternative for petroleum diesel fuel. Biodiesel produced by a transesterification process and this process explained in detail at following section. Biodiesel also called as fatty acid methyl ester (FAME).

Currently, biodiesel is mainly produced from oil-plants such as canola oil, palm oil, corn oil or soybean (Chisti, 2007). On the other hand, algae most desirable for feedstock for biodiesel production due to the high carbon and hydrogen contents, and low oxygen content. (Zhu et al., 2014).

Biodiesel production processes from microalgal source and from food crops source are similar and involves two main steps: algal oil extraction, which can be divided two sub steps, and transesterification (Huang et al., 2010).

2.3.1 Transesterification process

Biodiesel compose of fatty acid/alkyl esters which can be produced by oil species mainly triglycerides, diglycerides, monoglycerides, free fatty acids and phospholipids (Chisti, 2007)

Transesterification is a multiple step reaction and consist of three simple steps. Firstly, triglycerides converted to diglycerides. After that, diglycerides converted to monoglycerides. Finally, monoglycerides converted to esters (biodiesel) and glycerol (Chisti, 2007, Mata & Martins et al., 2009).

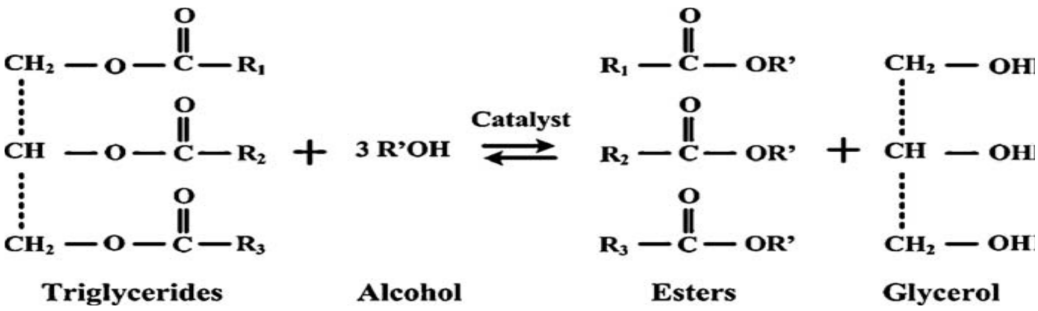


Figure 2.2: Transesterification of triglycerides (Mata & Martins et al., 2009).

In addition to oil species, which is used in transesterification process, also alcohols are used as a reactant in the transesterification process, Methanol, ethanol, propanol and butanol are mostly used alcohols for transesterification process. However, due to its cost-efficiency and high physical benefits, mainly methanol is used as an alcohols in the transesterification process. Acids, bases and enzymes are used as catalysts for transesterification. Alkali catalysts have 4000 times higher conversion rate than acid catalysts and in addition to this, alkali catalysts have higher reaction efficiency than acid catalysts (Fukuda et al., 2007).

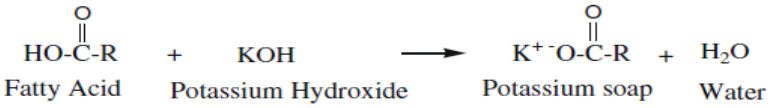


Figure 2.3: Transesterification by alkali catalyst.

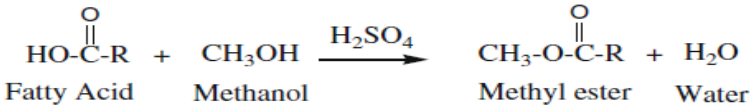


Figure 2.4: Transesterification by acid catalyst (Huang et al., 2009).

By transesterification process, less viscous product is produced than its precursor. Forwhy, microalgal oil is highly viscous and because of this, it cannot be used in engines and industrial processes. (Canakci & Vangerpen et al.1999)

Type of transesterification	Advantages	Disadvantages
Chemical catalysis	(a) Reaction condition can be well controlled (b) Large scale production (c) The cost of the production process is cheap (d) The methanol produced in the process can be recycled (e) High conversion of the production	(a) Reaction temperature is relative high and the process is complex (b) The later disposal process is complex (c) The process need much energy (d) Need a installation for methanol recycle (e) The waste water pollutes the environment
Enzymatic catalysis	(a) Moderate reaction condition (b) The small amount of methanol required in the reaction (c) Have no pollution to natural environment	(a) Limitation of enzyme in the conversion of short chain of fatty acids (b) Chemicals exist in the process of production are poisonous to enzyme
Supercritical fluid techniques	(a) Easy to be controlled (b) It is safe and fast (c) Friendly to environment	(a) High temperature and high pressure in the reaction condition leads to high cost of production and wastes energy

Figure 2.5: Application of transesterification technologies (Huang et al., 2009).

2.4 Microalgal Biodiesel: Advantages and Challenges

2.4.1 Advantages of using microalgae

Microalgae seem to be the only biodiesel feedstock that can completely displace fossil diesel when oil yields and land areas of microalgae and other biodiesel sources are compared. (Chisti, 2007).

Table 2.2: Comparison of microalgal with some first generation biodiesel feedstock in terms of needed land area to meet 50% of all transport fuel needs of USA. Taken from Chisti, 2007.

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

[a] For meeting 50% of all transport fuel needs of the United States.
[b] 70% oil (by wt) in biomass.
[c] 30% oil (by wt) in biomass.

Microalgae have very short harvesting life than first generation biodiesel feedstock. Microalgae biomass is doubled within 24 h, and even some spices doubling time can be as short as 3.5 h during exponantiel growth (Vijayaraghavan and Hemanathan, 2009). Their light energy capturing efficency make microalgae superior for lipid productivity. Microalgae's oil content can be as high as 70-80% of dry biomass by weight (Chisti, 2007; Vijayaraghavan and Hemanathan, 2009). Biodiesel, which is produced by using microalgael oil, has high levels of saturation, because of that, microalgae is suitable feedstock for biodiesel production (Demirbas, 2011). Cultivation of microalgae reguire less less freshwater and much more less land (estimated that, 2% of the land, which is used by oil bearing crops to produce biodiesel, required to produce the same amount of biodiesel from oil bearing crops) unlike terrestrial plants. Also, microalgae do not require arable lands for cultivation, and in

addition to this, cultuvation of microalgae does not require chemicals such as herbicides or pesticides and most case fertilizers. Microalgae removes phosphates and nitrates from water when growing in wastewater, and because of the this properties, microalgae can be used for bioremediation of wastewaters and prevent the eutrophication. Thus, wastewater can be ideal substrate for the cultivation of microalgae for biofuels production. Some high value products such as acids, carotenoids and anti-oxidant produced by microalgae as by-products and this by-products can be used for commercial or pharmaceutical purpose (Rawat et al., 2013). Producing this by-products also increase the cost-effectivness of microalgael biodiesel production.

2.4.2 Challenges of using microalgae

Open systems, such as open pond or raceway reactor, used for production of microalgeal biomass are sensitive to contamination. Contaminant can compete with microalgae for nutrients and oxidise organic matter or even more problematically, organism such as protozoa and zooplankton can directly consume microalgae. In both cases, algal concentration and biomass yield decrease in the open systems. Even the loss of entire biomass can be considered. Mostly unicellular structure of lipid storing microalgae cause low density in medium, so harvesting of microalgae are challenging (Rawat et al., 2013). Also, drying process of harvested algal biomass from high volume of water is time and energy-consuming process. In addition to this, cell wall of algae represents big barrier and make more difficult the process during oil extraction process (Tabatabaei et al., 2010). 80-90% of the whole cost of the production of biodiesel from microalgae is originated from harvesting and oil extraction process (Liu et al., 2011).

To get rid of the dependency of fossil fuel, microalgae biodiesel must be economically favorable. Algal biodiesel must be cheaper than petroleum diesel. Cost of producing microalgal biodiesel can be reduced substantially by using a biorefinery based production strategy.

In this thesis, in order to reduce the cost of algae biofuels by using a biorefinery based production strategy, *Synechocystis sp. PCC 6803* is used to clean wastewater while producing oils, which are precursor of biodiesel.

2.5 Cyanobacteria

Cyanobacteria, which is also known as Cyanophyta, is a phylum of bacteria that obtain their energy through photosynthesis (Cyanobacteria: Life History and Ecology, 2012). The name "cyanobacteria" comes from the color of the bacteria (Greek: κυανός (kyanós) means blue). Formerly, cyanobacteria are often called blue-green algae (sometimes still that name is used), but some consider that name a misnomer, due to the cyanobacteria are prokaryotic and term of microalgae should be eukaryotic, although other definitions of microalgae encompass prokaryotic organisms, so cyanobacteria can be classified in microalgae if the comprehensive definition take into account.

Being considered that, cyanobacteria, which they produce gaseous oxygen as a byproduct of photosynthesis, is converted the early reducing atmosphere into an oxidizing one. This process entitle as "rusting of the Earth" and dramatically changing the composition of early life forms on Earth by triggering biodiversity and cause to the near-extinction of oxygen-intolerant organisms. In addition to this, according to endosymbiotic theory, the chloroplasts which found in plants and eukaryotic algae and organelle responsible from photosynthesis evolved from cyanobacterial ancestors via endosymbiosis.

General properties of Cyanobacteria are (each of them described in detail later):

- Though that, cyanobacteria exist in the earth for a very long time and cyanobacteria were the first organisms to able make oxygenic photosynthesis, in which molecular oxygen is produced and by this way cyanobacteria changed the biosphere from anaerobic to largely aerobic.
- Cyanobacteria are a morphologically diverse group, even more diverse than any other group of prokaryotes, and some spices of cyanobacteria show unique patterns of cell differentiation.
- Many cyanobacteria species habitat is spread all over the planet, and because of that to investigate questions of microbial biogeography and evolution, cyanobacteria are excellent model organisms.

- Major contributors to the primary production of the oceans is cyanobacteria. Moreover, cyanobacteria are one of the most important groups that tfix molecular nitrogen.
- The ability to adapt to the environment of cyanobacteria is very high; Many species of cyanobacteria can actively move toward more habitable areas; they adapt their pigmentation according to color of the available light and sometimes also to the intensity of light; some species of cyanobacteria demonstrate staggering adaptation toward a life under anaerobic conditions; many types can grow at extremes of temperature, salinity, and pH; and some species of cyanobacteria can survive in adverse conditions for long periods when growth conditions are not suitable,.
- To grow most types of cyanobacteria in the laboratory is relatively easy, and in addition to this, many spices of cyanobacteria isolated and studied in axenic culture.

2.5.1 Occurrence in nature

Generally, cyanobacteria can found in almost every terrestrial and aquatic habitat such as oceans, fresh water, damp soil, temporarily moistened rocks in deserts, bare rock and soil, and even Antarctic rocks in the planet. They can observe as planktonic cells or form phototrophic biofilms. Cyanobacteria found in almost every endolithic ecosystem (Grube et al., 2007). Few of them are endosymbionts in lichens, plants, various protists, or sponges and provide energy for the host. Even, some spices of cyanobacteria live in the fur of sloths, providing a form of camouflage (Terry Vaughan, 2011).

The major habitats of cyanobacteria are limnic and marine environments. They flourish in almost all types of water such as salty, brackish or fresh, cold or hot springs, and even in environments where no other microalgae can exist. Most marine forms of cyanobacteria (Humm and Wicks, 1980) grow along the shore as benthic vegetation in the zone between the high and low tide marks. The cyanobacteria enclose a large component of marine plankton with global distribution (Wille, 1904; Gallon et al., 1996). A number of freshwater species of cyanobacteria (such as *Synechocystis sp. PCC 6803*) are also able to grow in relatively high concentrations of sodium chloride. It be clear that many cyanobacteria which are isolated from coastal environments can

tolerate saline environments (i.e. are halotolerant) rather than require salinity (i.e. are halophilic). As frequent colonisers of very saline (euryhaline) environments, cyanobacteria are found in salt works and salt marshes, and they are capable to grow at combined salt concentrations as high as 3-4 molar mass (Reed et al., 1984). Freshwater environment with diverse trophic states are the main habitats for cyanobacteria. Countless species of cyanobacteria characteristically inhabit, and they can occasionally dominate, both near-surface epilimnic and deep, euphotic, hypolimnic waters of lakes (Whitton, 1973). Others colonise surfaces by attaching to rocks or sediments, sometimes they forming mats that may tear loose and float to the surface.

Infertile substrates such as volcanic ash, desert sand and rocks can be colonise by cyanobacteria due to the impressive colonization capacity (Jaag, 1945; Dor and Danin, 1996). Cyanobacteria are exceptional excavators, boring hollows into limestone and special types of sandstone (Weber et al., 1996).

Another remarkable feature of cyanobacteria is their ability to survive extremely high and low temperatures. Cyanobacteria can inhabit hot springs (Castenholz, 1973), mountain streams (Kann, 1988), Arctic and Antarctic lakes (Skulberg, 1996a) and snow and ice (Kol, 1968; Laamanen, 1996). Some spicies of cyanobacteria run through the entire range of water types, from polysaprobic zones to katharobic waters (Van Landingham, 1982).

Also, cyanobacteria form symbiotic relations with animals and plants. Symbiotic relations exist with such as, fungi, bryophytes, pteridophytes, gymnosperms and angiosperms (Rai, 1990). The hypothesis, which explain the endosymbiotic origin of chloroplasts and mitochondria, should be talk about in this context. According to this theory, a cyanobacteria being engulfed and codeveloped by a phagotrophic host cause the evolutionary formation of a photosynthetic eukaryote (Douglas, 1994).

2.5.2 Characteristics

All cyanobacteria characterized by the ability to carry out oxygenic photosynthesis with two photosystems, similarly to plants. Cyanobacteria, due to their photoautotrophic growth property, can live in poor environments with only light, air, water and some salts. Many strains of cyanobacteria are able to fix atmospheric

dinitrogen into ammonium and thus they do not need the presence of combined nitrogen in the medium, which makes their cultivation inexpensive.

