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INVESTIGATION OF DIFFERENT MEDIUM ADDITIVES FOR MICROALGAE GROWTH

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LIST OF SYMBOLS

N_Re	Reynold's number
V	Fluid velocity (m/s)
P	Fluid density (kg/L)
μ	Fluid viscosity (m2/s)
N	Impeller speed (rpm)
Ne^'	Modified newton number in shaken flask
t_m	Mixing time (s)
g	Gravity (m/s2)
D_i	Impeller diameter (m)
D	Tank diameter (m)
Y	Depth of the liquid (m)
OD	Optical Density
OUR	Oxygen Uptake Rate
CDW	Cell Dry Weight (g)
C_L	Dissolved oxygen concentration
k_L	Local mass transfer coefficient
a	Specific interfacial area (m2)

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ABSTRACT

INVESTIGATION OF MEDIOM ADDITIVES FOR MICROALGAE GROWTH

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MSc. Thesis

Adviser: Assoc. Prof. Dr. Didem ÖZÇİMEN

Microalgae are a promising source of biofuels and other valuable chemicals. The low cell density and slow growth rate that have traditionally characterized microalgal cultures, however, have resulted in a reduced economic feasibility. To develop a sustainable microalgal process it is required to increase culture productivity, maximize production yield, and reduce production costs. To achieve these goals it is necessary to improve the current understanding of the dynamic behavior of microalgal cultures.In this thesis, investigation of different medium additives for microalgae growth was performed.

For this case, *C.vulgaris* and *C.minutissima* microalgae species were used with separately culture. The temperature, light source, mixing rate and pH values were reached in literature and fixed in each strains. Also the medium was preferred Bold Basal Medium according to literature. On the other hand, different type of three extract water were used for medium additives which are Beech, Oak wood extract water mixture and Iroko wood extract water.

These three extract waters were added in BBM solution with different concentration. The aim of this project was understanding the effects of wood extract water on microalgae production. For this case, biomass growing and glycose production were observing during process. All optical density data were measured and evaluated after finishing the process.

Also scale-up process was performed using obtained data in shaker flask production. Then, scale-up strategies were determined and applied experimentally with using calculations. As a result, experimentally perspective on different kind of medium additives is developed.

Key words: Microalgae, *C.vlgaris*, *C.minutissima*, medium additive, wood extract water, production characterization, scale-up process.

ÖZET

MİKROALG ÜRETİMİNDE FARKLI ORTAM KATKI MADDELERİNİN İNCELENMESİ

Burak Özgür DEMİRER

Biyomühendislik Bölümü

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Mikroalgler, biyoyakıt ve diğer değerli kimyasalların gelecek vaat eden bir kaynağıdır. Geleneksel mikroalg kültür üretimi, düşük hücre yoğunluğu ve yavaş büyüme oranı nedeni ile ekonomik açıdan uygun değildi, ancak karakterizasyon yöntemleri sayesinde, kültür verimliliğini artırmak, üretim verimini en üst düzeye çıkarmak ve üretim maliyetlerini azaltmak için gerekli olan sürdürülebilir bir mikroalg üretimi sağlanmaktadır. Bu hedeflere ulaşmak için mikroalg kültürlerinin dinamik davranışının mevcut anlayışını geliştirmek gereklidir.

Bu tez çalışmasında, mikroalg üretimi için farklı ortam katkı maddelerinin büyümeye olan etkisi araştırılmıştır. Bunun için, *C.vulgaris* ve *C.minutissima* mikroalg türleri ayrı ortamlarda üretim için kullanılmıştır. Üretimlerde, sıcaklık, pH değeri, karıştırma hızı ve ışık kaynağı literatürde yapılan araştırmalara göre her üretimde sabit bir değer alınmıştır. Ayrıca üretim ortamı için 'Bold Basal Medium' tercih edilmiştir.

Diğer yandan, kayın ve meşe odun özü karışımı ve iroko ağacı özü ortam için katkı maddeleri olarak kullanılmıştır. Bu üç ağaç ekstre suyu farklı konsantrasyonlarda BBM çözeltisi ile karıştırılıp ortam oluşturulmuştur.Bu projenin amacı mikroalg üretiminde ağaç özü suyunun etkilerinin araştırılmasıdır. Bunun için, biyokütle büyümesi ve glikoz üretim süreçleri gözlemlenmiştir. Üretim sürecinde belirli aralıklarla optik yoğunluk verileri ölçülmüş ve üretim sonunda bu elde edilen değerler ışığında farklı ortam katkı maddelerinin mikroalg üretimine etkisi konusunda deneysel olarak farklı bir perspektif geliştirilmiştir.

Ayrıca, elde edilen sonuçları kullanarak ölçek büyütme stratejisi belirlenmiştir. Elde edilen teorik hesaplamalarla doğrultusunda, deneysel çalışmalar yapılmıştır.

Anahtar Kelimeler: Mikroalg, *C.vulgaris, C.minutissima,* ağaç özütü suyu, üretim karakterizasyonu, ölçek büyütme yöntemleri.

CHAPTER 1

INTRODUCTION

1.1 Literature Rewiew

Commercial microalgae production is an industry based on solid foundations. Different designs of closed photobioreactors that will provide commercial production of new species and the production of such commercial microalgae has shown significant improvement in recent years. This design can also be seen to be commercially viable in the near future foreground. Closed systems located within the tubular photobioreactors, in various forms, is composed of transparent tubes or hoses of different sizes and lengths. These types of bioreactors, tube diameter suitable for 2 - to 4 cm [1].

Despite the success of *Spirulina* and *Dunaliella* as a selective growth medium open in the cultured microalgae stack culture system *Haemetococcus* and *Phaeodactylum* as commercially used but the production in a closed system microalgal species not having selective environments are more successful. But high production costs have prevented these systems until recently, and commercial applications. The basic principle of the flat panel reactor and tubular photobioreactor design; by cells being cultured is to provide more efficient utilization of light.

Thus, the accumulation of harmful gases away from the culture system by providing an appropriate mix and the benefit of the cells more efficiently than light, it is possible to achieve higher biomass density. Consequently, this system is suitable to run in continuous production. Maximum intensity of the continuous production of algae and growth medium conditions, depending on the effective use of production allows the ability to determine in advance. Thus, the total costs are reduced and the smaller space is needed [2].

1.2 Objective of the Thesis

This research aims to investigate the effect of wood extract water on the production of microalgae (*C.vulgaris* ve *C.minutissima)*. For this case, all optical density data were measured to observe biomass growing and glycose production during process. As a result, experimentally perspective on different kind of medium additives is developed. Scale-up strategies will determined and applied experimentally with using calculations.

1.3 Original Contribution

Although there are studies reported before about microalge production with different media additives, there is not any study on the investigation of the utilization of tree extract in medium. *C.vulgaris ve C.minutissima* microalgae species were affected in positive way by tree extract additive. The results have shown that the usage of tree extracts in algae medium will be helpful for microalgae product.

CHAPTER 2

MICROALGAE

Microalgae are microscopic algae which are generally found in freshwater and marine ecosystem. They are unicellular organism and can be observed in chain or group. Cell size can be changed according to its species between a few micrometers to hundreds micrometers. They can photosynthesis with their chlorophyll. Unlike higher plants, microalgae do not have any roots or stems. They can be adapted to the environment. That's why, they become approximately half of the total amount photosynthetic organism. Also huge rate of atmospheric oxygen is created from these microscopic factories. Because of their ability such as fast growing, high amount of nutrition content and low production costs, microalgae become a popular row material in industrial, medical, food and bioenergy areas [3,4].

2.1 Applications of Microalgae

The popularity of microalgae in commercial production is based on their potential virtually inexhaustible raw material. Due to there are millions of microalgae species, they can be used as a source in any commercial area. The first commercial and large-scale production of microalgae was performed in the 1960s in Japan. In this production, *Chlorella* culture was used to make a food additive. From that time to present day, production of microalgae is increased with an unprecedented level [5,6].

Table 2.1 Microalgal production of previous years [7].

Microalgae	Annual production	Producer county	Application and product	Price (€)
Spirulina	3000 tones dry weight	China, India, USA, Myanmar, Japan	Human Nutrition Animal Nutrition Cosmetics Phycobiliproteins	36 kg^{-1}
Chlorella	2000 tones dry weight	Taiwan, Germany, Japan	Human Nutrition Cosmetics Aquaculture	11 mg^{-1}
Dunalei salina	1200 tones dry weight	Australia, Israel, USA, Japan	Human Nutrition Cosmetics B-carotene	36 kg^{-1} 50 kg^{-1}
Aphanizamenon floss-aquae	500 tones dry weight	USA	Human Nutrition	215-2150 kg^{-1}
Haematococcus pluvialis	300 tones dry weight	USA, India, Israel	Aquaculture Astaxanthin	501 kg^{-1} 7150 kg^{-1}
Crypthecodininun cohnii	240 tones DHA oil	USA	DHA oil	43 g^{-1}
Shizochytrium	10 tones DHA oil	USA	DHA oil	43 $^{-1}$

2.1.1 Nutrition

Nowadays, nutrition of microalgae is very popular and marketed in different forms such as tablets and liquid for food additions for both human and animals. Because of their huge amount of lipid, protein and vitamins, microalgae biomass is become an important row material as nutrition source. Also their various chemical structure, they can act as a nutritional supplement or represent a source of natural food colorants [1,2]. The commercial applications are dominated by four strains: *Arthrospira, Chlorella, D. salina* and *Aphanizomenon flos-aquae*.

2.1.2 Cosmetics

Likely food industry, microalgae can also be used in this area. Some microalgal species produce curative components that are used in anti-agent items, regenerate cream, refreshing and face and skin care products. Microalgae extract has an important role for production of cosmetics because of their natural ability and low growing cost. Even they can be used in some sun protection product [8].

2.1.3 Bioenergy

It is understood that microalgae can be used as an energy source because rich content of lipid, glycose and carbon. Microalgae are started to use as an alternatively renewable energy source for diesel, gasoline, coal and flammable gases. Lipid content from microalgae biomass can be converted to biodiesel with transesterification process [9,10]. And also their carbohydrate content can be utilized for bioethanol production by fermentation process.

2.2 Microalgae Cultivation Systems

Cultivation is started with microalgae strain selection according to production aim. After selection of strain to obtain the product of interest, theoretical and practical production information is maintained in the production of light. There are two different part in this process. One of them is biomass production and other is harvesting [11].

In production part, cultivation area can be changed according to product quality and amount. In this case, there are two product area that can be explained which are open pound system and bioreactors. This areas volume also can be small or large which depends on production strategies and time periods of purpose. In this case, process designed is very important factor for reaching aim [12].

Before selecting cultivation area, media which microalgae grow, optimization should be determined for increasing biomass amount while degreasing process cost. This part is generally performed in small scale production in early process [13].

2.2.1 Media Optimization

To determine the content of media depends on literature researches and practical trials. First of all, selected microalgae strain growing media is determined in experiments and researches that has been done before. Then practical trials are started with considering the manufacturing cost. Some additional nutrition can be added into media and biomass growing rate or production volume target are observed and examined for if these rate are affected in good or bad ways. There are many sources such as, molasses, waste water and tree water have impact on production [14]. These items have some elements or components that can play a role directly

in growing pathways which are called enhancers. On the other hand, some of them can be act security which means protect microalgae from negative effects of substances on growing. Media optimization takes a long time and it is a process that requires patience but if we think of the importance of efficiency, this part of process is a deal values results. After media optimization, the process is continued with selection of production type and area system [15]

2.2.2 Open pond system

Open ponds are the simplest and low cost for mass cultivation for microalgae. Most large-scale, commercial microalgae biomass production is done in open ponds. It is a large open water raceway track where algae and nutrients are pumped around by a motorized paddle. The algae culture will grow continuously and part of the algae will be removed during the growing process. Carbon dioxide also has to be added to the pond for the use of microalgae. The algae culture will grow continuously and part of the algae will be removed during the growing process. The biggest advantage of these open ponds is their simplicity, yielding low production costs and low operating costs. However, not all algae species can be grown in these ponds, due to contamination of other algae and bacteria. Also the process conditions, like temperature and the amount of light, are hard or impossible to control [16].

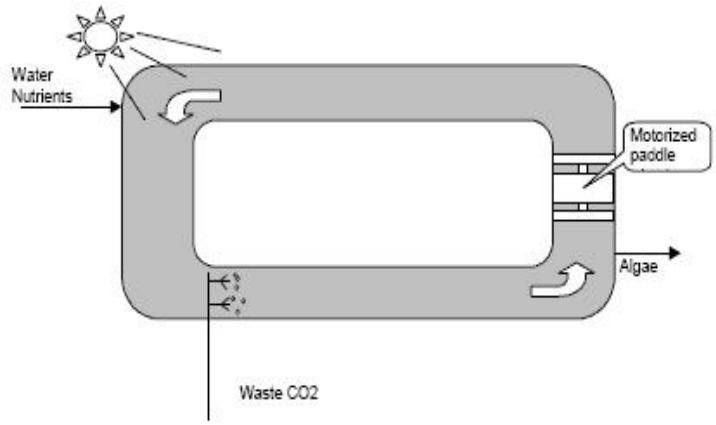
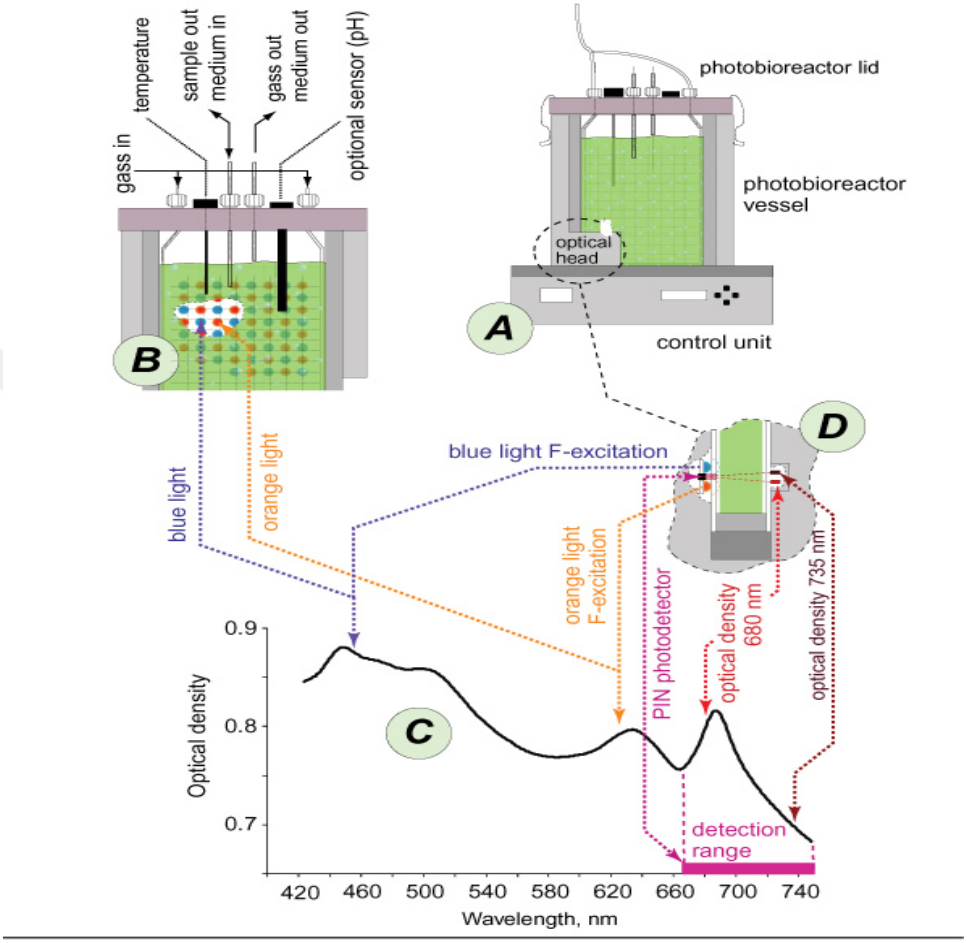


Figure 2.1 An open pond system for microalgae production [17].

2.2.3 Photobioreactors

Photobioreactor systems are more prevalent, primarily because they are well suited to small-scale production. They are most commonly used by government and university labs, and small projects for research and demonstration purposes [18]. Photobioreactor systems are closed, self-

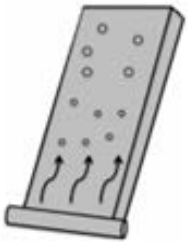
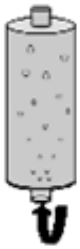
contained systems that continuously pump water containing algae through clear tubes, spheres or plastic bags of various sizes, where algae are exposed to light for growth. Because of they are closed system, control of production is easier and unexpected production environment changings are avoidable [19].



A: Photobioreactor configuration
B: Sensors and LEDs configuration
C: Measured spectrum
D: Detectors (optical head)

Figure 2.2 A simple photobioreactor for microalgae [20].

Table 2.2 Advantages and limitations of various microalgae culture systems [21].

Culture System	Advantage	Limitations
Open systems 	Relatively economical Easy to clean up Easy maintenance Utilization of non-agriculture land Low energy cost	Little control of culture conditions Poor mixing, light and CO_2 utilization Difficult to grow algal cultures for long periods Poor productivity Limited to few strains Cultures are easy contaminated
Tubular PBR 	Relatively cheap Large illumination surface area Suitable for outdoor culture Good biomass productivities	Gradients of pH, dissolved oxygen and CO_2 along the tubes Fouling Some degree of wall growth Requires large land space Photoinhibition
Flat PBR 	Relatively cheap Easy to clean up Large illumination surface area Suitable for outdoor culture Low power consumption Good biomass productivities Good light path Readily tempered Low oxygen build-up Shortest oxygen path	Difficult scale-up Difficult temperature control Some degree of wall growth Hydrodynamic stress to some algal strains Low photosynthetic efficiency
Column PBR 	Low power consumption Readily tempered High mass transfer Good mixing Best exposure to light-dark cycles Low shear stress Easy to sterilize Reduced photoinhibition Reduced photo-oxidation High photosynthetic efficiency	Small illumination surface area Sophisticated construction materials Shear stress to algal cultures Decrease of illumination surface area upon scale-up Expensive compared to open ponds Support cost Modest scalability

CHAPTER 3

SCALE-UP PROCESS

The term of scale-up is that increasing the quantification of production without losing qualification of product. This process can be classified into three main classes which are small scale pilot scale and large scale. In industrial production, defined product is firstly progress in small amount. This basic production result gives an idea to producer with optimization for maximum product amount or minimum requirement for production. After determination of some production rates and results depending on purpose, usage area and using equipment & materials, production volume is increased from first small volume production with some intermediate scale to the main production volume. During this process, calculated rates at first production are measured and controlled for each intermediate scale and this experimental progress is followed until the final volume reached before the main production. This last production volume before the main scale is called pilot plant. In industry, pilot plant production and its results are the main determination factors for the production is acceptable depend on economical, marketing rate and viability cases of projects [22].

In biological production, scale-up process is more complicated when compare with others process. After the determination of product and microorganism, media optimization for biomass growth and production quality is determined and measured in the lab scale. Then scale-up strategies is determined. These strategies can be driven as many parameters changing or stabilizing. Generally, only one or two parameter is fixed for each production volume and depends on results, strategy is changed or applied for main production. Scale-up parameters are not only associated with microorganism but also with reactor type. These parameters are determined and calculated before the reactor running, then production is performed in a following of calculated values [23].

3.1 Scale-up Process Characterization

For reaching the optimum design and suitable scale-up approach, we must transfer the rates and values directly from lab scale to large scale. There are two mainly conditions should be provided which are;

- Geometrical shape in which fermentation takes place should be similar or near each other to get optimum result for both culture growing stagnant and product quality.
- Dynamic similarity of flow fields that determined ratio or value must be the same with both scale.