In contrast to eukaryotic microalgae, cyanobacteria are prokaryotic organism. It means that, they do not have membrane-bound sub-cellular organelles and they have no discrete membrane-bound nucleus. In addition to this, wall structure of cyanobacteria contain a peptidoglycan layer and their ribosomes consist of 70 S rather than 80 S subunits (Fay and Van Baalen, 1987; Bryant, 1994). Cyanobacteria have structure called thylakoids or thylakoid membrane, which is a folding of cell membrane inside the cell that function in photosynthesis.

The photosynthetic pigments of cyanobacteria are located in thylakoids. Thylakoids of cyanobacteria lie free in the cytoplasm near the cell periphery. Cell colors of cyanobacteria vary from blue-green to violet-red. Chlorophyll "a", which is color is green, is usually masked by carotenoids (e.g. beta-carotene) and accessory pigments such as phycocyanin, allophycocyanin and phycoerythrin (phycobiliproteins). The pigments of cyanobacteria are embodied in phycobilisomes, which are found in rows on the outer surface of the thylakoids (Douglas, 1994). All cyanobacteria contain chlorophyll a and phycocyanine.

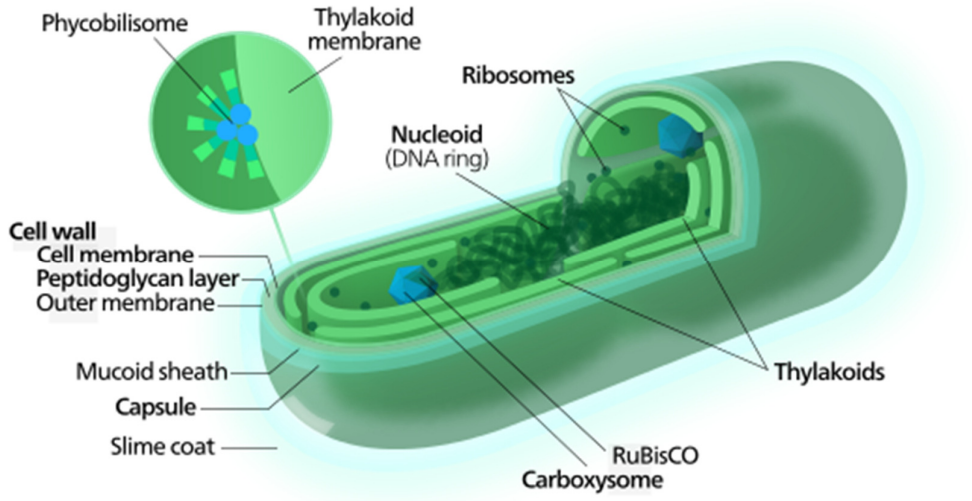


Figure 2.6: Typical cyanobacterial cell.

The basic features of photosynthesis in cyanobacteria have been well characterized (Ormerod, 1992). Cyanobacteria are oxygenic phototrophs own two kinds of reaction centers which are called as PS I and PS II, in their photosynthetic apparatus. They are

able to use effectively region of the light spectrum between the absorption peaks of chlorophyll a and the carotenoids with help of the accessory pigments mentioned above. Cyanobacteria able to colonize a wide range of ecological niches by helping of the ability of continuous photosynthetic growth in the presence of oxygen and together with having water as their electron donor for CO2 reduction (Whitton, 1992). Phycobiliprotein synthesis in cyanobacteria is especially responsive to environmental influences, particularly light quality. Chromatic adaptation is largely ascribable to a change in the ratio between phycocyanin and phycoerythrin in the phycobilisomes. Therefore, cyanobacteria are able to produce the accessory pigment, which needed to absorb light most efficiently in the habitat where they are present.

One of the fundamental metabolic process of cyanobacteria is dinitrogen fixation by this way they meet the simplest nutritional requirements of all living organisms. Some spices of cyanobacteria can convert N2 directly into ammonium (NH4) (a form through which nitrogen enters the food chain) by using the enzyme nitrogenase. They only need N2, CO2, water and mineral elements are needed for growth in the light. Nitrogen-fixing cyanobacteria are widespread among the filamentous, heterocyst forming genera (e.g. Anabaena, Nostoc) (Stewart, 1973). However, there are also several well proven examples of dinitrogen fixation among cyanobacteria not forming heterocysts (e.g. Trichodesmium) (Carpenter et al., 1992). Nitrogen fixing cyanobacteria may be favoured and gain growth and reproductive success under predominantly nitrogen limited conditions, but when other nutrients are available. Mass developments (often referred to as "blooms") of such species in lacustrine and marine environments (e.g. theBaltic Sea) are common circumstance world-wide.

Cyanobacteria have a spectacular ability to lay essential nutrients and metabolites within their in cytoplasm. For this purpose, notable cytoplasmic inclusions can be seen with the electron microscope (e.g. glycogen granules, lipid globules, cyanophycin granules, polyphosphate bodies, carboxysomes) (Fay and Van Baalen, 1987). Under conditions of an excess supply of particular nutrients, reserve products are accumulated. For example, the primary products of photosynthesis are channeled towards the synthesis and accumulation of glycogen and lipids, when the synthesis of nitrogenous cell constituents is halted because of an absence of a usable nitrogen source.

Many species of cyanobacteria own gas vesicles. These gas vesicles are cytoplasmic inclusions which structures are cylindrical that make possible buoyancy regulation and are gas-filled. Function of the gas vesicles is to give cyanobacterial species an ecologically important mechanism allowing them to adjust their vertical position in the water column (Walsby, 1987). Cyanobacteria use different environmental stimuli (e.g. photic, gravitational, chemical, thermal) as clues to optimize their position, and so to find a suitable niche for survival and growth. When light is reduced, gas vesicles become more abundant and the growth rate slows down. Increases in the turgor pressure of cells which is the result of the accumulation of photosynthate cause a decrease in existing gas vesicles and thus a reduction in buoyancy. By such buoyancy regulation, cyanobacteria can poise themselves within vertical gradients of physical and chemical factors. Other ecologically important mechanisms of movement demonstrated by some cyanobacteria are photomovement by slime secretion or surface undulations of cells (Häder, 1987; Paerl, 1988).

2.5.3 Morphology

The basic morphology of cyanobacteria include unicellular, colonial and multicellular filamentous forms.

Unicellular forms, for example in the order Chroococcales, have spherical, ovoid or cylindrical cells. When the daughter cells separate after reproduction by binary fission, they occur solo. The cells may aggregate in irregular colonies, during the growth of the colony these colonies held together by the slimy matrix secreted. More ordered colonies may be produced, by means of a more or less regular series of cell division, combined with sheath secretions.

Filamentous morphology is the result of repeated cell divisions occurring in a single plane at right angles to the main axis of the filament. The multicellular structure consisting of a chain of cells is called a trichome. The trichome may be straight or coiled. Among the filamentous cyanobacteria, cell size and shape show great variability. Species in the order Oscillatoriales, with unseriated and unbranched trichomes, are composed of essentially identical cells. The other orders with a filamentous organization (orders Nostocales and Stigonematales) are characterized are

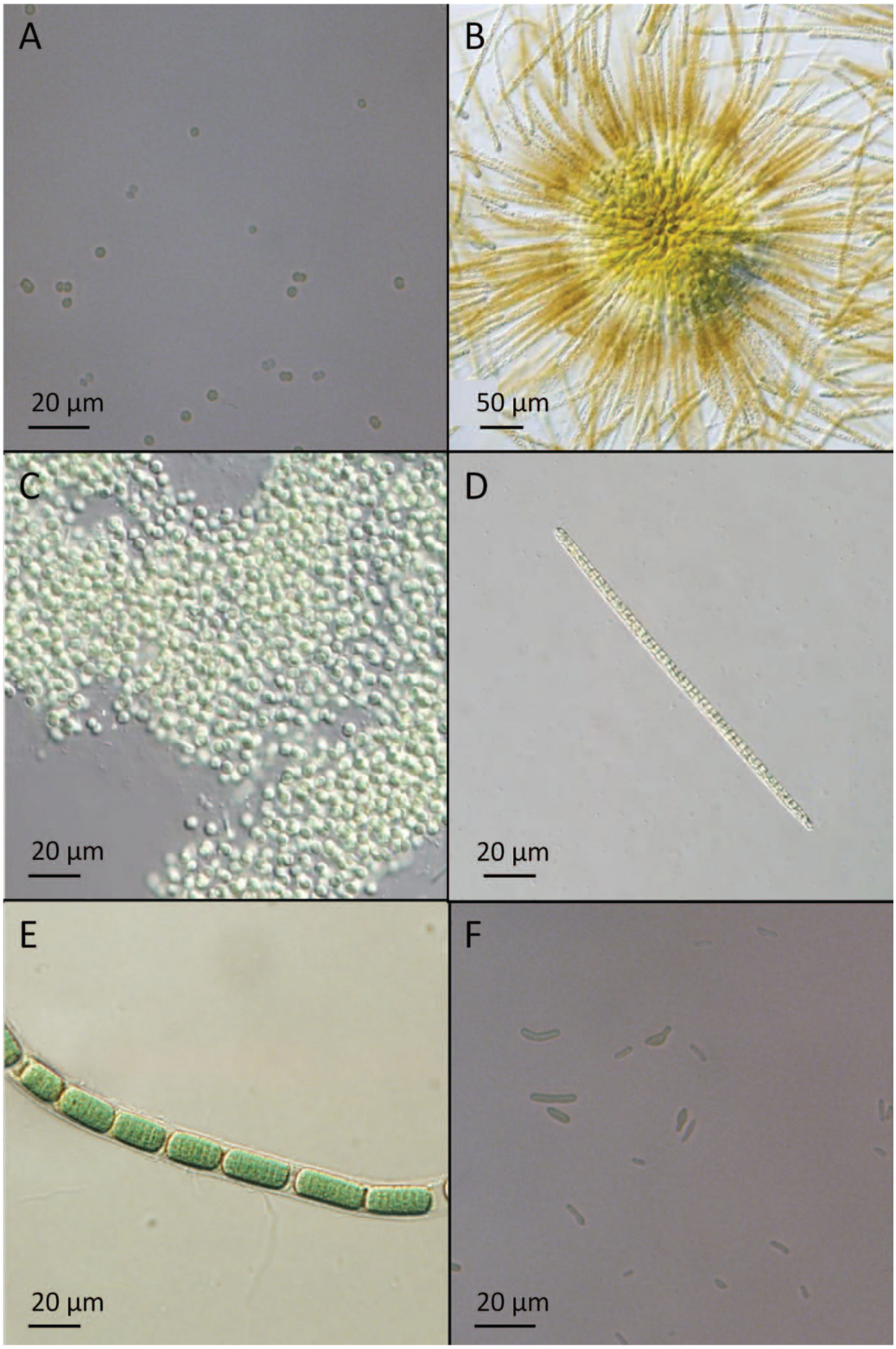


Figure 2.7: Light microscopy images of cyanobacteria. (A) *Synechocystis sp. PCC 6803*, (B) *Gleotrichia echinulata* colony, (C) Microcystis spp., (D) *Planktothrix suspensa*, (E) Lyngbya sp., (F) *Synechococcus elongatus PCC 7942*. Photo credit for images (B–E): Barry H

characterised with trichomes having a heterogeneous cellular composition. Vegetative cells may be differentiated into heterocysts (having a thick wall and hyaline protoplast, capable of nitrogen fixation) and akinetes (large thick-walled cells, containing reserve materials, enabling survival under unfavourable conditions). In the order Stigonematales, the filaments are often multiseriated, with genuine branching. Moreover, in the order Stigonematales show both heterocysts and akinetes cell type.

Many spices of cyanobacteria form motile filaments of cells, which are called hormogonia, that travel away from the main biomass to bud and form new colonies elsewhere. The cells in a hormogonium are often thinner than in the vegetative state, and the cells on either end of the motile chain may be tapered. A hormogonium often must tear apart a weaker cell in a filament, which are called a necridium, to break away from the parent colony,

Each individual cell of a cyanobacterium typically has a gelatinous, thick cell wall. They lack flagella, but hormogonia of some species can move about by gliding along surfaces. Many of the multicellular filamentous forms of Oscillatoria are capable of a wavin□ m□ti□n; the filament □□cillate□back and f□rth. Like archaea, □□me □pecie□□f cyanobacteria float by forming gas vesicle in water columns. It must appear on these gas vesicles are not organelles. These gas vesicles are not bounded by lipid membranes, but by a protein sheath.

Some of these organisms contribute significantly to global ecology and the oxygen cycle. More than half of the photosynthesis of the open ocean is carried out by the tiny marine cyanobacterium Prochlorococcus, which was discovered in 1986. Even, many cyanobacteria exhibit the circadian rhythms that were once thought to exist only in eukaryotic cells.