The first case is easy to accomplish but the second is more complex than the other. Also these parameters need to be calculated meticulously depends on culture type. If the chosen cell is sensitive for shear like animal cell culture, power consumption and impeller tip speed approach cannot be used for scale-up process because the force that is acted to fluid may give a harm on cell and both product level and biomass are degreased. On the other hand, if the cell that you choose for production, grow in high O_2 concentration condition, the oxygen transfer rate must be increased but at this time, in one case reactor cannot supply the calculated rate and other case, foam formation can occur which causes heterogeneity of medium [24].

Some proteins can be moved up from to this foam layer and amount of product quality losses it' stability. To prevent this foam formation, some chemical solvent and oil based solutions are present but these solutions mostly influence culture growing. On the other hand, mechanical foam breaker is used instead of chemical solvents. However, sterilization problems crop up during preparation reactor for following studies.

Briefly cell type, cell requirement for growing and reactor type play an important role for determining of scale-up strategies. Also, the best way that you consider all of cases and calculated in theoretically may not give an expected result in practical. In this case, more than one suitable parameter are fixed and tried on different small volume of reactor theoretically. This shows that scale-up process has a multidisciplinary formation and determination of parameter part must be worked with both theoretically and practically in real time [25].

3.2 Scale-up Parameters

3.2.1 Reynold's Number

Dimensionless numbers are often used in scale-up calculations in industrial fermentation process since this process is more complicated. In this parameter, calculation may not be satisfy so this parameter calculation is used for co-calculation to increase successful scale-up design. One of the most important and well known dimensionless number is Reynold's which is defined for fluid flow through the pipe as:

$$N_{Re} = \frac{D\,V\,\rho}{\mu} \tag{3.1}$$

Where; N_{Re} is the Reynold's number

D is the pipe diameter

V is the fluid velocity

ρ is the fluid density

μ is the fluid viscosity

Another Reynolds number formulation exists for a stirred tank and lab scale calculation that is more suitable than other equation for scale-up design. The terms that ρ and μ can be good choices for fermentation properties, but the problem start with selecting of D and V values. In lab scale production, either diameter of Erlenmeyer flask is used for D nor diameter of horizontal rotational for flask which is done by shaker [26].

On the other hand, there is some quest in reactor fermentation in usage of V value. The rotation of the impeller per second is chosen even though it is not a velocity of fluid. The equation of Reynold's number in scale-up can be defined as:

$$N_{Re} = \frac{Di^2\,N\,\rho}{\mu} \tag{3.2}$$

Where; N is impeller speed (rpm)

3.2.2 Power Consumption (P/V)

All fermentation process requires for energy. This requirement must be calculated before the experiment and process is followed by this direction. Power consumption calculation is one of the most important and efficient parameter in scale-up strategies. This calculation based on a determined period which is called volumetric power consumption. P/V ratio defined as given energy for movement generation of fluid within a vessel in a given period of time. This ratio is

calculated with different ways which contains some parameters. It depends on used cell culture, media component for shear stress, process volume and reactor type.

In general, power consumption can be classified into two main section which are the power directly drawn by the fluid and requirement for energy losses such as friction and consumption by motors. This approach is in terms of overall power consumption. However, volumetric power consumption represents to fluid flow and media circulation. Power consumption parameter has been started to apply in scale-up process in early industrial penicillin production. In these years, constant P/V ratio was able to use in fermentations with low power input but this method is limited by microorganism such as recombinant *Escherichia coli* for requiring high energy input. Also, it encountered with high cost and shear stress in large scale stirred tanks.

To make power consumption in large scale, P/V ratio be set a constant before calculations and experiment. This value is gotten from in small scale fermentation process in laboratory. In laboratory scale, characterization and optimization of parameters are performed. One of the process parameters is chosen and scale-up calculation is started. The most of the power consumption in lab scale is worked by the group of Jochen Büchs at RWTH Aachen [27].

Power consumption in shaken flask is measured by controlling of low and high viscosity. Torque and shaking speed is observed and measured and volumetric power consumption is calculated by this equation [28];

$$\frac{P}{V} = Ne'\rho\frac{N^3 d^4}{V^{\frac{2}{3}}} = C \quad \frac{N^3 d^4}{V^{\frac{2}{3}}} \tag{3.3}$$

Where; Ne' is modified newton number in shaken flask

Modified newton number is calculated both experimentally and theoretically. During calculation, all flow regimes are considered and generate a formulation that gives newton number for shaken flask. This combination is consists of laminar flow (Re^{-1}), turbulent flow ($Re^{-0.6}$) and transition flow ($Re^{-0.2}$) where;

$$Ne' = 70Re^{-1} + 25Re^{-0.6} + 1.5Re^{-0.2} \tag{3.4}$$

For example in stirred tank bioreactors, power consumption consists of two approach; power for stirred and compression power for aeration. First type is used for stirred tank reactors and the other for bubble columns and airlift reactors. Power input in stirred tank reactor is the major cost of aerobic fermentation and the calculation depends on various factors [29].

These important factors are stirrer rate (*N*), the impeller diameter (*di*), viscosity of the fluid (μ) and the density of the fluid (ρ). These factors can be associated with dimensionless number and power consumption for stirred tank N_P can be written as a formula which is [30];

$$N_p = \frac{P}{\rho N^3 d_s^5} \tag{3.5}$$

Also power input can be expressed by a formulation with dimensionless number Reynold's for stirring tank;

$$Re_s = \frac{\rho_l N^2 d_s^2}{\mu} \tag{3.6}$$

The relationship between power consumption for stirred tank (N_P) and Re_s makes a linear values when it is shown on a fig. 3.3 According to characteristics of flow, N_P value decreases from laminar to transition statement and finally, stabilized in turbulent stages.

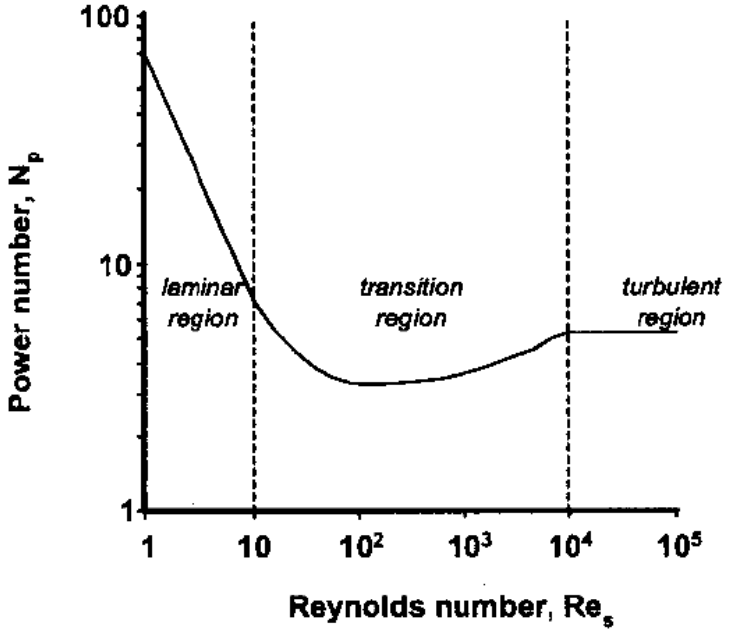


Figure 3.1 Figure shows variation of power consumption (N_P) depending on Reynold's number in stirring tank bioreactor [31].

In transition region between laminar and turbulent flow, power consumption becomes more complex to calculation. Internal and viscous forces must be considered. Power consumption can be calculated with a known power number [32];

$$P = N_S \rho_\iota N^3 d_s^5 \quad (3.7)$$

3.2.3 Impeller Tip Speed (rpm)

Impeller tip speed is not a main scale-up issue but it can be an important assistant and used together with other strategies. Like other cases, constant impeller tip speed has a several problems. In sensitive cell culture, impeller may damage on to culture while tip speed is increasing. Rotational movement of impeller is transferred to the fluid as a shear force. This force acts directly on cell surface. After a certain limit which depends on cell type, cell membrane starts to loss it's stability. Cell cannot keep its structural conformation and hence cell membrane is broken. As a result, cells burst. Increasing of cell viability casualties cause low production level and also death cells spatter the medium and survived cell also be influenced. On the other hand, fluid medium homogenization is not performed successfully and density differences can occur. Depends on the location of media, some cells obtain enough media component to grow while some cells do not [33].

Shear force resistant cell culture has other problem when impeller tip speed is increased. Although cell can survive in high mixing rate, foam formation can be occurred because of turbulent flow of fluid medium. These foams grow out from inside of media. While they are coming to the media surface, some protein based molecules are taken along with foam and transferred on the surface of media. This event causes a problem that media losses its rich component structure and cells aren't fed enough for optimum growing. To prevent this problem, there are many items can be used. Some oil based solution and chemicals prevent the foam formation during fermentation [34].

The impeller tip speed v_{tip} can be expresses in an equation in which D is a diameter of stirred tank;

$$v_{tip} = \pi N D \quad (3.8)$$

Constant impeller tip speed is acts on different ways in fermentation process. In the equations of 3.1, 3.2, 3.4 and 3.5, impeller tip speed can be used same as a constant value both small scale and large scale and other parameters are changed. After calculations, experiment is performed.

Even this parameter has widely used in scale-up process, it isn't enough for growing optimization and scale-up process itself, but this parameter is used with other parameter characterization for successfully scale-up.