2.5.4 Systematics and Classification

Cyanobacteria can defined to include all known prokaryote capable of oxygenic photosynthesis and they present a diverse group of Gram-negative bacteria. The cyanobacteria are a monophyletic group of organisms, they representing one of the more ancient evolutionary lineages among Bacteria (Aharon Oren, 2014). Phylogenetically (as based on the small-subunit ribosomal RNA-based tree of life) they are equiphase group within the domain Bacteria (Bonen, Doolittle, and Fox, 1979; Wilmotte, 1994; Wilmotte and Herdman, 2001). Still their phylogenetic position

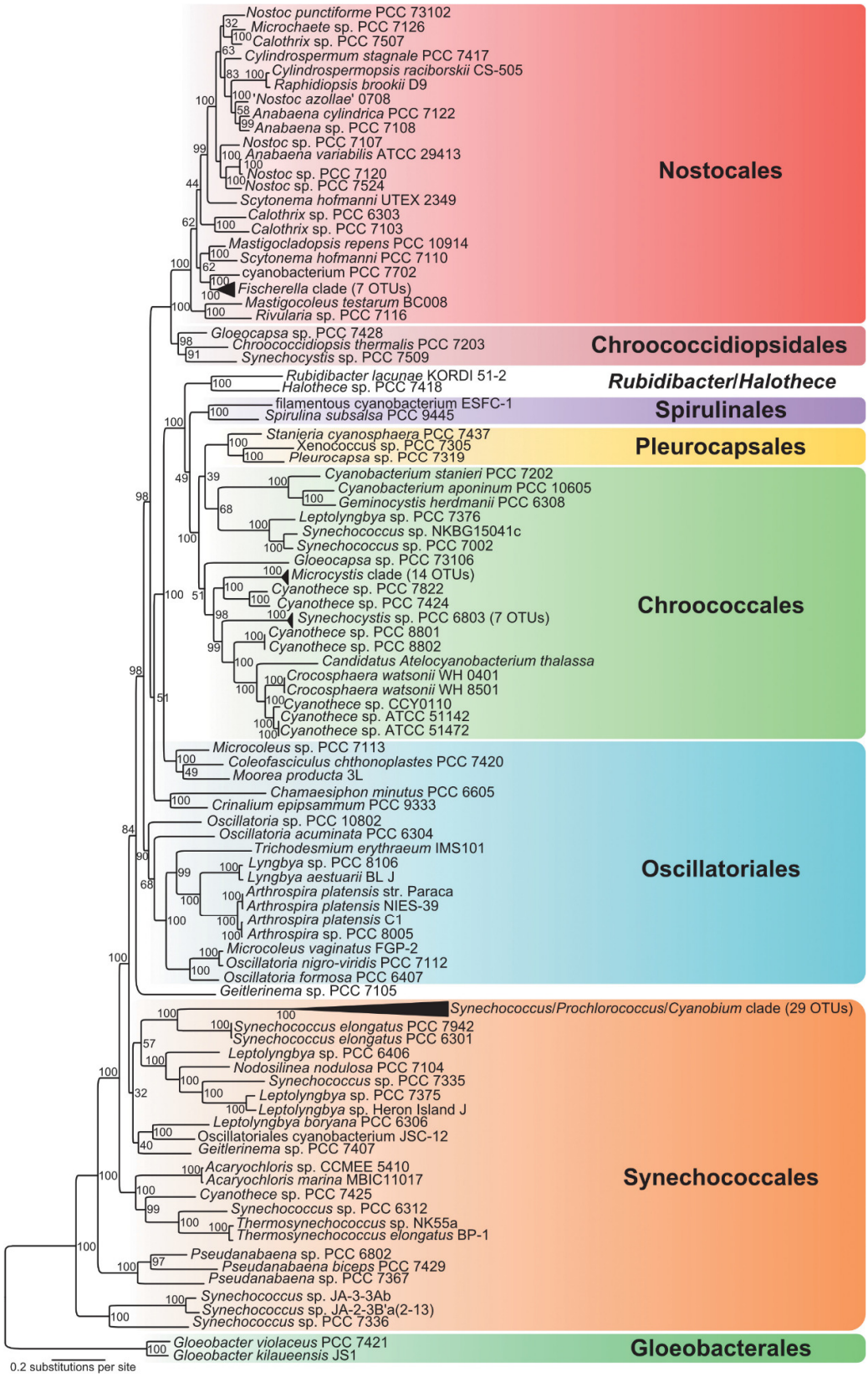


Figure 2.8: Classification of *Cyanobacteria*.

in the bacterial kingdom is uncertain, however recent analysis of ancient genes has indicated a genetic relationship with Gram-positive bacteria. The cyanobacterial lineage also includes the chloroplasts of the eukaryotic cells and plants (Giovannoni et al., 1988).

Chloroplasts are evolutionarily related to cyanobacteria as they have emerge by an ancient endosymbiotic incident, in which a heterotrophic eukaryote engulfed a cyanobacterium like organism. All nowadays chloroplasts sprout up from a single endosymbiotic incident that happened around 1.5-1.2 billions years ago. However, evolution of chloroplast included organisms is complex, and many algal groups, such as diatoms, brown algae or Euglenophytes have sprout up by secondary endosymbiosis, in which a chloroplast-less eukaryote engulfed a chloroplast containing eukaryote (Aharon Oren, 2014). It is believed that cyanobacteria are liable in great part for the increase in oxygen content in the atmosphere of our planet that happened around 2.2 billion years ago, and that oxygenic atmosphere is necessary entailment for the evolution of complex life forms with aerobic metabolism (Aharon Oren, 2014).

Taxonomic schemes of cyanobacteria are still largely based on morphological characters because of morphologically cyanobacteria are much more diverse than any other group within the prokaryotes, Bacteria and Archaea combined.

Traditionally the cyanobacteria were classified by morphology into five sections, referred to by the numerals I-V. The first three – Chroococcales, Pleurocapsales, and Oscillatoriales – are not supported by phylogenetic studies. However, the latter two – Nostocales and Stigonematales – are monophyletic, and constitute the heterocystous cyanobacteria. The members of Chroococales are unicellular and they usually aggregate in colonies. The classic taxonomic criterion has been the cell morphology and the plane of cell division. In Pleurocapsales, the cells have the capacity to form internal spores (baeocytes). The rest of the sections include filamentous species. In Oscillatoriales, the cells are arranged in one line and do not form specialized cells (akinetes and heterocysts). In Nostocales and Stigonematales, the cells have the capacity to develop heterocysts in certain conditions. Stigonematales, unlike Nostocales, include species with truly branched trichomes.

Most taxa included in the phylum or division Cyanobacteria have not yet been authentically published under the Bacteriological Code, except:

- The classes Chroobacteria, Hormogoneae, and Gloeobacteria
- The orders Chroococcales, Gloeobacterales, Nostocales, Oscillatoriales, Pleurocapsales, and Stigonematales
- The families Prochloraceae and Prochlorotrichaceae
- The genera Halospirulina, Planktothricoides, Prochlorococcus, Prochloron, and Prochlorothrix

However, there are recent attempts to classify the cyanobacteria mainly on the basis of morphological traits while incorporating as much “polyphasic” information as possible by including other characteristics was made by the editors and authors of the last edition of Bergey’s Manual of Systematic Bacteriology, as outlined by Castenholz (2001).

2.5.5 Biotechnology and Applications

Cyanobacteria have a long history of use by man. Because of high protein content Spirulina is used as a source of protein and vitamins. Spirulina platensis in Africa and Spirulina maxima in America have been used as food since antiquity. Other type of cyanobacteria are used as food in India, China, and the Philippines. Daily eating of Spirulina by the Kamemba tribe in Chad is epidemiologically linked to reduced rates of HIV infection.

Cyanobacteria is used to investigate biological systems. During the past half century, cyanobacteria have been used increasingly to study, such as photosynthesis and its genetic control; photoregulation of genetic expression; cell differentiation and N2 fixation; metabolism of nitrogen, carbon, and hydrogen; resistance to environmental stresses; and molecular evolution.

Cyanobacteria produce a large variety of secondary metabolites, which are has many potential use. Many of these metabolites have complex and unique structures and stereospecificity, which makes their chemical synthesis expensive or impossible. Thus the cyanobacterium itself must be used as a "cell factory". The pathway for the synthesis of some of these metabolites has been illuminated, and genes responsible the production of these metabolites are isolated. Therefore, improve their production

through the use of transgenic cyanobacteria is potentially possible. The molecular genetics tools developed for cyanobacteria have been used for the study of many aspects of their biology. A large amount of knowledge on photosynthesis has originate from studies in cyanobacteria. Detailed excellent reviews on molecular genetics tools for cyanobacteria have been published.

Today, there are more than 15 cyanobacteria genomes sequenced, and many more in progress (http://www.kazusa.or.jp/cyano/; http://img.jgi.doe.gov/pub/main.c^i). The availability of genome sequences, which facilitates transcriptomic, proteomic and metabolomic studies is a powerful tool for the development of cyanobacteria as biotechnological tools.

The availability of powerful genetic techniques allows the biotechnological application of cyanobacteria to produce specific products (including photosynthetic pigments and molecular hydrogen), to biodegrade organic pollutants in surface waters, to control mosquitoes, and for other purposes.

2.6 Synechocystis sp. PCC 6803

Synechocystis sp. PCC 6803 is a unicellular non-nitrogen (N_2) fixing cyanobacterium and a ubiquitous inhabitant of fresh water. To date, from of all cyanobacteria, *Synechocystis sp. PCC 6803* is the best-characterized species and, given its robust growth characteristics (Angermayr et. al., 2009).

'Synechocystis' describe as a unicellular coccoid, or spherical cyanobacterium lacking gas vesicles or a sheath. They divide by binary fission at two or three successive planes. Based on their GC contents, many cultured strains of Synechocystis can be classified into three groups; the marine group, the low GC group and the high GC group (Holt et al. 1998).

Strain PCC 6803 belongs to the latter group, whose members, including PCC 6714, have been mostly isolated from freshwater. Some sub-strains of *Synechocystis sp. PCC 6803* also have the ability to utilize glucose (Rippka et al. 1979). A phylogenetic tree based on 16S rRNA sequences suggests that high GC content species are more closely related to Microcystis aeruginosa, which is a unicellular spherical cyanobacterium with gas vesicles, than to other Synechocystis groups (Honda et al. 1999).

Property	Organism	Value	Units	ID	Details
Elemental composition of biomass	Cyanobacteria Synechocystis PCC 6803	51.4% C, 6.8% H, 11.3% N, 27.5% O, and 3.3% others	N/A	105530	Shastri AA, Morgan JA....
Doubling time	Cyanobacteria Synechocystis PCC 6803	12	hours	111252	Bernstein et al., Effect...
Final composition of mid-exponential photoautotrophically growing Synechocystis	Cyanobacteria Synechocystis PCC 6803	51% proteins, 19% carbohydrates, 10% lipids, and 20% nucleic acids	N/A	105531	Shastri AA, Morgan JA....
Total number of iron atoms per cell	Cyanobacteria Synechocystis PCC 6803	9.3e+6 (±7.5×10^5)	unitless	100838	Keren N, Aurora R, Pakrasi...
Iron atoms per photosystem I unit	Cyanobacteria Synechocystis PCC 6803	12	unitless	100836	Keren N, Aurora R, Pakrasi...
Number of iron atoms in photosystem I units per cell	Cyanobacteria Synechocystis PCC 6803	1.2e+6	unitless	100837	Critical roles of ba...
Fastest generation time	Cyanobacteria Synechocystis PCC 6803	5.13	hours	111335	Zavřel T., Sinetova M. A....
Photosystem 2 effective fluorescence cross-section values	Cyanobacteria Synechocystis PCC 6803	Table - link	Å^2	107404	Maksimov EG, Kuzminov FI...
Intracellular iron content in wildtype	Cyanobacteria Synechocystis PCC 6803	9.3e+6 (±7.5e5)	atoms/cell	108818	Keren N, Aurora R, Pakrasi...
Number of chlorophyll molecules per cell	Cyanobacteria Synechocystis PCC 6803	1.4e+7	unitless	100834	Keren N, Aurora R, Pakrasi...

Figure 2.9: Properties of Synechocystis sp. PCC 6803 – Part I.

There are four sub-strains of Synechocystis ('PCC', 'ATCC', 'GT' (glucose-tolerant) and 'Kazusa'), all of which were derived from the Berkeley strain 6803, which was isolated from freshwater in California by R. Kunisawa at 1968 (Stanier et al. 1971). It was originally believed that these sub-strains were the same. For this reason, they were grouped together under the name of *Synechocystis sp. PCC* strain number 6803 (Rippka and Herdman 1992). However, the four sub-strains show certain differences in phenotype.

Property	Organism	Value	Units	ID	Details
isoelectric point of plastocyanin	Cyanobacteria Synechocystis PCC 6803	6	Unitless	104022	De la Cerda B, Navarro JA...
Cell diameter	Cyanobacteria Synechocystis PCC 6803	1.75	µm	105535	Shastri AA, Morgan JA....
Generation time	Cyanobacteria Synechocystis PCC 6803	6.93-9.9	hours	105522	Shastri AA, Morgan JA....
mRNA lifetime psbA (D1) gene under illumination	Cyanobacteria Synechocystis PCC 6803	12	minutes	100784	Redox Control of psbA...
mRNA lifetime psbA (D1) gene under conditions of darkness	Cyanobacteria Synechocystis PCC 6803	90	minutes	100783	Redox Control of psbA...
Cell diameter	Cyanobacteria Synechocystis PCC 6803	1	µm	100530	Sarcina M, Mullineaux...
Number of predicted protein coding genes	Cyanobacteria Synechocystis PCC 6803	3661	Genes	107222	Pisareva T, Kwon J, Oh J...
Number of c-rings in ATPase (equivalent to number of protons translocated/rotation)	Cyanobacteria Synechocystis PCC 6803	14 (Table - link)	Unitless	106285	Pogoryelov D, Reichen C...
Generation time	Cyanobacteria Synechocystis PCC 6803	6	Hours	100289	Yukako Hihara, Ayako Kamei...

Figure 2.10: Properties of *Synechocystis sp. PCC 6803* – Part II.

Synechocystis sp. was reported to have relatively high biomass productivity (about 200 $mg/L^{-1}*d^{-1}$) (Kim et al., 2011), but low lipid content (18.4%) (Seefeldt et al., 2011), which may result in a comparable lipid productivity about 37 $mg/L^{-1}*d^{-1}$. (Cai et al., 2013).

Perhaps most significantly, *Synechocystis sp. PCC 6803* was the first photosynthetic organism which the entire genome sequence was determined. In 1996, Dr. Satoshi Tabata and coworkers at the Kazusa DNA Research Institute finished the genomic sequence of this organism and made the information available in very useful format on a website named CyanoBase (http://www.kazusa.or.jp/cyano/cyano.html). This information, coupled with the easy genetic manipulation strategy that is available for *Synechocystis sp. PCC 6803*, has made *Synechocystis sp. PCC 6803* the cyanobacterium of choice for research in many groups. Deletion mutants lacking a specific gene have been created and analyzed for many different genes. For example, Synechocystis is used to study pigment (carotenoid and chlorophyll) synthesis and its

regulation, tocopherol synthesis, carbon metabolism, respiration, photosynthesis and a host of other processes. The information gained not only is valuable for understanding the physiology of cyanobacteria, but also for that of other organisms such as plants (http://synechocystis.asu.edu/).