3.2.4 Liquid Mixing Time and Recirculation (t_m)

Alternatively, constant mixing time can be used for scale-up process and also this method is applied with other important parameter such as volumetric power consumption and constant oxygen transfer rate. The importance with mixing time is that low mixing time causes low OTR and this may influence with bad effective on fermentation process. Low OTR may not be enough for cell culture growing and both the amount of product and biomass is quantitatively decreased. Also, mixing time is correlated with fluid flow and impeller speed. In large scale, mixing time is determined with consideration of geometrical information of reactor such as diameter and volume. The basic function of mixing time can be defined as [35];

$$\mathrm{f(t)} = t_m (N D_i^2)^{2/3} g^{1/6} D_i^{1/2} Y^{1/2} D_t^{3/2} \tag{3.9}$$

Where; t_m is mixing time

g is gravity

D_i is impeller diameter

D_t is tank diameter

Y is the depth of the liquid

The relationship between small and large scale production can be defined with mixing time as;

$$\frac{t_{mL}}{t_{mS}} = \left[\left(\frac{N_S}{N_L} \right)^4 \left(\frac{D_{iL}}{D_{iS}} \right) \right]^{1/6} \tag{3.10}$$

3.2.5 Oxygen Mass Transfer Coefficient ($k_L a$)

Oxygen is the most important supplement of the aerobic fermentation process. Cells required an oxygen during growing, generating fermentation product and cell metabolism. Generally,

oxygen is used for generation of energy in ATP to use cell metabolism. In aqueous fermentation, oxygen is dissolved in liquid media and cell use it. Oxygen transfer coefficient is based on given oxygen and used oxygen in the system. The source of oxygen is generally sterile air. Air can be given in the system with different ways. For example, sparger is mainly used stirred tank, air-lift and bubble column reactors which is located at the bottom of the impeller and air bubbles are formed. These bubbles are spread homogenously in media and oxygen diffuses equally at each point of media. OTR calculation is very difficult, because of consideration lots of cases. Oxygen requirement is changed with cell type. Air system must supply this amount of requirement. Amount of air that given in the system is calculated according to cell requirement and also pressure in reactor is optimized to oxygen dissolving in liquid medium. Impeller diameter is determined according to reactor volume for homogenization. In some cases these calculations are not enough so pure oxygen or gas mixing that high oxygen concentration is used. Also, reactor pressure can be increased to provide more oxygen dissolves. During these experimental and theoretical optimization, cell viability and media homogeneity are strongly considered and some complications must be prevented such as foam formation [36,37].

OTR is calculated with oxygen uptake rate which is microbial oxygen demand. With using these two parameter, it is possible to access the rate limiting step of a microbial process such as mass transfer or reaction limited, through the use of a modified Damköhler number (*Da*). This number can be calculated as [38];

$$Da = \frac{OTR_{max}}{OUR_{max}} \tag{3.11}$$

When $Da = 1$, low diffusivity leads and the process is mass transfer limited. On the other hand high diffusivity leads and the process is limited by biochemical reaction rate where $Da \geq 1$. OTR is an important parameter for optimization of fermentation process. The simple equation for oxygen transfer rate is based on the dissolved oxygen concentration (C*) and dissolved oxygen concentration (C_L) in the bulk liquid which is;

$$OTR = k_L a\ (C^* - C_L) \tag{3.12}$$

The oxygen transfer coefficient $k_L a$ is combination of the local mass transfer coefficient in the liquid phase k_L, and is the specific interfacial area a. Calculation of these two terms one by one

is more difficult and take long time than when consider single value of these value so most of the fermentation process design, k_La value is assumed according to similar previous experimental result or calculated as possible as simple.

Although enhancement factor *E* can be used for k_La calculation which is depended on experimental changing such as concentration of biomass or cell concentration according to different strain. A factor of *E* influences the transportation due to oxygen uptake by microorganism. During calculation, this factor is multiplied with k_La and OTR rate is determined. In most cases, this factor can be assumed at 1 because usage biomaterial and media component may not chance efficiently to OTR. In this case this factor is negligible. Application of enhancement factor can be expressed as [39];

$$k_La = Ek_La \tag{3.13}$$

Determination of volumetric mass transfer coefficient in stirred tank reactor has a different approach. In this calculation, reactor diameter and power consumption play an important role in determination of k_La because increasing of density of liquid media, addition of ions, hydrocarbons or temperature increase the k_La value and it becomes a more complicated function and has less tolerance [40].

On the other hand, k_La can be calculated with other measurable parameters. The equation can be written as [41];

$$k_La = C_1(\frac{P}{V})^{C_2}(v_g)^{C_3} \tag{3.14}$$

Where *P* is the power input, *V* is the liquid volume, v_g the gas superficial velocity and *C*1, *C*2 and *C*3 are constants which can be changed [42];

3.2.5.1 Measuring Methods of k_La Without Biological Consumption of Oxygen

If the biomass is not present or non-respiring cell is used, biochemical reaction is not occurred. So the OUR value becomes zero. In this situation, k_La can be defined as[43];

$$\frac{dC}{dt} = k_La\ (C^* - C_L) \tag{3.15}$$

3.2.5.2 Chemical Method

In early years, chemical methods were widely used. However, this method is not useful to measure volumetric mass transfer coefficient for sparger bioreactors, because of the changes in physical and chemical properties of liquids and incorporations produced with addition of some chemicals such as antifoams. Due to this, chemical method cannot give real value which is mostly higher than the real result. If the liquid media are not kept within certain conditions or stabilized, the absorption result of media may be enhanced by these added chemical to go into a reaction [44].

3.2.5.3 Sodium Sulfide Oxidation Method

This method is based on reaction between sodium sulfide and oxygen. Sodium sulfide acts as a reducing agent and sulfide is formed as a result of reaction. The oxygen level can be determined with measurement of this produced sulfide. This measurement can be done with stoichiometry of this reaction. In this measurement, colorimetric determination of iodine concentration is performed. Increasing the amount of sulfide represent high amount of dissolved oxygen in liquid media and reaction takes place in this way [45];

$$O_2 + 2\,SO_3^{-2} = 2\,SO_4^{-2} \tag{3.16}$$

3.2.5.4 Absorption of CO_2

Microorganisms use oxygen to generate energy. In this reaction, CO_2 is produced as a refuse product. The amount of CO_2 product has a direct correlation with oxygen so we can make a discussion and relation about dissolved oxygen by measuring amount of produced CO_2. If the amount of refuse CO_2 is measured, we can calculate the used oxygen value due to biochemical metabolism of microorganisms [46].

This method was developed by Danckwerts and Gillham (1966). The principle of this technique is that absorption of carbon dioxide measuring with an alkaline solution. The reaction of carbon dioxide and alkaline solution takes place as this formula [47];

$$HCO_3^- - CO_2 - OH^- = 0 \tag{3.17}$$

$$HCO_3^- + H^+ - CO_2 - H_2O = 0 \tag{3.18}$$

3.2.5.5 Physical method

The physical methods for measurement of dissolved oxygen uses a probe which is sensitive in oxygen concentration changes in liquid medium under non-stationary conditions. Todays, this method is the most commonly used method in industrial fermentation. The mechanism of probe is based on the measurement of dissolved oxygen concentration in the liquid media during fermentation and also measurement the absorption or desorption of oxygen in the solution [48].

CHAPTER 4

TREE EXTRACT WATER

Commercial microalgae production is an industry based on solid foundations. Different designs of closed photobioreactors that will provide commercial production of new species and the production of such commercial microalgae has shown significant improvement in recent years. This design can also be seen to be commercially viable in the near future foreground. Closed systems located within the tubular photobioreactors, in various forms, is composed of transparent tubes or hoses of different sizes and lengths. These types of bioreactors, tube diameter suitable for 2 - to 4 cm [1]

Despite the success of *Spirulina* and *Dunaliella* as a selective growth medium open in the cultured microalgae stack culture system *Haemetococcus* and *Phaeodactylum* as commercially used but the production in a closed system microalgal species not having selective environments are more successful. But high production costs have prevented these systems until recently, and commercial applications. The basic principle of the flat panel reactor and tubular photobioreactor design; by cells being cultured is to provide more efficient utilization of light.

Thus, the accumulation of harmful gases away from the culture system by providing an appropriate mix and the benefit of the cells more efficiently than light, it is possible to achieve higher biomass density. Consequently, this system is suitable to run in continuous production. Maximum intensity of the continuous production of algae and growth medium conditions, depending on the effective use of production allows the ability to determine in advance. Thus, the total costs are reduced and the smaller space is needed [2].

The trees are also living beings as well as all the water circulation in the tissue. This continuous and sufficient amount of water to ensure the circulation of the tree needs water. The amount of water needed for each tree species show differences. An adult beech, dry and warm 250 liters a

day, while a small sunflower consumes 1 liter of water. Eucalyptus trees are useful in drying of the marshes that they spend an average of 400 liters of water per day [49].

The bark is a very effective water-proof armor. A tree, until the beginning of the foot is shielded against leakage of water vapor. In this way, with rich ingredients that contain water in the wood it is suitable for use in many areas [50].

Water can be obtained by the conventional dry tree. However, analyzing the results of a newly developed vacuum drying liquid samples from the timber made by using radio frequency technology according to the fluid samples obtained from normal drying of similarities with the sap of the tree was found to be much higher. When this drying technology developed as a result of examining the content of the water obtained from wood; hormones, minerals, organic acids and amino acids have been identified [51].

Because the three water contains hormones and other rich nutrients, it can be used with again the feeding. Also it can accelerate algae growth due to nutrient content (fat, carbohydrates and so on). Because of this, the three water is expected to increase enrichment additive. Also tree water contained hormones and organic acids, it mainly used for plant feed, foliar and direct application to do the ground "ready solution" raw materials, it can be said on the basis of content obtained from the analysis performed can be used as semi-finished and finished products [52].

Hormones in the tissues and organs that make up a regulatory function they reached effect and are very low amounts of organic compounds which have a significant impact by showing tasks. Hormone first time the term was used in 1902 by Bayliss and Starling. Harmon's Latin = warn (activation) means to revive [53].

Some hormones found effective in promoting plant is located in another part of the inhibiting effect. Therefore, hormones only "encouraging chemicals" rather than evaluate the "chemical regulators" would be a more accurate description to call [54].

Studies showed that water trees in gibberellic acid, salicylic acid, indole 3-acetic acid (IAA) and abscisic acid (ABA) has been found to be found. These hormones are referred to as plant growth regulating hormones. In hormone, gibberellic acid has the highest rate of water in the tree at least abscisic acid [54].