Synechocystis sp. PCC 6803 has developed into a model cyanobacterium that scientists around the world are using. The strain was isolated from a fresh water lake, and was stored in the Pasteur Culture Collection (PCC) in 1968. Then, due to the work of Prof. Sergey Shestakov and coworkers in Moscow and Dr. John Williams (Michigan State University and Du Pont) in the early 1980s, the strain was known to be spontaneuosly transformable, it means that train has the ability to integrate foreign DNA into its genome by homologous recombination (allowing targeted gene replacement). In addition, *Synechocystis sp. PCC 6803* able to survive and grow in a wide range of conditions. For example, *Synechocystis sp. PCC 6803* can grow in the absence of photosynthesis if a suitable fixed-carbon source such as glucose is provided.

The cyanobacterium *Synechocystis sp. PCC 6803* (PCC 6803) is an excellent candidate for large-scale biomass production because of its resiliency toward a wide range of environmental conditions, including salt concentration, pH, temperature, UV light, and carbon dioxide (CO_2) level (Ogawa and Kaplan, 2003; Ohkawa et al., 2000). *Synechocystis sp. PCC 6803* has its highest lipid content when it has a high specific growth rate during optimal photosynthesis, because the lipids of *Synechocystis sp. PCC 6803* are mainly originate from thylakoid membranes, (Hu et al., 2008; Sakamoto and Bryant, 1999; Wada et al., 1992). Although *Synechocystis sp. PCC 6803* has relatively low lipid content comparing the other microalgae such as *chlorella vulgaris* or *Nannochloropsis salina*, correlation of between increased growth rate and increased lipid content may compensate this disadvantages in terms of lipid productivity. This combination of properties means that PCC 6803 has the potential to generate lipid yields per hectare that are higher than from high-lipid plants (Chisti, 2007; Huber et al., 2006; Rittmann, 2008). Therefore, the key challenge is to find an efficient means to grow and harvest *Synechocystis sp. PCC 6803* at mass scale with high yields for producing a range of renewable bioproducts (Chisti, 2007, 2008; Huber et al., 2006; Rittmann, 2008; Rodolfi et al., 2009).

However, Synechocystis sp. was mainly cultivated in freshwater medium using commercial nutrients (Kim et al., 2010, 2011; Seefeldt et al., 2011; Sheng et al., 2011).

2.6.1 Influential Factors for Synechocystis sp. PCC 6803 Cultivation

Cyanobacteria can grow very large pH scale but generally found in alkaline environment and preferably grow at pHs ranging from neutral to 11 (Yolo et al., 2012). Cyanobacteria are among the most alkalotolerant or alkalophilic microbes (Lopez-Archilla et al., 2004). Moreover, cyanobacteria growth in neutral to slightly alkaline medium for optimum growth under laboratory conditions according to report (Kurian et al., 2006). On the other hand, cyanobacteria extend their distribution to pH values as low as 4 in natural environment (Steinberg et al., 1998). Cyanobacteria are absent in extremely acidic environments and according to Brock (Brock, 1973) this situation may be associated with their acid-sensitive chlorophyll molecules.

Synechocystis sp. PCC 6803 is commonly cultivated at pH around 7~8 (Yu et al., 2013). However, *Synechocystis sp. PCC 6803* can grow at larger pH scale from 6 to 11 (Lopo et al., 2012). At pH 7 or under below this value until the pH 6, growth rate of *Synechocystis sp. PCC 6803* is inhibited and under pH 6, growth can not observed (Kallas and Castenholz, 1982). However, *Synechocystis sp. PCC 6803* cells can retained a higher intracellular pH when they were exposed to pH 4.8 (Lopo et al,, 2012). When *Synechocystis sp. PCC 6803* placed in acid stress situation at a tolerable pH (pH≥4.4), *Synechocystis sp. PCC 6803* cells are able to increase their surrounding pH to 6 and above within a few minutes (Lopo et al., 2012). In the other hand, in alkaline medium, *Synechocystis sp. PCC 6803* cells can maintain growth (Yu et al., 2013). From pH 7.5 to 11, growth rate of the *Synechocystis sp. PCC 6803* cells almost similar (Lopo et al., 2012).

Cyanobacteria can be found in environments where temperature changes in a wide range (Lopo et. al., 2012) Most of the cyanobacteria species are mesophilic and live on environments where temperature may range from freezing to 40°C (Waterbury, 2006). However, some species can grow high temperature up to 75°C (Castenholtz, 1969). Generally, cyanobacteria grow between 20 and 35 ° C optimally (Lopo et. al., 2012).

Synechocystis sp. PCC 6803 is mesophilic organism and it generally grow temperature around 30°C in laboratory conditions (Yu et al., 2013). When the temperature higher than 40°C or lower than 25°C, *Synechocystis sp. PCC 6803* cells seem to suffer from low or high temperature stress (Berry and Bjorkman, 1980). According to Berry and

Bjorkman, this observed stress associated to PS II sensitivity, with a complete stop of growth at 15 and 45°C. *Synechocystis sp. PCC 6803* grow best at 30°C, but *Synechocystis sp. PCC 6803* grow at a quite similar rate in temperatures ranging from 25 to 40°C, which correlates with the rate of photosynthesis at these temperatures (Inoue et al., 2001). Growth limitation at high temperatures seems to be owing to limitation of PS II activity, on the other hand, growth limitation at low temperatures seems to cause by increase in the desaturation level of thylakoid membranes fatty acids (Los et al., 1993). A increase in the desaturation level of fatty acids in the thylakoid galactolipids of *Synechocystis sp. PCC 6803* increases the resistance of the *Synechocystis sp. PCC 6803* cells to low temperatures (Wada et al., 1994).

Like other organisms *Synechocystis sp. PCC 6803* also needs to C, N, P as a macronutrients. Elemental analysis *Synechocystis sp. PCC 6803* dry biomass shows that chemical composition of *Synechocystis sp. PCC 6803* dry biomass is $CH_{1.6}O_{0,40}N_{0,22}P_{0,01}$ (Yu et al., 2013).

Many species of cyanobacteria are able to use nitrate, nitrite and ammonium as nitrogen sources but ammonium is the preferred nitrogen source (Flores and Herrero, 2005). Some strains of cyanobacteria are able to fix dinitrogen gas, and in addition to this a number of cyanobacteria may also have the ability to use urea, cyanate and amino acids as additional nitrogen sources (Valladares et al. 2002). Nitrogen compounds which are obtained by cyanobacteria are converted to ammonium and subsequently assimilated for biosynthesis (Krasikov et al. 2012).

Synechocystis sp. PCC 6803 use urea, ammonium, nitrate, and nitrite as the sources of nitrogen (Wang et al., 2004). However, for *Synechocystis sp. PCC 6803*, ammonium is the preferred nitrogen source (Drath et al., 2008). However, high ammonia concentration causes the ammonia induced photodamage on photosystem II (PS II) apparatus of *Synechocystis sp. PCC 6803* and because of that high ammonia concentration causes stress in *Synechocystis sp. PCC 6803* via damage (Dai et al., 2014). Moreover, ammonia induced photodamage on PSII also increase with increased light intensity (Dai et al., 2014). *Synechocystis sp. PCC 6803* is able to store ammonium nitrogen inside of the cell by producing cyanophycin, which is a polypeptide containing multiple arginine and aspartate residues (Yu et al., 2013). This polymer can be degraded by cyanophycinase, which is a hydrolytic enzyme, under nitrogen limitation condition (Richter et al., 1999). In addition, *Synechocystis sp. PCC*

6803 cannot fix $N_{2(gas)}$ naturally, *Synechocystis sp. PCC 6803* doesn't have nitrogenase (Yu et al., 2013). In case of nitrogen starvation, *Synechocystis sp. PCC 6803* exhibit many different response. These are up-regulation of nitrogen assimilation, visible chlorosis (the pigment degradation process) or 'yellowing' of the cells with a transcriptional repression of the phycobilisome genes, degradation of phycobiliproteins, alteration of the ratio of phycocyanin to allophycocyanin, degradation of thylakoid membranes, a decrease in chlorophyll, an increase in carotenoid content or carotenoid/chlorophyll ratio, down-regulation of enzymatic activities and decreasing the rate of photosynthetic carbon fixation (i.e., RuBisCO encoding genes are strongly down-regulated) (Osanai et al., 2006, Lopo et al., 2012, Krasikov et al., 2012). In addition, under the nitrogen limiting conditions, *Synechocystis sp. PCC 6803* cells start to accumulate glycogen (Yoo et al., 2007). According to all data given above nitrogen supplements are crucial for *Synechocystis sp. PCC 6803* cultivation, whether to aim is to produce biomass, whether to modify the chemical composition of the *Synechocystis sp. PCC 6803* cells.

Phosphate is least abundant naturally occurring mineral in earth (Wassen et al., 2005). Phosphorus starvation cause the partial loss of pigment of phycobilisome, which is the light-harvesting antenna typical of most cyanobacteria, in cyanobacteria (Collier & Grossman, 1992). Various cyanobacteria synthesize the nitrogen and energy storage compound cyanophycin (multi-L-arginyl-poly-L-aspartic acid) under conditions of phosphorus starvation (Allen, 1984).

Phosphorus other crucial nutrient for *Synechocystis sp. PCC 6803*. Approximately, %1,32 of molar weight of *Synechocystis sp. PCC 6803* is consist of phosphorus. *Synechocystis sp. PCC 6803* in is able to accumulate inorganic polyphosphate (polyP) inside the cell (Yu et al., 2013). Also according to Yu et. al., *Synechocystis sp. PCC 6803*, which modifed with proper genetic modification, can be used for Pi removal from wastewater. The growth rate of *Synechocystis sp. PCC 6803* is significantly impaired in phosphate-depleted conditions and in addition to this, Green color of medium of *Synechocystis sp. PCC 6803* is noticeable reduced (Fuszard et al., 2013). In addition, phosphorus starvation harms photosynthetic systems of *Synechocystis sp. PCC 6803* and slows down both energy and nucleotide sugar generation (Yu et al., 2013). For example, according to data published by Fuszard et al (2013), In In case of lack of phosphate, the growth rate of *Synechocystis sp. PCC 6803* was significantly

impaired with noticeable reduction of colour of medium of *Synechocystis sp. PCC 6803*. Also their data showed that while the control culture reached an OD730 of 7 at the end of the 60-day cultivation, whereas the phosphate-limited cultures only reached a maximum OD_{730} of 0.9 for contain about 0,1674 mg/L phosphorus and 0,5 for contain about 0,0167 mg/L phosphorus. In given light above, Pi supplements are crucial for *Synechocystis sp. PCC 6803* cultivation.

Carbon is main nutrient for all organisms. For oxygenic photosynthesis, inorganic carbon is an essential and often limiting inorganic substrate (Wang et al., 2004). For cyanobacteria and other aquatic photosynthetic species, carbon is acquired either as HCO_3^- or as dissolved CO_2 (Wang et al., 2004). In addition to this, some species of cyanobacteria is able to use organic carbon as well.

Synechocystis sp. PCC 6803 is able use inorganic carbon spices, CO_2 and HCO_3^- as carbon source. In addition to this, *Synechocystis sp. PCC 6803* can able use different organic carbon as well such as glucose, fructose, maltose, ethanol, pyruvate, succinate and acetate (Panda et al., 2005, Yu et al., 2013). Due to the Synechocystis sp. PCC 6803 has the ability of perform photosynthesis, it can uses inorganic carbon as carbon source. *Synechocystis sp. PCC 6803* is able to fix inorganic carbon via Ribulose-1,5-bisphosphate carboxylase (RuBisCO), which catalyzes the first step of the Calvin Cycle for inorganic carbon fixation (Yu et al., 2013). However, RuBisCO is sensitive to O_2. To solve this problem, *Synechocystis sp. PCC 6803* has evolved organelle called as carboxysome, which a unique organelle that encapsulates RuBisCO and concentrates inorganic carbon to prevent he binding of O_2 to RuBisCO (Yu et al., 2013). Thus, *Synechocystis sp. PCC 6803* has the capability to grow under very low CO_2 concentrations (Battchikova et al., 2010). On the other hand, increasing CO_2 concentration increase the growth rate of *Synechocystis sp. PCC 6803*. According to data published by Zavrel et al., (2015) growth rate of *Synechocystis sp. PCC 6803* increase with increasing CO_2 concentration, however they did not observe increase in growth rate, after the CO_2 concentration reach the 1667 ppm. In addition to this they point out that there is no negative effect of CO_2 concentration as high as 15,000 ppm. Also they stated that under CO_2 concentration equal the CO_2 concentration in the air, growth rate of *Synechocystis sp. PCC 6803* was limited. While *Synechocystis sp. PCC 6803* performing photosynthesis, usage of HCO_3^- amount always higher than CO_2 usage (Benschop et al., 2003). Growth of *Synechocystis sp. PCC 6803* can occur when

using organic carbon as carbon source regardless of usage inorganic carbon by *Synechocystis sp. PCC 6803*. *Synechocystis sp. PCC 6803* can grow under mixotrophic conditions or heterotrophic conditions but, pulse of white light has to be given culture of *Synechocystis sp. PCC 6803* (Yu et al., 2013). According to Wang et al, (2002), under mixotrophic conditions, growth rate higher than growth rate under the autotrophic conditions. On the other hand, under heterotrophic conditions (with daily pulse of light, which in sufficient for photosynthesis), doubling time of *Synechocystis sp. PCC 6803* is time more than doubling time of *Synechocystis sp. PCC 6803* under photoautotrophic conditions. Choosing the right type of carbon spices and define the right amount of them are import for cost-effective *Synechocystis sp. PCC 6803* cultivation.

Light plays a vital role in all photosynthetic organisms from cyanobacteria to plants by regulating growth, altering gene expression, and resetting circadian rhythms, among others [Gill et al., 2002; Mullineaux, 2001, Montagud et al., 2010], but light can also be harmful to the photosynthetic unit of the cell (Lopo et al., 2012).

Light is essential component for cultivation of *Synechocystis sp. PCC 6803*. However, Optimum light intensity for *Synechocystis sp. PCC 6803* or cyanobacteria in general still are arguably consensual in scientific community (Lopo et al., 2012). Depending on the culture volume, flask geometry and inoculum, amount of Optimum light intensity for *Synechocystis sp. PCC 6803* differentiate (Lopo et al., 2012). Number in literature change between 20 $\mu E/m^{-2}*s^{-1}$ (Lopo et al., 2012) to 300 $\mu E/m^{-2}*s^{-1}$ (Allakhverdiev and Murata, 2004). Also, according to Allakhverdiev and Murata (2004), light might play a double role in growth of *Synechocystis sp. PCC 6803* that inducing damage to PS II when it is strong and inducing repair of the photodamaged PS II when it is weak. Thus, optimal light intensity must be defined according to photobioreactor condition.

2.7 Production of Microalgae on Wastewater

Many species of microalgae are able to effectively grow in wastewater conditions because of their ability to utilize abundant organic carbon and inorganic N and P in the wastewater. The use of microalgae in wastewater treatment has been long developed (Oswald et al., 1957), but still the conventional treatment method is used processing of waste or the generation of activated sludge. Although the application of microalgae

in the wastewater industry is still fairly limited, algae are used throughout the world for wastewater treatment on a relatively minor scale. This is either through the use of conventional oxidation (stabilization) ponds or the more developed suspended algal pond systems such as high-rate algal ponds which are shallow raceway-type oxidation ponds with mechanical mixing, and it was shown to be highly effective for wastewater treatment (Green et al., 1995; Hoffmann, 1998).

A major necessity of wastewater treatment is the need to remove high concentrations of nutrients especially N and P, which otherwise can lead to risks of eutrophication if these nutrients accumulate in rivers and lakes. P is especially difficult to remove from wastewater. P is precipitated from the effluent with the use of chemicals to form a solid insoluble fraction or is converted into an activated sludge by microbial activity in most commercial wastewater processing (Hoffmann, 1998). However, the P recovered by these methods is not fully recyclable and the P precipitate is either buried in landfill or further treated to generate sludge fertilizer. Microalgae are effective in removing N, P and toxic metals from wastewater (Ahluwalia and Goyal, 2007; Mallick, 2002) and thus they have potential to play an important remediation role especially during the final (tertiary) treatment phase of wastewater. Indeed algae-based treatments are found to be as efficient at removing P from wastewater as compared to chemical treatment (Hoffmann, 1998). The significant advantage of algal processes in wastewater treatment over the conventional chemical-based treatment methods is the potential decrease in the cost and requirement of low level technology usage. Thus, this approach more attractive to developing countries. For example, the significant O_2 generation from photosynthetic microalgae will negate the need for aeration and therefore high operational cost of mechanical aeration of the treatment pond is reduced if used mix culture with heterotrophic aerobic bacteria (Mallick, 2002). Efficient bioremediation of organic and inorganic compounds by heterotrophic aerobic bacteria to be ensured by oxygenation of treatment ponds (Munoz and Guieysse, 2006). Furthermore, an algal method of remediation is more environmentally plausible and sustainable as it does not generate additional pollutants such as sludge byproducts and provides an opportunity for efficient recycling of nutrients. For example, recovered N and P-rich algal biomass can be used as low-cost fertiliser or as animal feed (Munoz and Guieysse, 2006; Wilkie and Mulbry, 2002).

Most of the research on algal wastewater treatment has come from the analysis of laboratory-based small scale and pilot pond scale cultures, and from experimental high-rate algal ponds. A wide range of studies have analysed the growth of microalgae under a variety of wastewater conditions, mainly growth in municipal (urban) sewage wastewater and agricultural manure wastewater. These studies have principally been focused on evaluating the potential of algae for removing N and P, and in some instances metals from wastewater. These initial experimental studies, particularly those that have also assessed variables for maximal algal biomass production and methods for harvesting algal biomass from wastewater, will be of significant benefit for the evaluation of wastewater-grown microalgae as a biofuel.

3. METARIALS AND METHODS

3.1 Metarials

3.1.1 Synechocystis sp. PCC 6803 Culture Media

In this work BG-11 medium used as culture media to grow *Synechocystis sp. PCC 6803* on it. Medium BG-11 is used for strains of freshwater, soil or thermal origin, and for those isolated from a marine environment which do not display the ionic requirements characteristic of true marine strains (Waterbury & Stanier, 1981).

Composition of BG-11 medium given in table 3.1.

Table 3.1: Composition of medium BG-11.

Ingredient	Concentration	
	g. L-1	mM
$NaNO_3$	1.5	17.67
K_2HPO_4. $3H_2O$	0.04	0.18
$MgSO_4$. $7H_2O$	0.075	0.30
$CaCl_2$. $2H_2O$	0.036	0.25
Citric acid	0.006	0.029
Ferric ammonium citrate	0.006	0.030
EDTA K_2 Mg. $2H_2O$	0.001	0.0024
Na_2CO_3	0.04	0.38
Trace Metal (see A5+Co)	Add 1mL	
Deionized water	To 1 L	

Composition of of Trace metal A5+Co given in table 3.2.

Table 3.2: Composition of Trace metal A5+Co.

Ingredient	Concentration
	g. L-1
H_3BO_3	2.86
$MnCl_2$. $4H_2O$	1.81
$ZnSO_4$. $7H_2O$	0.222
$Na2MoO_4$. $2H_2O$	0.390
$CuSO_4$. $5H_2O$	0.079
$Co(NO_3)_2$. $6H_2O$	0.0494
Deionized water	To 1 L

3.1.2 Chemicals

Chemical used in this work given in table 3.3.

Tablo 3.3: List of chemical used in this work.

Chemical	Company
DOTAP	Avanti Polar Lipids
DOPE	Avanti Polar Lipids
Kloroform	ReAgent
Tris	AppliChem
Kanamisin	Merck
Tripsin	Merck
PBS (tablet)	Merck
Ethanol	J.T.Baker
OPTİ-MEM	Clonagen
Methanol	J.T.Baker
Formaldehit	AppliChem
DAPI	Merck
H_3BO_3	Merck
$MnCl_2$. $4H_2O$	Merck
$ZnSO_4$. $7H_2O$	Merck
Na_2MoO_4. $2H_2O$	Merck
Na_2MoO_4. $2H_2O$	Merck
CuSO4. $5H_2O$	Merck
$Co(NO_3)_2$. $6H_2O$	Merck
$NaNO_3$	Merck
K_2HPO_4. $3H_2O$	Merck
$CaCl_2$. $2H_2O$	Merck
Citric acid	Merck
Ferric ammonium citrate	Merck
EDTA K_2 Mg. $2H_2O$	Merck
Na_2CO_3	Merck
n-Hexane	ReAgent

3.1.3 Laboratory Equipment

Laboratory Equipment used in this work given in table 3.4:

Table 3.4: List of equipment used in this work.

Laboratory Equipment	Firma / Model
Micropipettes	Biohit, m10, m100, m1000, m5000
pH-meter	İno-lab pH 720
Magnetic Shaker	Yellow Line MSH Basic
Laminar Flow	Faster BH-EN 2003
Fume	Waldner N-TA 1500x900-900
Deep Freeze (-80 °C)	New Burnswick Scientific U410 Premium
Fridge (-20 °C)	Bosch KIL38A41NE
Fridge (4 °C)	Beko 7125 A+
Vortex	Stuart Vortex mixer SA8
Orbital Mixer Incubator	Certomat S-2
Nanodrop	Thermo Scientific Nanodrop 2000
Sonicator	ELMA TranssonicTP690
Serological Pipettes	Axygen Scientifec, 5 ml, 10 ml, 25 ml
Pasteur Pipettes	İsolab Pasteur Pipette Glass-225 mm
Centrifuge (big)	Beckman Coulter Allegra 25 Centrifuge
Centrifuge (small)	ScanSpeed Scanspeed Mini Blue
Centrifuge (small)	CMS Harmony
Shaker	Thermo electron Corporation Forma orbital shaker
Rotary Evaporator	Heirdolp laborata 4000
Microscope	Leica TCS SP5 X
İnverted Microscope	Accu-scope 3032 Inverted BrightField Microscope

3.2 Methods

3.2.1 Strains, seed culture, and pre-treated landfill leachate

Synechocystis sp. PCC 6803 was ordered from The Pasteur Culture Collection of Cyanobacteria, which is located in Paris, France. Sample of *Synechocystis sp. PCC 6803* has reached in our hands as planted on solid media in a plastic tube.

The our strain were cultured at 32±2°C with a continuous white led illumination with intensity of 100-300 $\mu E/m^{-2}*s^{-1}$. Our cultures are aerated with air that aeration speed is 1 L/min.

The medium used in this study was the pre-treated landfill leachate collected from Kemerburgaz. Pre-treated landfill leachate was stored at room temperature, and had been used for growing *Synechocystis sp. PCC 68*03 in the study. The characteristic of

pre-treated landfill leachate to use to growth *Synechocystis sp. PCC 68*03 with different dilution rate given in table 3.5.

Table 3.5: Characteristic of pre-treated landfill leachate.

Cultivation Medium Characteristic Parameter	Non-diluted PLL	%20 PLL	%40 PLL	%60 PLL	%80 PLL
pH	8,08	7,92	7,96	7,96	7,96
Alkanite (mg/L CaCO3)	346	69,2	138,4	207,6	276,8
TKN (mg/L)	1005	201	402	603	804
Ammonium as N (mg/ml)	1380	126	252	378	504
Organic nitrogen (mg/L)	375	75	150	225	300
Ortaphosphate as P (mg/L)	0,41	0,082	0,164	0,246	0,328

3.2.2 Batch and semi-continuous cultivation conditions

Cultivation methods used in this study were described below:

- Cultivations were performed in BG-11 and leakage water diluted with different ratio in 500 ml volume under 24 h of continuous white led light with illumination intensity of 100-300 µE/m–2*s-1 at 32±2°C.
- An ambient air flow of 1000 ml/min was provided continuously to each bottle.
- The reactors were inoculated with *Synechocystis sp. PCC 6803* so that each had an initial optical density (OD) of 0.1 at 680 nm, and the final working volume was adjusted to 500 ml. Water loss due to evaporation was made up daily by adding culture media before sampling.
- Batch experiments were carried out for 21d with different leakage water loading ratios (20%, 40%, 60%, 80%, and 100%).
- The OD of each reactor was measured daily.
- At the beginning and the end of batch cultivation, samples were taken and centrifuged in a Sorvall RC 6 Plus centrifuge (Thermo Scientific, Waltham, MA, USA).

- The supernatants were subjected to TAN, TN and TP assays.
- At the end of cultivations, biomass pellets separated by the centrifugation were used for fatty acid analysis.
- Each bottle's growth rate, lipid productivity and removal efficeny of N and P was determined.
- The optimal leakage loading ratio was then determined based on the biomass and lipid productivity.
- All cultivations were performed in duplicate and average values were recorded.

3.2.3 Analytical methods

3.2.3.1 Obtaining the Growth Chart

The OD at 680 nm was measured with a Biomate 3 spectrophotometer (Thermo Fisher Scientific, Madison, WI, USA).

A linear relationship between the biomass concentration (ash-free dry weight, AFDW) and the OD_{680} was found. Biomass-OD_{680} standard curves were used to predict their biomass concentration (dry weight).

4 ml sample taking throughout each day 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.

3.2.3.2 Nitrogen Measurment

The sample volume for the total kjeldahl nitrogen mesasurment (TKN) and ammonia measurment is chosen according to the table below:

Table 3.6: Appropriate sample volume for different TKN concentration.

TKN in sample (mg/L)	Sample Volume (mL)
0-1	500
1-10	250
10-20	100
20-50	50
50-100	25

Table 3.7: Appropriate sample volume for different ammonia-N concentration.

NH_3-N in sample (mg/L)	Sample Volume (mL)
5-10	250
10-20	100
20-50	50
50-100	25

a) Digestion step of total kleldahl nitrogen

- For TKN measurment, appropriate sample volume chosed from the table above and added to the distillation flask.
- If the sample volume is smaller than 300 mL, diluted it up to 300 mL with distilled water.
- Then, added a few glass beads, carefully add 50 mL of digestion reagent.
- After mixing, heated under a fume hood to remove acid fumes.
- Samples boiled until the sample volume was reduced to about 25 to 50 mL and copious white fumes are observed. As digestion continues, colored or turbid samples was become transparent and pale green.
- After digestion, samples cooled and dilute to 300 mL with distilled water then mix.
- Then, carefully added sodium hydroxide- thiosulfate reagent.

b) Distilation

- For TKN measurment, after digestion, added 25 mL borate buffer solution and adjust to pH 9.5 with 6N NaOH using phenolphthalein indicator.
- For ammonia-N measurment, appropriate sample volume chosed from the table above and added to the distillation flask. Then, added 25 mL borate buffer solution and adjust to pH 9.5 with 6N NaOH using phenolphthalein indicator.
- After that, either TKN measurment or ammonia-N measurment same step was followed.
- Then, assemble the sample flask to the distillation apparatus.
- Next, samples distilled at a rate of 6 to 10 mL/min with the tip of the delivery tube below the surface of acid receiving solution.
- After distillation, we collected distillate in a 250-mL Erlenmeyer flask containing 50 mL indicator boric acid solution.

- Distillation was ended, when 250 ml sample is collected in 250-mL Erlenmeyer flask.

c) Titration step

- Titrate ammonia in distillate with standard 0.02 N H_2SO_4 titrant until indicator turns to a pale lavender.
- Carried a blank through all steps of the procedure and apply the necessary correction to the results.

3.2.3.3 Phosphorus Measurement

Total phosphorus were determined using a HACH 3900 spectrophotometer (Düsseldorf, Germany)

First of all, preliminary sample treatment was made. One drop phenolphthalein indicator was added to 100 mL sample containing not more than 200 µg P and free from color and turbidity. If the sample turns pink, add from the strong acid solution dropwise to remove the color. If more than 5 drops (0.25 mL) is required, take a smaller sample and dilute to 100 mL after first discharging the pink color with acid.