Gibberellic acid is isolated from the first time in Japan has been noticed because of fungus Gibberella fujikuroi causing excessive height growth of the fungus on rice. Today, 76 different types of gibberellin, isolated from plant species of the fungus Gibberella and specifications are determined. Also in agriculture and in particular to obtain the gibberellin hormone used in horticulture mainly from fermented fungus Gibberella. Although there are known today nearly 100 GA series, are found in over 50 plant seeds thereof. Ga3't commercial purposes, with the most widespread use [54].

Indole 3-acetic acid (IAA), the only known naturally occurring auxin. Tryptophan ester. IAA show ends in plant growth (coleoptile tip, bud, leaf and stem end) are quite high [54].

Abscisic acid (ABA) is a substance which sesquiterpene structure. Auxin, a natural antagonist of growth hormone accelerator such as gibberellin and cytokinin. And always they are found in almost every part of the plant. Changeable only in environmental conditions with reduced or increased. This condition also varies depending on the physiological effects. Normally they engaged in high levels of seed dormancy and bud dormancy case is considered to be a maintainer effects. Moreover, they also make the leaves, stem and fruit [55].

The analysis made in the tree water tree water has been found to contain many organic acids. Other organic acids and water in the tree up with 37,8% oxalic acid and maleic acid is contained at least 0.77% [56].

In the tree with water present a wide range of significant use of organic acids. Especially when they affect the development of all vegetation period the plant, the plant made with the leaves or soil application of physical, chemical and biological are likely to affect many functions. Organic acids agriculture, fertilizers, food and cosmetics as well as there are different uses [56,57].

Development of Plant Growth Regulators Using Algae Growth

This patent study of the year 2011 was carried out by William McCaffrey and colleagues. Details about the patent is shown in Figure 3.4. This patent covered by plant growth regulators (indole acetic acid, growth hormone, etc.) And hormone mimetics (phenoxy acetic components) using autotroph algae, grow heterotrophically and photoheterotrophic provided. Also, according to this method alkyne content in the fat or carbohydrate ratio can be increased as desired purpose. In this study, *Scenedesmus obliquus* and *Chlorella protothecoides* algal species are used [59].



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Rüçhan tarihi ⓘ	13 Oca 2009
Şu şekilde de yayınlandı:	CA2748917A1, Daha Fazla (5) »
Buluş Sahipleri	William Mccaffrey, Robert Edward Burrell, Mark Stephen Burrell, Brett Kotelko
Başvuru sahibi	Alpha-J Research Limited Partnership
Alıntıyı Dışa Aktar	BiBTeX, EndNote, RefMan

Patent Harici Atıflar (8), Sınıflandırma (5), Yasal Etkinlikler (6)

Dış Bağlantılar: Espacenet, EP Kaydı

Figure 4.1 EP EP 2387304 Al [60].

CHAPTER 5

MATERIALS AND METHOD

In this project *Chlorella vulgaris* and *Chlorella minutissima* culture was used for understanding the effect of processing parameters on biomass production. In this case, cell growing rate and glucose production parameters were followed up periodically after different components were added into media. Total production took 3 weeks until stationary and dead phase were seen on production process.

For understanding the effect of different components on production, different concentrated media solutions were prepared. For this reason, beech and oak mixture wood extract water (BK samples) and iroko wood extract water (I samples) were used as additive component of media. The rate of these wood water were prepared from %5 wood water, %5 stock culture and %90 Bold Basal Medium to %50 wood water, %5 stock culture and %45 BBM solution. There were 2 control sample prepared for comparison of cell growing rate between all samples and at the end of the experiment, optical density of samples at 540 nm and glucose amount were compared and discussed.

5.1 *Chlorella*

Chlorella for unicellular algae that live in freshwater body's scientific name. Sun *Chlorella* also known as green algae. Green its name comes Greek word for small and *Chlorella* means common or ordinary ella. *Chlorella* by an antioxidant containing lutein may be some cataracts and other eye diseases associated with aging should be useful in protecting against. Also considered a probiotic, the cell is damaged fighting substance [61].

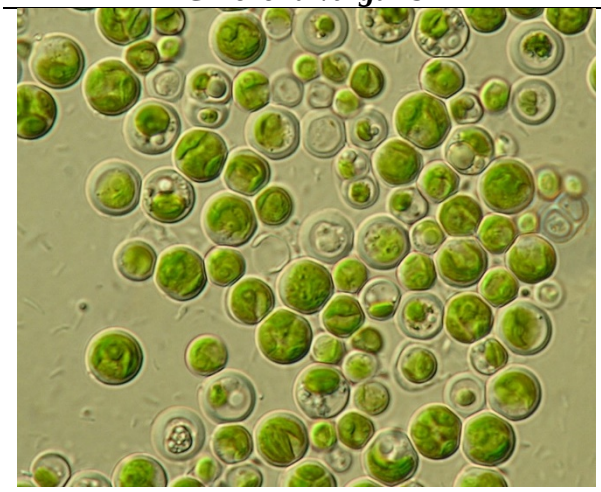
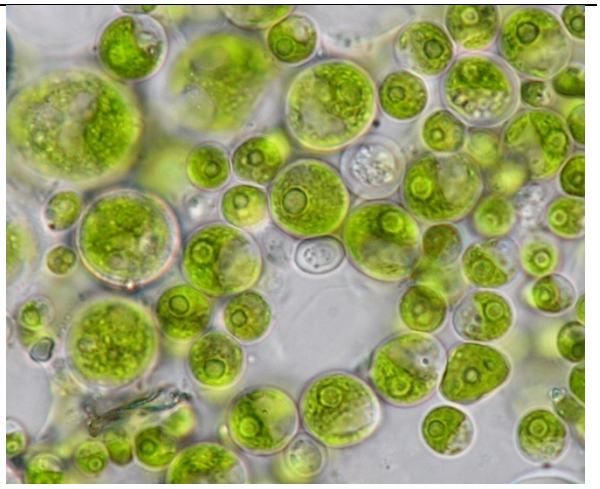
Chlorella vulgaris	*Chlorella minutissima*
	

Figure 5.1 Microscopic image of *Chlorella* species.[62]

Some proponents of Chlorella is believed that probiotic properties help relieve the side effects of chemotherapy and may even play a role in reducing the risk of cancer. There are laboratory studies, 1985 was perceived as a part of their daily diet is dated cancerous tumors in mice shrink this report. More recent experiments have highlighted some strains of Chlorella probably inhibit the human cancer cells in a petri dish vulgaris. It currently shows no clinical effects and advantages, in addition to ingestion of algae in humans.

Chlorella species are considered useful for increasing the digestive system. In addition, intestinal disorders such as ulcers, which are thought to help, colitis or Crohn's disease, often severe and painful inflammation of the gastrointestinal tract. While the good bacteria called in the intestines help develop, Chlorella can also reduce bad bacteria is considered responsible for bad breath or halitosis. In addition, it is considered by some to strengthen the immune system to fight harmful bacteria and viruses [61].

Chlorella that cleanse the blood and detoxify the system, you may want to take a statement. The chlorophyll algae, the green pigment in plants creates and contains a substance that allows them to absorb solar energy. This process is known as photosynthesis. Chlorophyll some assistance, such as mercury and lead is thought to help the body eliminate environmental pollutants. It has also been reported to help remove the body and broken pesticides.

Chlorella nutritional supplement capsules may be taken as a capsule or powder. Some possible side effects are sensitivity to sunlight, nausea or chest tightness, as this may cause allergic reactions Chlorella get shortness of breath. Benefits of claimed not yet evaluated by the Food and Drug Administration (FDA). A doctor should always be consulted before taking additional.[61]

Table 5.1 Taxonomic information of *C. vulgaris* and *C. minutissima* from NCBI [63]

Organism Name	Chlorella minutissima cv. UTEX2341
Genus	Chlorella
Species	Chlorella minutissima
Domain	EUKARYAL
Phylum	PLANTS-CHLOROPHYTA
NCBI Taxonomy ID	3081
NCBI Kingdom	Eukaryota
NCBI Phylum	Chlorophyta
NCBI Class	Trebouxiophyceae
NCBI Order	Chlorellales
NCBI Family	Chlorellaceae
NCBI Genus	Chlorella
NCBI Species	Chlorella minutissima
Is Public	Yes
Related Sequencing Projects	1

Organism Name	Chlorella vulgaris UTEX 395
Genus	Chlorella
Species	Chlorella vulgaris
Strain	UTEX 395
Domain	EUKARYAL
Phylum	PLANTS-CHLOROPHYTA
NCBI Taxonomy ID	3077
NCBI Kingdom	Eukaryote
NCBI Phylum	Chlorophyta
NCBI Class	Trebouxiophyceae
NCBI Order	Chlorellales
NCBI Family	Chlorellaceae
NCBI Genus	Chlorella
NCBI Species	Chlorella vulgaris
Related Sequencing Projects	1

5.2 Microalgae and Inoculant Preparation

Firstly, microalgae, *Chlorella vulgaris* and *Chlorella minutissima* was put into 1/50 diluted stock Bold Basal Medium and grown by making about 30 days. For inoculation, 70 ml sample in culture was taken and transferred 5 ml separately as a %5 inoculant into each BK, I and control samples.



Figure 5.2 *C. vulgaris* and *C. minutissima* cultured with different wood water concentration.

5.3 Bold Basal Medium Preparation

For preparation BBM solution for microalgae production, literature researches were performed and chemicals and their ratios were determinate. After the stock BBM stock solution was prepared, solution was diluted 50-fold and stored at 4C°. Chemical contents and ratios are shown in Table 5.3

Table 5.3 Preparation of stock Bold Basal Medium.