After that, color development was observed. Added, by thoroughly mixing after each addition, 4.0 mL molybdate reagent and 0.5 mL (10 drops) stannous chloride solution. We maintained constant that the temperature of the reagents and samples are constant and between 20°C and 30°C throughout the experiment because temperature affects both the rate and intensity of color development.

Next, color of the sample were measured 10 min later (but before 12 minutes) after the color development, and by using the same specific interval for all the measurements/analyses determinations, record the absorbance (color) at 690 nm wavelength in 1 cm pathlength cuvette and compare with a calibration curve, using distilled water as the sample blank.

Always run a blank on reagents and distilled water. Because the color first develops progressively and later fades, maintain equal timing conditions for samples and standards. Prepare at least one standard with each set of sample or once each day the tests are made. The calibration curve may deviate from a straight line at the upper concentrations of the 0.3 to 2.0 mg/L P range. Prepare individual calibration curves from a series of six standards within the phosphate ranges indicated above. Plot absorbance vs mg P.

4. RESULTS AND DISCUSSION

4.1 Algal growth

Synechocystis sp. PCC 6803 strain grown on mediums, of which dilution rate differ from each other, for 21 days. Growths of the culture was determined via optical density measurement. For each different medium, which has different dilution rate, optical density time graps was prepared.

Before giving graps of cultication of *Synechocystis sp. PCC 6803* on PLL with different dilution rate, *Synechocystis sp. PCC 6803* growth on synthetic medium BG - 11 must be given. In this medium, *Synechocystis sp. PCC 6803* or many other type of algae has high growth rate. To determine the capacity of pre-treated landfill leachate to fulfill our aims, growth rate of *Synechocystis sp. PCC 6803* on BG – 11 must be compared.

Avarage optical density change during cultication of *Synechocystis sp. PCC 6803* on BG – 11 given in "Figure 4.1". Cultivation was maintained 350 hours, until the culture reach the stationary phase. Maximum OD_{680} value which was seen is 11. This amount roughly equal the 2 g dry weight/L. This value is the optimal value which is given literature (Chisti, 2007). Only 23 hours lag phase was seen. This little lag phase may caused by majority of the cells at the time when they were transferred to the culture media were already in stationary phase. Spesific growth rate of culture was calculated as a 0,01544017 and with help of this value, doubling time of the culture was calculated as 44,8924618 hours. Even if the maximum OD_{680} value is high, growth rate of culture was lower than other value which is given in literature. Mean doubling time of *Synechocystis sp. PCC 6803* on BG – 11 is given 7-14 hours in literature, moreover some work, shows that this value can as low as 5,14 hours. After the finalized the culture, orthophosphate value was measured but orthophosphate was not detected.

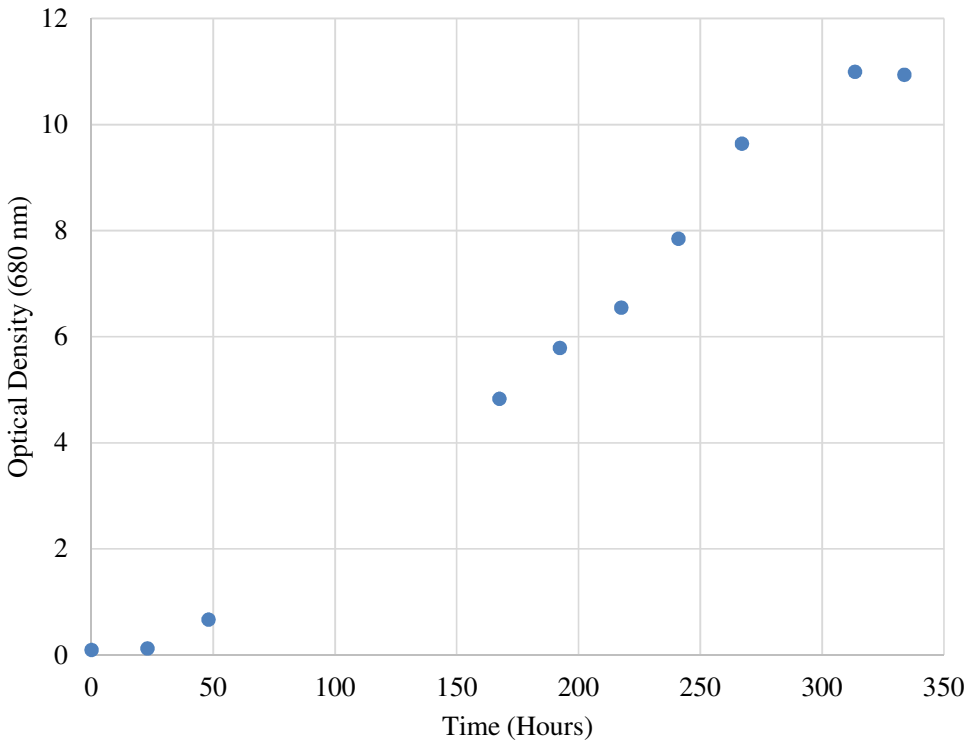


Figure 4.1: Avarage optical density change during cultication of *Synechocystis sp. PCC 6803* with synthetic medium, BG – 11.

Avarage optical density change during cultication of *Synechocystis sp. PCC 6803* on %20 pre-treated leachate water given in "figure 4.2". Cultivation was maintained 500 hours. Culture and other culture was ended simultaneously, not the culture reach stationary phase. Maximum OD_{680} value which was seen is 1,816. This amount roughly equal the 316 mg dry weight/L. Lag phase did not observed. It is thought that there are reason of this. First of all, this caused by majority of the cells at the time when they were transferred to the culture media (and other dilution of pre-treated landfill leachate) were already in log phase. Second of all, conditions of medium are somewhat similir to BG-11, which seeds taken from. However, if culture conditions is similar, a question that why the maximum OD_{680} value is limited must be answered. Spesific growth rate of culture was calculated as a 0,007537702 and with help of this value, doubling time of the culture was calculated as 91,95736322 hours.

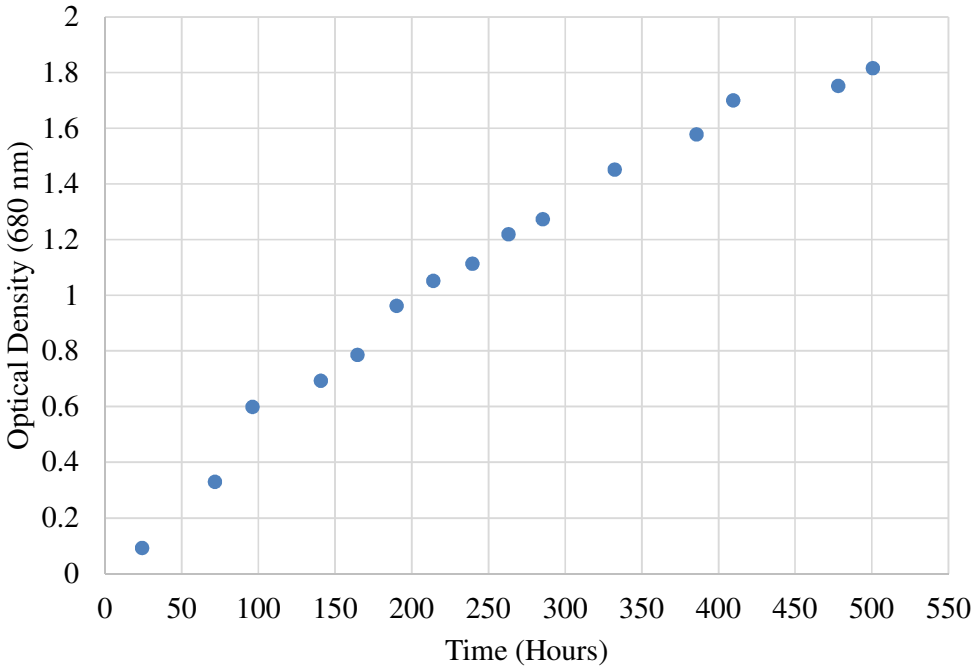


Figure 4.2: Avarage optical density change during cultication of *Synechocystis sp. PCC 6803* with %20 PLL.

Avarage optical density change during cultication of *Synechocystis sp. PCC 6803* on %20 pre-treated leachate water given in "figure 4.3". Between 4 and 6 days (80-130 hours) of lag phase was seen. The exponential growth in culture media lasted for 13–15 days, followed by a stationary phase until the finisiatition of culture. Maximum OD_{680} value which was seen is 1,0893. This amount roughly equal the 189,784 mg dry weight/L. Spesific growth rate of culture was calculated as a 0,00887 h^{-1} and with help of this value, doubling time of the culture was calculated as 91,95736322 hours. While the culture was grown, some reaction, which caused by stress, of *Synechocystis sp. PCC 6803* was observed. Cell of *Synechocystis sp. PCC 6803* was pelleted, but these pellet are not observed all time. This pellet is small and some of them can stay suspended in water. From time to time they formed and then they disappeared. However, must of the time of growth and end of the cultivation these pellet was observed.

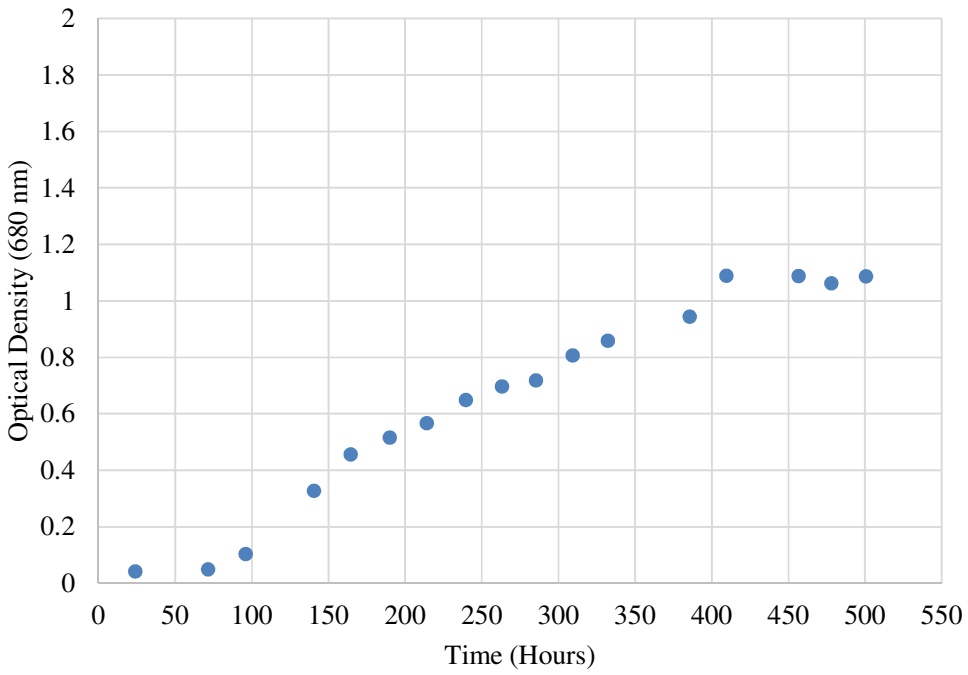


Figure 4.3: Avarage optical density change during cultication of *Synechocystis sp. PCC 6803* with 40% PLL.

Figure 4.4 shows the growth curves of cultication of *Synechocystis sp. PCC 6803* on %60 pre-treated landfill leachate. Between 3 and 4 days (60-90 hours) of lag phase was seen. The exponential growth in culture media lasted for 12–13 days, followed by a stationary phase until the finisiatition of culture. Maximum OD_{680} value which was seen in log phase is 0,749333333. This amount roughly equal the 141,9312267 g dry weight/L. Spesific growth rate of culture was calculated as a 0,009373757 and with help of this value, doubling time of the culture was calculated as 73,945506 hours. While the culture was grown, it is obserev that cells of of *Synechocystis sp. PCC 6803* was pelleted like cells of *Synechocystis sp. PCC 6803,* which growth in 40% pre-treated landfill leachate with one little modifications. Amoun of these flocculations was higher than amount of flocculation, which found in 40% pre-treated landfill leachate.

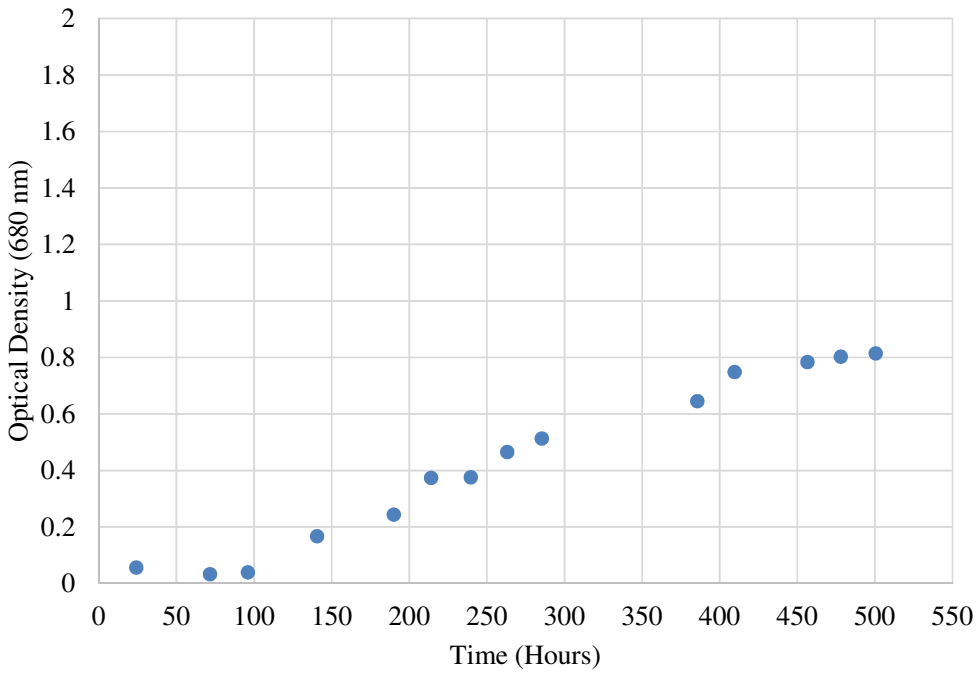


Figure 4.4: Avarage optical density change during cultication of Synechocystis sp. PCC 6803 with %60 PLL.

Avarage optical density change during cultication of *Synechocystis sp. PCC 6803* on %80 pre-treated leachate water given in "figure 4.5". Very long lag phase have been observed. Lag phase last between 3 and 4 days (60-90 hours). Howver, the exponential growth in culture media lasted for only 5-6 days, followed by a stationary phase until the finisiatition of culture. Maximum OD_{680} value which was seen in log phase is 0,578. This amount roughly equal the 100,69916g dry weight/L. Spesific growth rate of culture was calculated as a 0,014088404 and with help of this value, doubling time of the culture was calculated as 49,19983835 hours. Even if the culture has lowest maximum OD_{680} value, doubling time, which calculated by only using log phase data, is very close the doubling time of *Synechocystis sp. PCC 6803* which grown in BG - 11. In addition, all the time of cultivation, all cells of *Synechocystis sp. PCC 6803* flocculate at the bottom of erlenmayer flash. Even if the flash shake vigoruly they settled after some time. Even if, color of medium not clealy green.

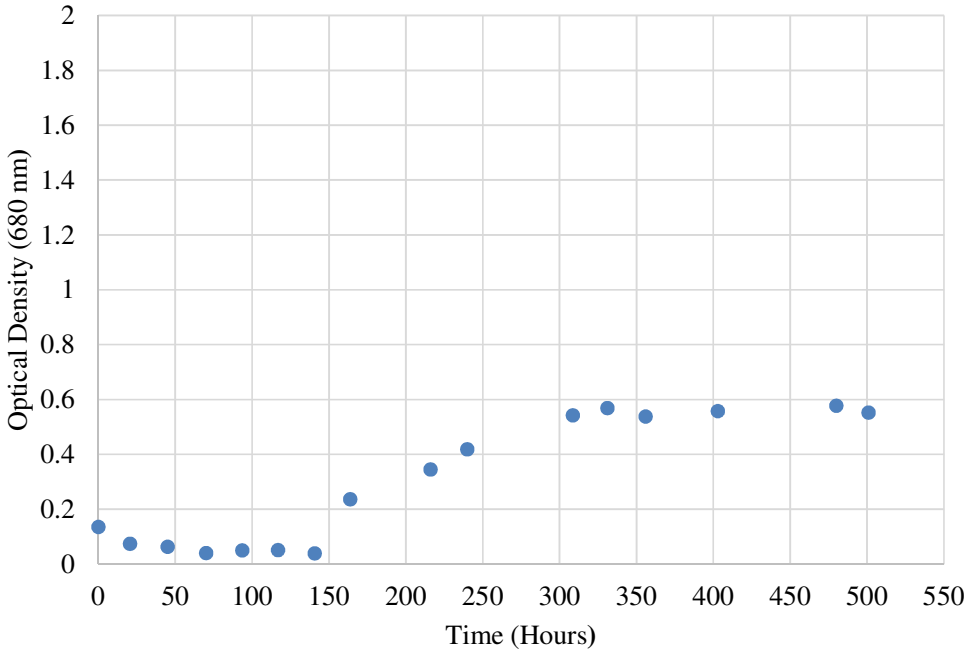


Figure 4.5: Avarage optical density change during cultication of *Synechocystis sp. PCC 6803* with %80 PLL.

In figure 4.6, comparasion of the growth curves of *Synechocystis sp. PCC 6803* on different pre-treated landfill leachate can be seen.

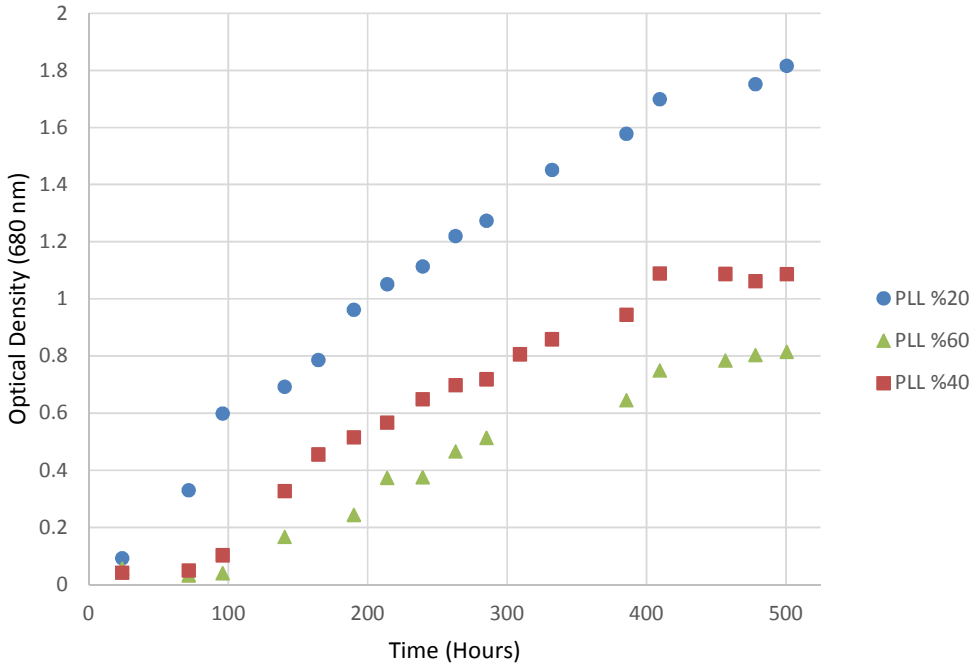


Figure 4.6: Comporision of the curves of *Synechocystis sp. PCC 6803* growth on different PLL dilution.

In table 4.1, Comporasion of the value of the spesific growth rate and doubling time of of *Synechocystis sp. PCC 6803* on PLL with different dilution rate and BG-11 medium

Table 4.1: Comporasion of the value of the spesific growth rate and doubling time of of *Synechocystis sp. PCC 6803* with different cultivation medium.

	20% PLL	40% PLL	60% PLL	80% PLL	BG - 11
μ ($Hours^{-1}$)	0,00754	0,00887	0,00937	0,01409	0,01544
Doubling Time (Hours)	91,96	78,35	73,95	49,20	44,89

To determine expected biomass value, optical density-TSS grap was prepared In figure 4.7, optical density (680 nm)-TSS (mg/L) can seen. According to data, relation between optical density-TSS was found as y=175,35x-0,645. In here, x define optical density value and y define TSS value.

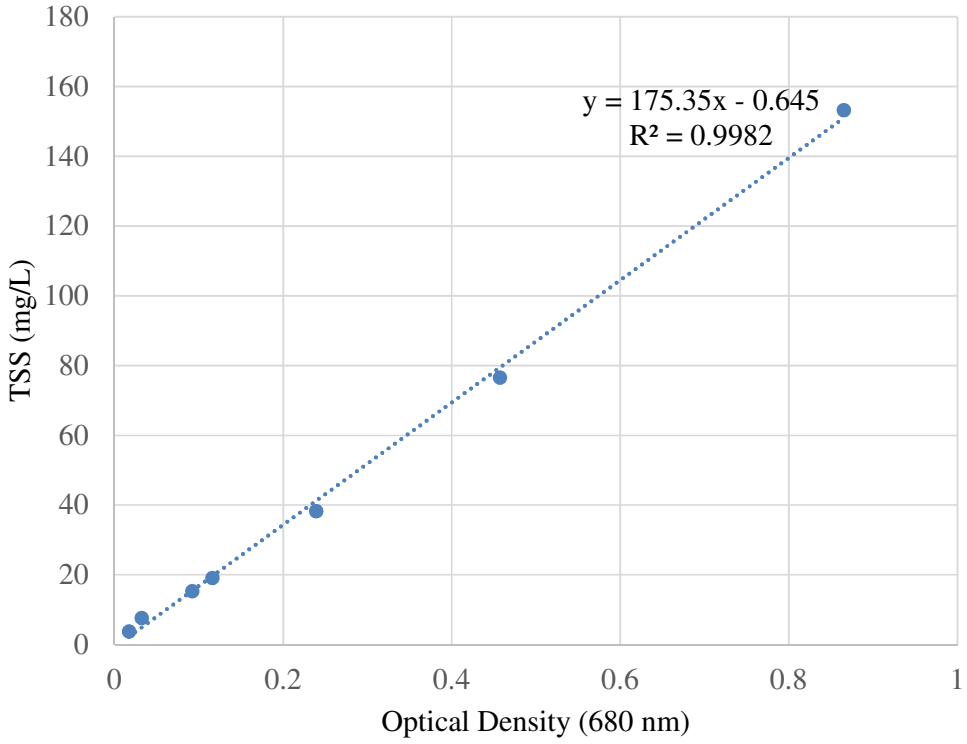


Figure 4.7: Total suspended solids change according to different optical density (680 nm) value.

In table 4.2, maximum TSS value which can obtained from medim after the termination can seen. In addition, expected TSS value of BG-11 can seen to comparation purpose. Maxsimum TSS value which can obtained from medim after the termination was calculated by helping of equation which give above.

Table 4.2: Expected TSS value of mediums after the terminated according to formula given figure 4.7

	%20 PLL	%40 PLL	%60 PLL	%80 PLL	BG -11
VSS (mg/L)	317,7906	190,3696	142,2068	101,3523	1928,205

4.2 Characteristic of Medium After Algal Cultivation

After 500 hours incubation, changing in the parameter of the cultivation medium is given tablo 4.2.

Table 4.3: Cultivation medium characteristics after the 500 hours cultivation of *Synechocystis sp. PCC 6803.*

Cultivation Medium Characteristic Parameter	%20 PLL	%40 PLL	%60 PLL	%80 PLL
TSS (g/L)	0,52	0,665	0,74	0,665
VSS (g/L)	0,34	0,37	0,37	0,2
Fixed Solids (g/l)	0,18	0,295	0,37	0,59
pH	6,44	6,64	6,75	4,68
Alkanite (mg/L $CaCO_3$)	64	53	48	ND
TKN (mg/L)	97	223	358	642
Ammonium as N (mg/ml)	90	203	321	466
Organic nitrogen (mg/L)	7	20	37	176
Ortaphosphate as P (mg/L)	0,006	0,007	0,027	0,039

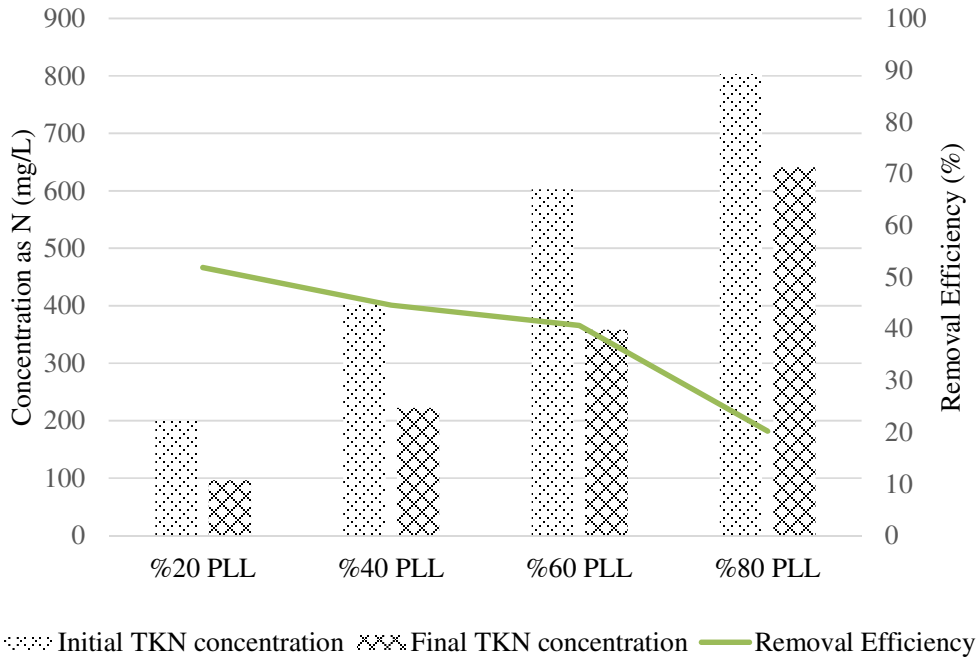


Figure 4.8: Comparasion of initail and final TKN concentration of cultivation medium of *Synechocystis sp. PCC 6803*.

In figure 4.7, comparasion of initail and final TKN concentration of cultivation medium of *Synechocystis sp. PCC 6803* can be seen. According to our data, with increased concentration of pre-treated landfill leachate TKN removal efficeny was drop. Especially, medium %80 PLL, removal efficeny drop significanly. Removal efficeny of %20 PLL, %40 PLL, %60 PLL and %80 PLL are about %51, %44, %40 and %20, respectively.

In figure 4.8, comparasion of initail and final ammonia-N concentration of cultivation medium of *Synechocystis sp. PCC 6803* can be seen. According to our data, with increased concentration of pre-treated landfill leachate ammonia-N removal efficeny was drop. However, ther is no quick drop observed from grap. Removal efficeny of %20 PLL, %40 PLL, %60 PLL and %80 PLL are about %28, %19, %14 and %7, respectively.