Stock Solution (SL)	Volume	Component	Concentration in SL	Conc. in final Medium
SL 1	10 mL	$NaNO_3$	2.50 g · 100 mL^{-1}	2.94 · 10^{-3} M
SL 2	10 mL	$MgSO_4 \cdot 7H_2O$	0.75 g · 100 mL^{-1}	3.04 · 10^{-4} M
SL 3	10 mL	NaCl	0.25 g · 100 mL^{-1}	4.28 · 10^{-4} M
SL 4	10 mL	K_2HPO_4	0.75 g · 100 mL^{-1}	4.31 · 10^{-4} M
SL 5	10 mL	KH_2PO_4	1.75 g · 100 mL^{-1}	1.29 · 10^{-3} M
SL 6	10 mL	$CaCl_2 \cdot 2H_2O$	0.25 g · 100 mL^{-1}	1.70 · 10^{-4} M
For 3N-BBM add triple quantity of $NaNO_3$, i.e. 30 mL of SL 1.				
SL 7 Trace elements solution	1 mL	$ZnSO_4 \cdot 7H_2O$	8.82 g · L^{-1}	3.07 · 10^{-5} M
		$MnCl_2 \cdot 4H_2O$	1.44 g · L^{-1}	7.28 · 10^{-6} M
		MoO_3	0.71 g · L^{-1}	4.93 · 10^{-6} M
		$CuSO_4 \cdot 5H_2O$	1.57 g · L^{-1}	6.29 · 10^{-6} M
		$Co(NO_3)_2 \cdot 6H_2O$	0.49 g · L^{-1}	1.68 · 10^{-6} M
Combine all trace elements in one SL. Dissolve each component completely one after the other. It may need autoclaving to dissolve. Trace elements solution should **not** be stored in glass containers, but instead in teflon or polycarbonate containers to prevent adsorption of metals to container surface.				
SL 8	1 mL	H_3BO_3	1.14 g · 100 mL^{-1}	1.85 · 10^{-4} M
SL 9 EDTA-KOH solution	1 mL	EDTA · Na_2	5.0 g · 100 mL^{-1}	1.71 · 10^{-4} M
		KOH	3.1 g · 100 mL^{-1}	5.53 · 10^{-4} M
SL 10 Ferric solution	1 mL	$FeSO_4 \cdot 7H_2O$	4.98 g · 1 L^{-1}	1.79 · 10^{-5} M
		H_2SO_4 conc.	1 mL (to acidify)	
This results in the original Bold's Basal Medium (BBM) according to Bischoff & Bold (1963). The pH-value will be about 6.4 to 6.8 at a conductivity of 1.4 mS cm^{-1}.				

5.4 Sample Preparation

Into 250 ml Erlenmeyer flasks, medium components were prepared according to Table 5.4.1, 5.4.2 and 5.4.3. The total volume should be 95 ml. 5 ml inoculant was added into each Erlenmeyer flask. They were put to shaker which was set to 150 rpm at 27°C.

Table 5.4 Preparation of Beech and Oak Wood Extract Water samples.

Sample	Bold Basal Medium (%, v/v)	Beech and Oak Wood Extract Water (%, v/v)	Inoculation Ratio (%, v/v)	Total Working volume in 250ml shaker flask (ml)
KM5	90	5	5	100
KM10	85	10	5	100
KM20	75	20	5	100
KM30	65	30	5	100
KM40	55	40	5	100
KM50	45	50	5	100

Table 5.5 Preparation of Iroko Wood Extract Water samples.

Sample	Bold Basal Medium (%, v/v)	Iroko Wood Extract Water (%, v/v)	Inoculation Ratio (%, v/v)	Total Working volume in 250ml shaker flask (ml)
I5	90	5	5	100
I10	85	10	5	100
I20	75	20	5	100
I30	65	30	5	100
I40	55	40	5	100
I50	45	50	5	100

Table 5.6 Preparation of Control samples.

Sample	Bold Basal Medium (%, v/v)	Inoculation Ratio (%, v/v)	Total Working volume in 250ml shaker flask (ml)
C1	95	5	100
C2	95	5	100

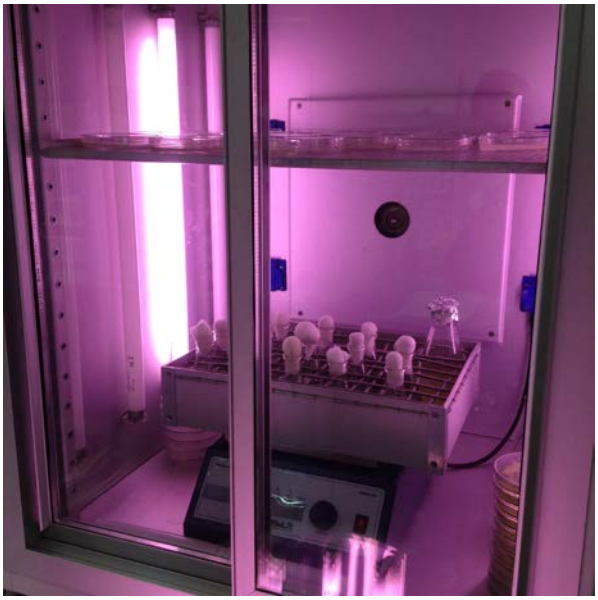


Figure 5.3 Cultured microalgae in incubator with shaker.

5.5 Optical Density Measurement

Absorbance of solutions was measured at 540 nm by taking 1.5 ml of solution to cuvettes. For this purpose, Blank samples were prepared for each different concentrated solution. Preparation of blank samples were similar to production samples except for adding inoculant. They were used for reference of absorption measurements are taken before putting flasks to shaker and for 4^{th}, 8^{th} 12^{th}, 24^{th}, and 36^{th} Day.

5.6 Total Glucose Analysis

A 1 mL of supernatant is mixed with firstly 1 mL 5% phenol solution then 5 mL concentrated sulfuric acid (H_2SO_4) and homogenized gently. This mixture is incubated at a room temperature for 10 minutes then sample is placed into the water bath at 30 C^0 for 20 minutes. After the water bath incubation, sample mixture is warmed with ice for a few second. The spectrophotometric measurement is performed at 490 nm and the reference of absorption is double distilled H_2O.

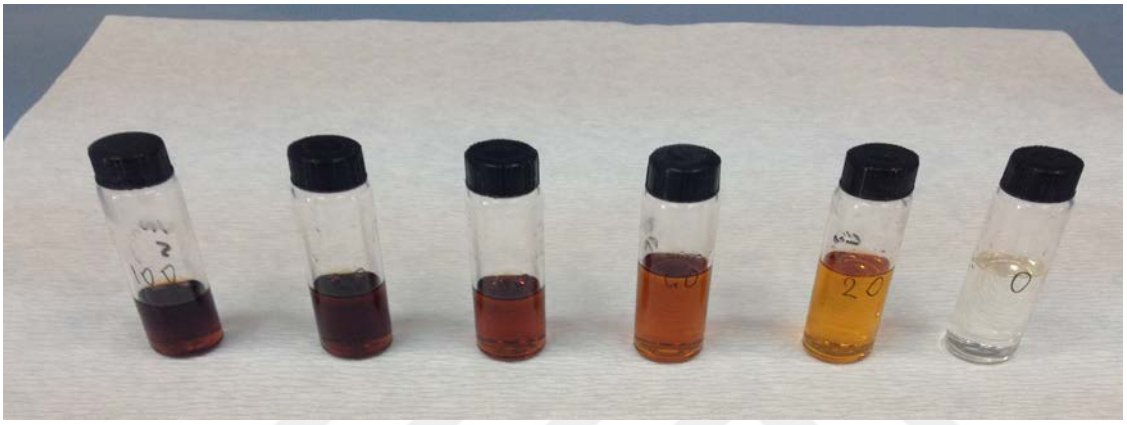


Figure 5.4 Glucose solution in different concentration for reference.

CHAPTER 6

RESULTS AND DISCUSSION

6.1 Optical Density Measurement

The optical density measurement was performed with UV spectrophotometer at 540 nm. Each sample was incubated at the same conditions and cellular growing rate was observed periodically.

All numbered production sample had a different medium concentration. C1 samples were control of process which had a same concentration with C2 sample. At measurement time, samples were taken from each shaker flask and incubation process was continued.

First set of production flask were measured at zero time. Spectrophotometric values were recorded and prepared in Table and Figure. In the beginning of incubation, absorbance of all sample were not assumed zero because all sample inoculant volume contain different amount of cell. All higher value upper than 3 were also set as 3 due to maximum limit of spectrophotometer absorbance value is 3.

6.1.1 Beech and Oak Wood Extract Water Mixture Samples OD Results

In the period of 21 days, Beech and Oak wood extract water mixture (KM) samples were incubated in shaker. Optical density measurement was performed during process regularly. The logarithmic phases were observed in clearly in *C. vulgaris* culture but OD results were higher in *C. minutissima* culture. Results are shown below;