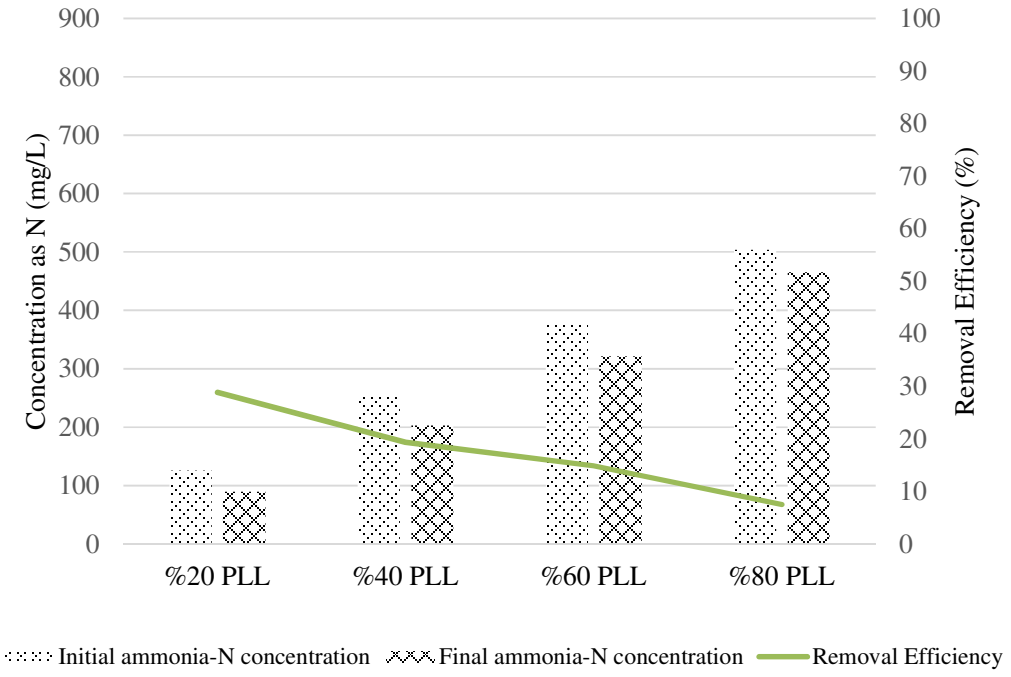


Figure 4.9: Comparasion of inital and final ammonia-N concentration concentration of the cultivation medium of *Synechocystis sp. PCC 6803*.

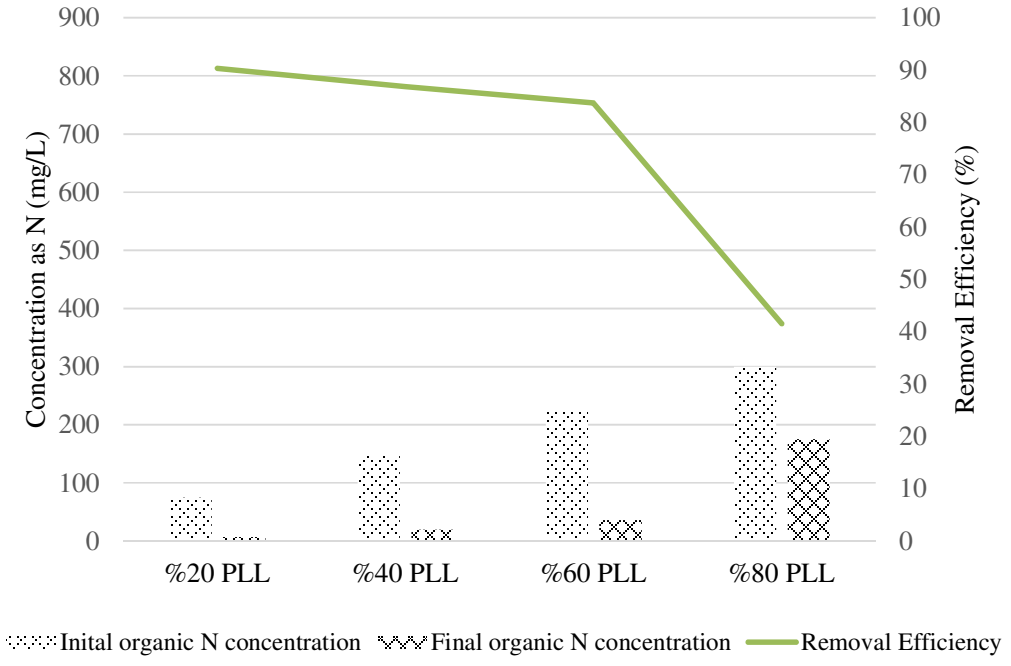
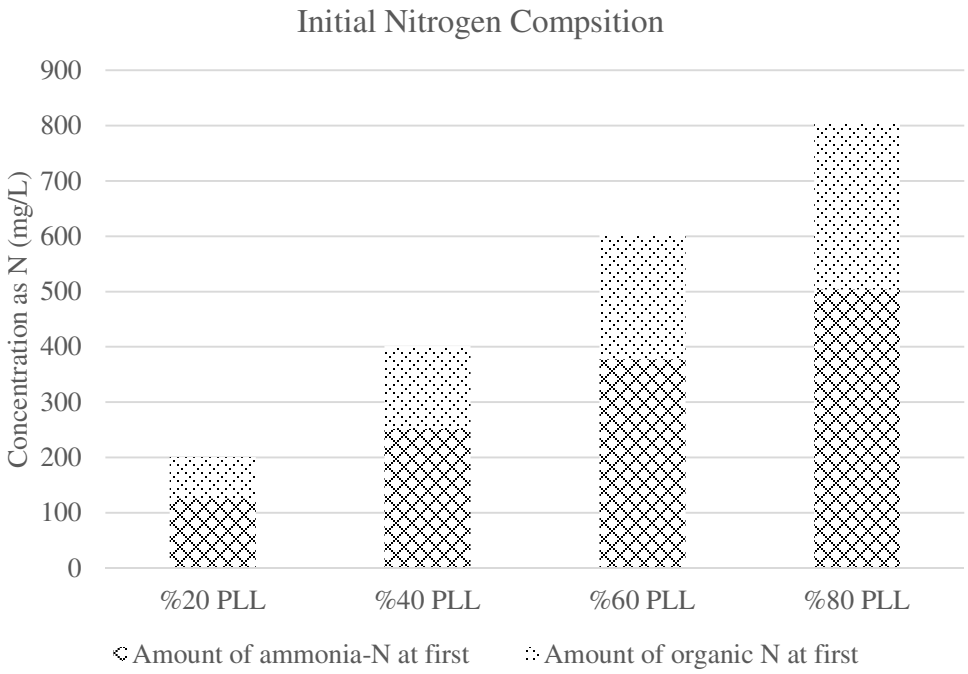


Figure 4.10: Comparasion of initial and final organic nitrogen concentration of the cultivation medium of *Synechocystis sp. PCC 6803*.

In figure 4.9, comparasion of initail and final organic N concentration of cultivation medium of *Synechocystis sp. PCC 6803* can be seen. According to our data, with increased concentration of pre-treated landfill leachate organic N removal efficeny was drop. Especially, medium %80 PLL, removal efficeny drop significanly. Removal efficeny of %20 PLL, %40 PLL, %60 PLL and %80 PLL are about %90, %86, %83 and %41, respectively.

In figure 4.10, comparasion of inital and final nitrogen composition of cultivation medium of *Synechocystis sp. PCC 6803*. Changing the rate of the organic nitrogen to ammonia-N can be seen clearly. According to our data, with increased concentration of pre-treated landfill leachate rate of N species in the medium was changed. Especially, N spices composition of medium %20 PLL is significanltly differ from intial value. However, N spices composition of medium %80 PLL is much similar the initail N spices compositions. Initail ratio of ammonia-N/TKN value is about %62 and initail ratio of organic-N/TKN value is about %38. However after the 21 day incubation, ratios was changed. Final ratio of ammonia-N/TKN value is %92, %91, %89 and %72 for %20 PLL, %40 PLL, %60 PLL and %80 PLL respectively. Final ratio of organic-N/TKN value is %8, %9, %11 and %28 for %20 PLL, %40 PLL, %60 PLL and %80 PLL respectively.

In figure 4.11, comparasion of inital and final orthophosphate concentration concentration of the cultivation medium of *Synechocystis sp. PCC 6803*. We know that our initail P concentration was low. We think that culture may be limited by phosphorus limitation. We saw that temperory chlorosis in the culture. Chlorosis is the indicator of the nutrient limitations. However, according to data given the literature even if the PCC 6803 fed with only 0,1674 mg/L can reach OD_{730} 0,9. In addition to this, with increased PLL ratio and increased P concentration naturally, there is no significant growth increase not observed. Removal efficeny of orthophosphate of medium %20 PLL, %40 PLL, %60 PLL and %80 PLL are about %94, %96, %89 and %88, respectively.



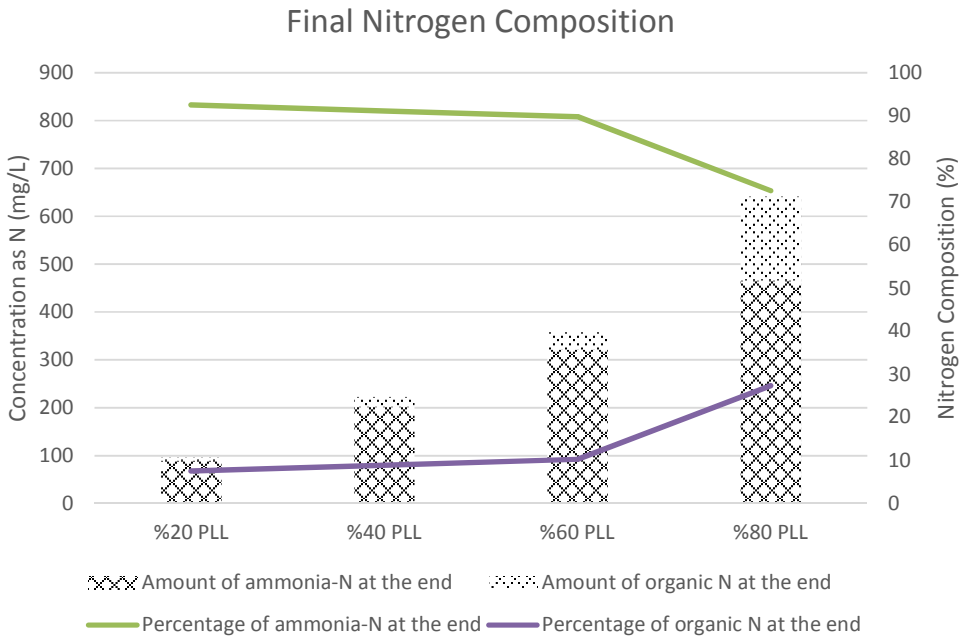


Figure 4.11: Comparasion of inital and final nitrogen composition of cultivation medium of *Synechocystis sp. PCC 6803*. Changing the rate of the organic nitrogen to ammonia-N can be seen clearly.

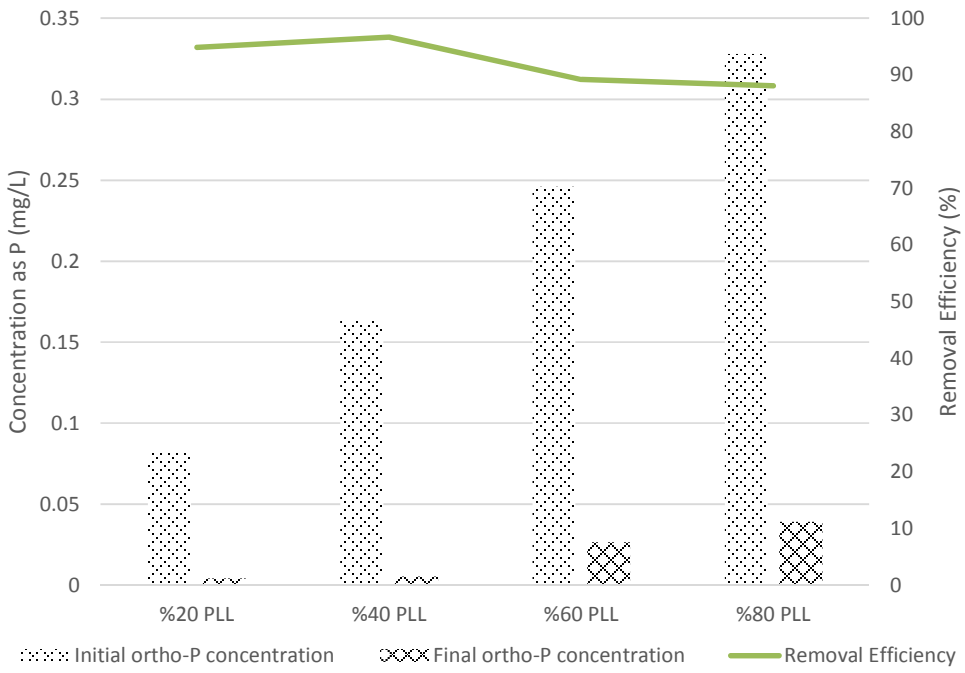


Figure 4.12: Comparasion of inital and final orthophosphate concentration concentration of the cultivation medium of *Synechocystis sp. PCC 6803*.

5. CONCLUSIONS AND RECOMMENDATIONS

Our data shows that our spices can not grow well in pre-treated landfill leachate water. These are main reason which thought by us:

- Low phosphorus concentration of pre-treated landfill leachate
- High ammonia concentration of pre-treated landfill leachate
- Weak sturucture *Synechocystis sp. PCC 6803*. Our culture was not accilimate pre-treated landfill leachate. Also, it is know that laboratory starin is fragile versus harsh conditions.
- Heterotrophic organisms contamination.

To overcome to this problem this approach may be applied

- Culture media, which contain appropriate amount and type of nutrient for *Synechocystis sp. PCC 6803* can be used to growth *Synechocystis sp. PCC 6803*
- *Synechocystis sp. PCC 6803* can be accimalate to pre-treated landfill leachate via natural or artificial methods.
- To prevent contaminations, sterile conditions can be achieved

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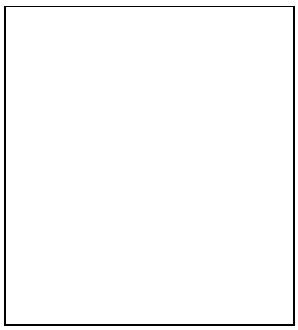

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