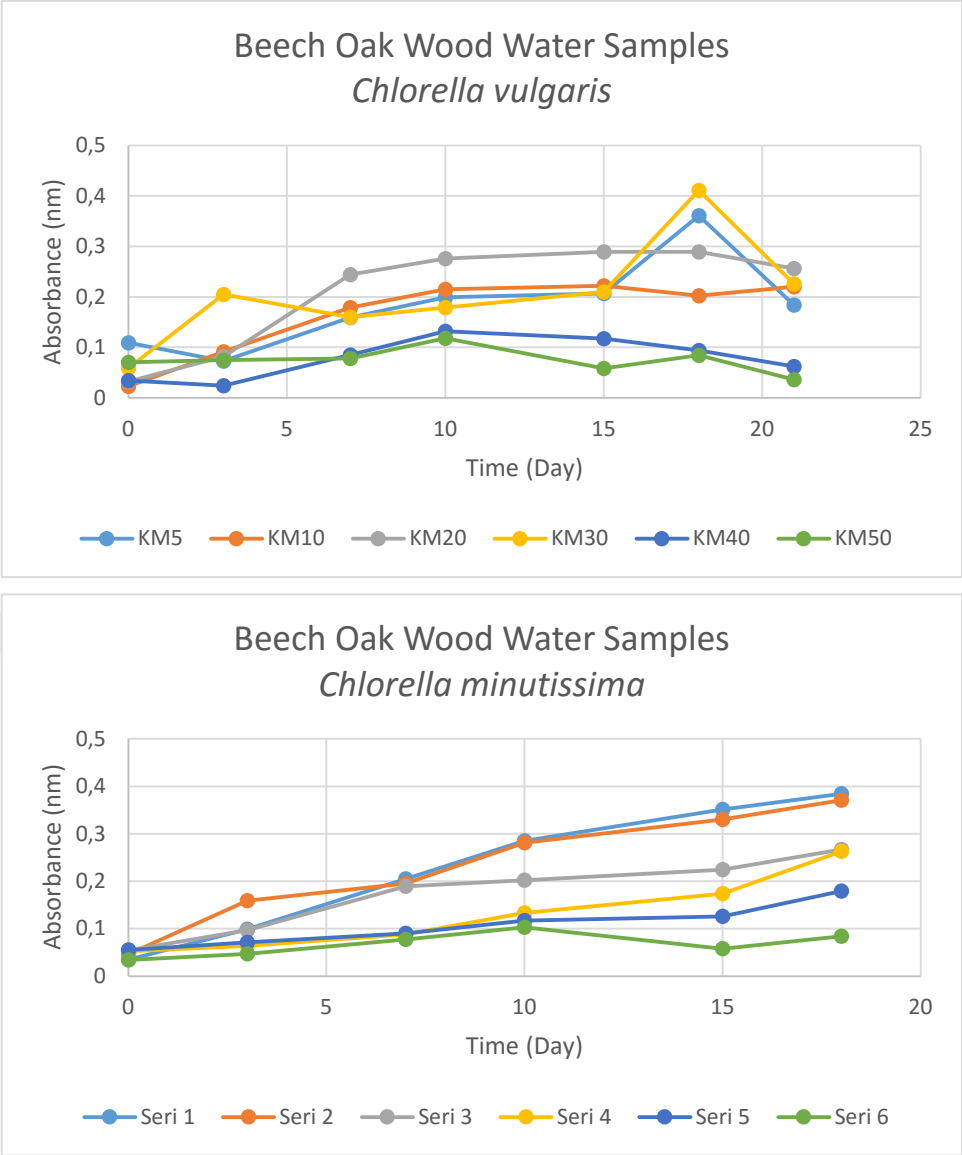


Figure 6.1 Absorbance of all KM samples according to time.

In KM5 samples, both two culture optical results continuously increased on their Figure. *Chlorella minutissima* culture growth seemed to be still in log phase. Growing stage didn't stop yet at the end of production process. This shows, 20 day of production is not enough for observation the all microorganism growing stage in *Chlorella minutissima* culture. On the other hand, the death phase can be observed easily after the sixth optical density measurement in *Chlorella vulgaris* culture. This may occurred with normal microorganism growing process. After a certain time which is cell growing and concentration reach the top level, cells start to death.

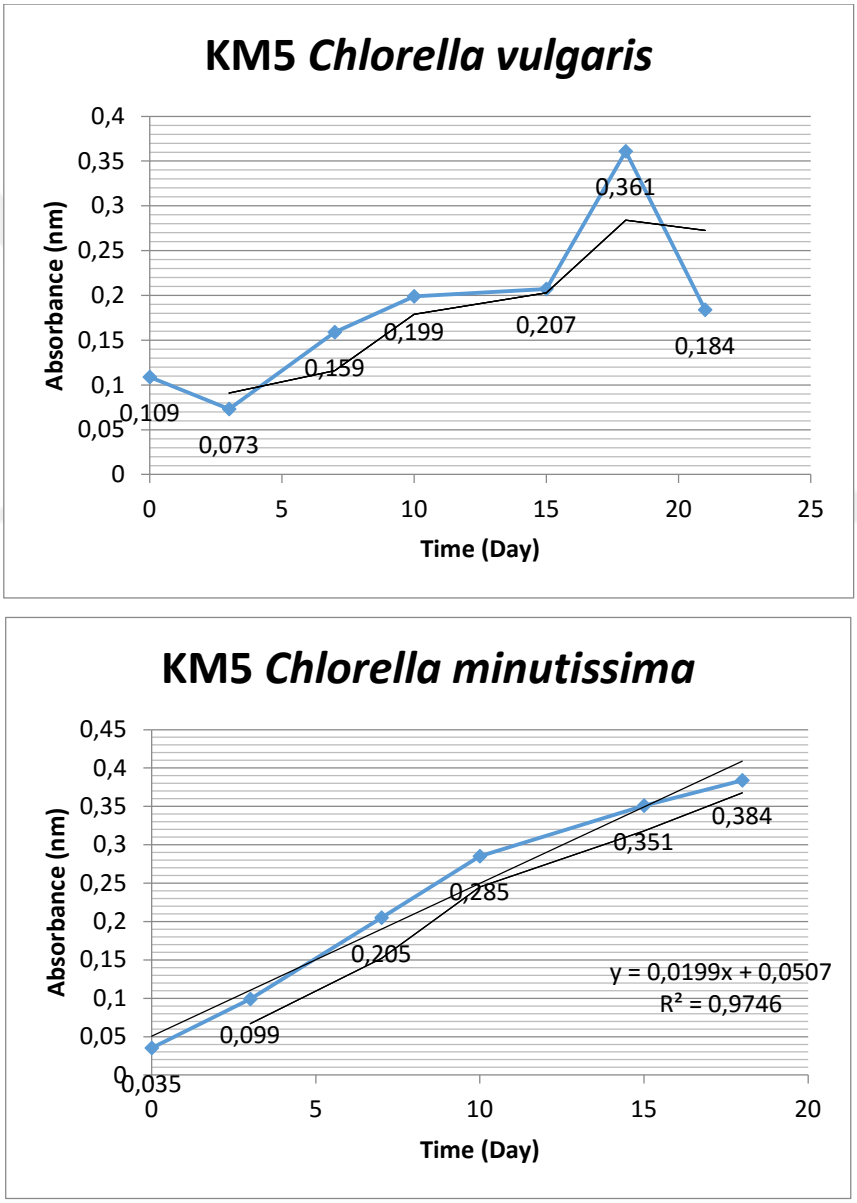


Figure 6.2 Absorbance of KM5 sample according to time.

In KM10 samples, the results were very similar with KM5 samples. Because of the wood water concentration in medium was lower level, the effects of this medium additives couldn't be observed clearly. Thus, KM5 and KM10 sample results were near with control group.

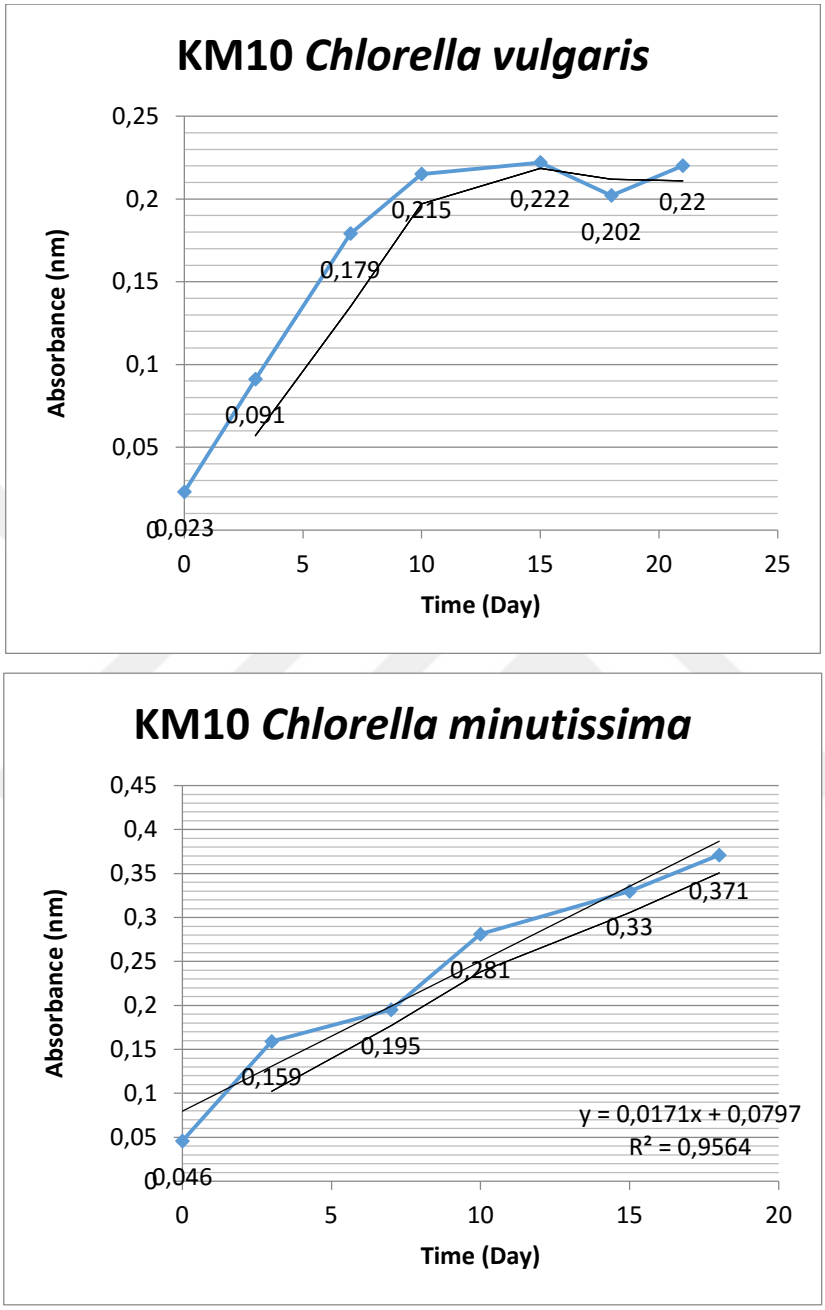


Figure 6.3 Absorbance of KM10 sample according to time.

In KM20 samples, *Chlorella minutissima* culture results were more linear and acceptable when compare *Chlorella vulgaris* culture. That means, Beech, Oak wood mixture is more affective medium additives for *Chlorella minutissima* and also for biomass production.

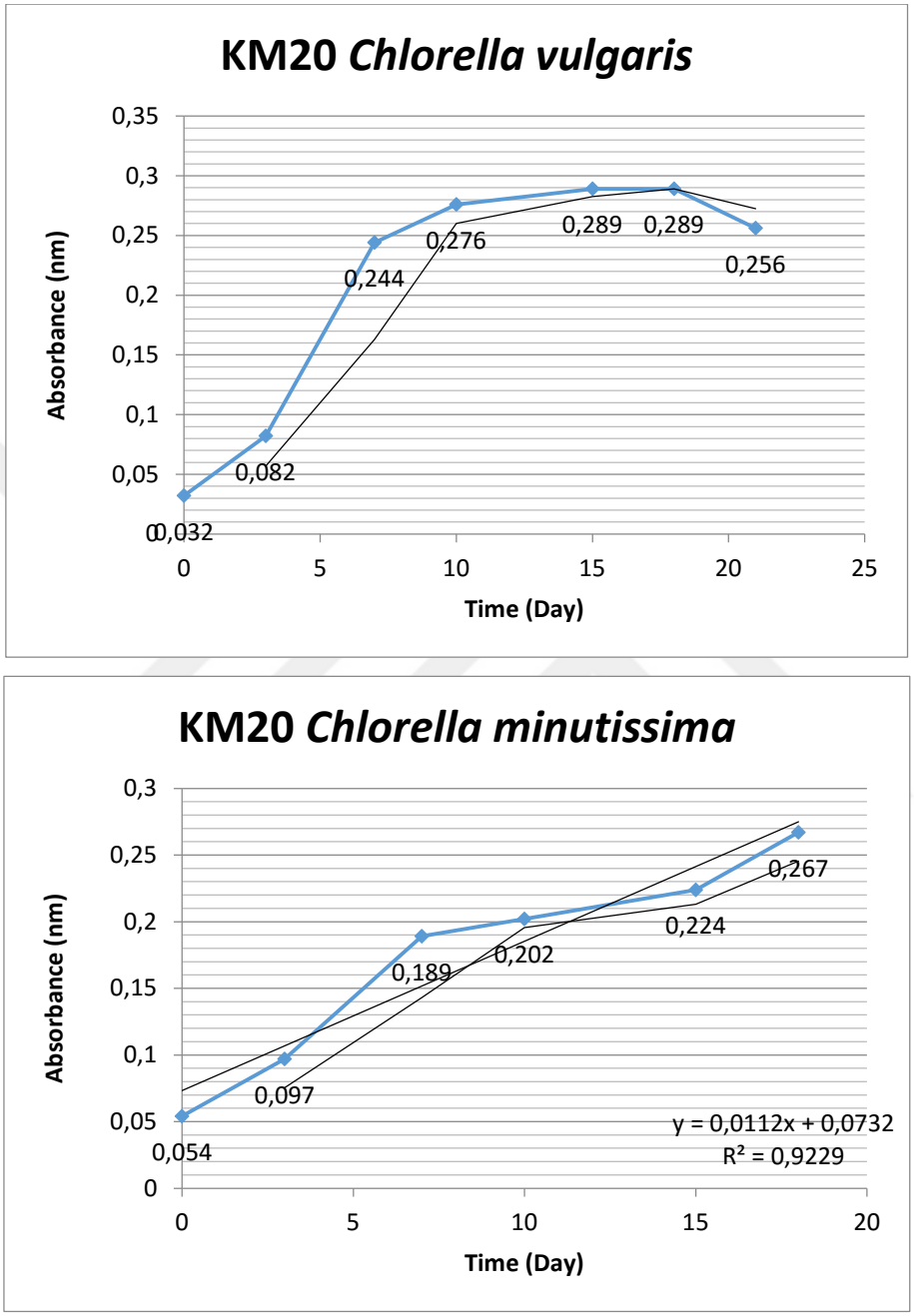


Figure 6.4 Absorbance of KM20 sample according to time.

In KM30 samples, *Chlorella minutissima* culture results were still linear and continuously when compare *Chlorella vulgaris* culture. That means, Beech, Oak wood mixture is more affective medium additives for *Chlorella minutissima* and also for cell growing and biomass production. In addition, this sample gave the best result for *Chlorella minutissima* culture in all KM samples.

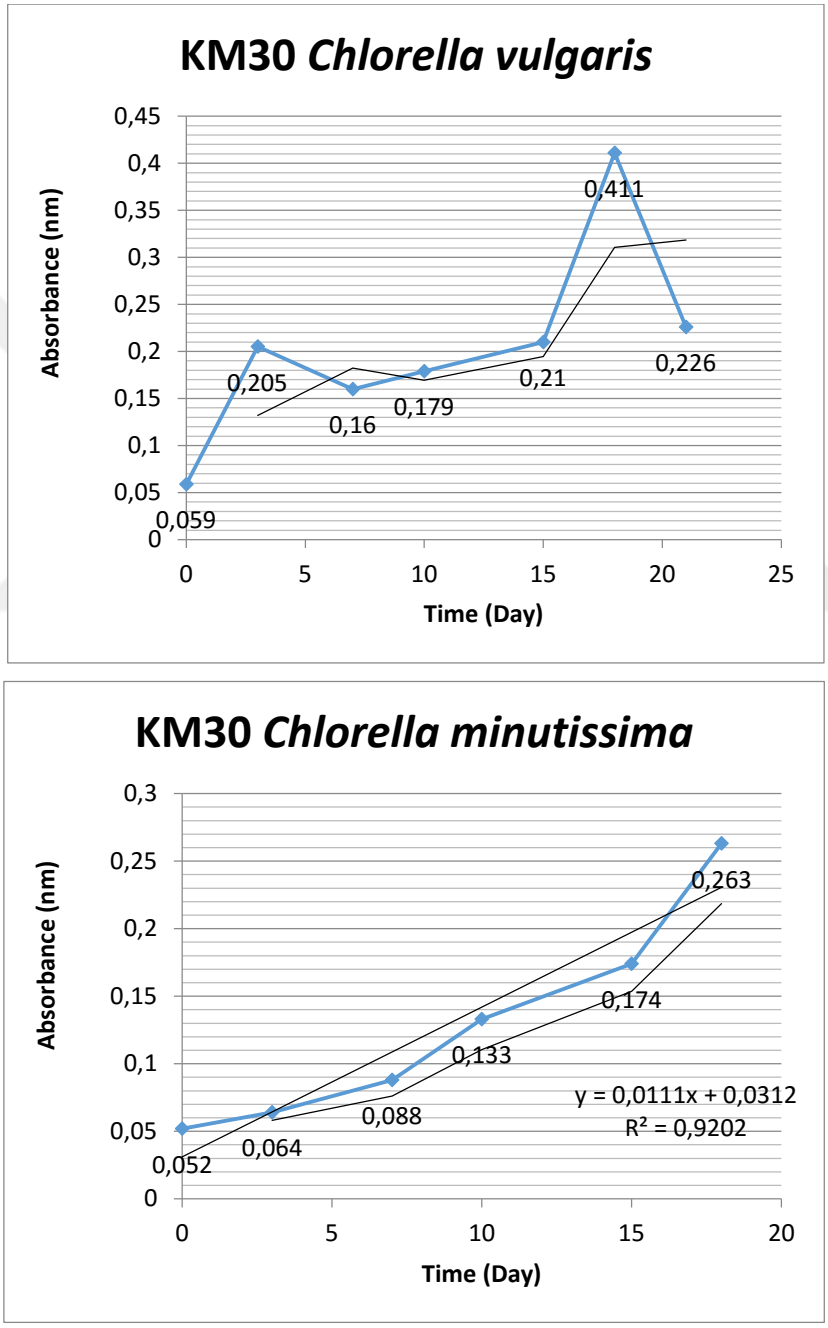
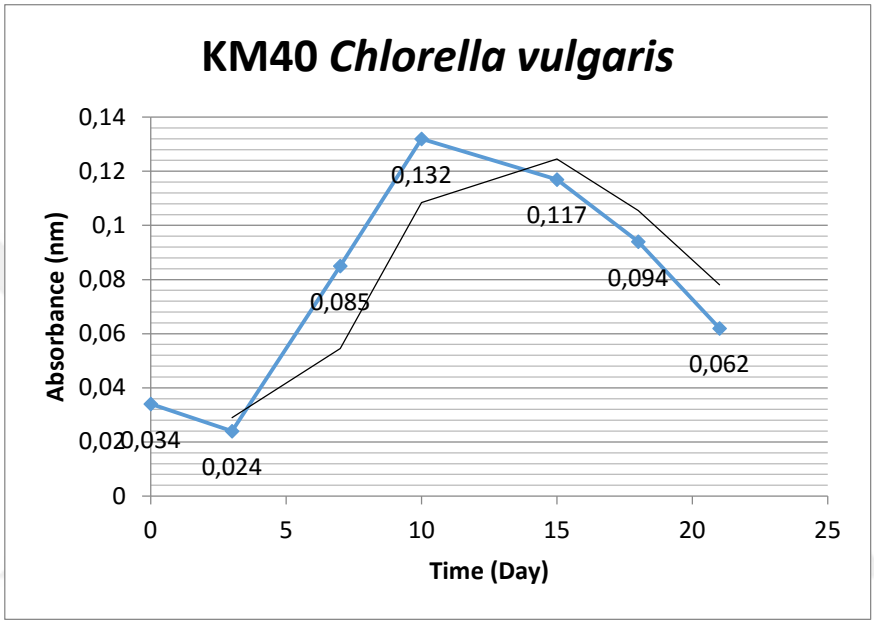


Figure 6.5 Absorbance of KM30 sample according to time.

In KM40 samples, optical measurement results were different with previous samples. After second and third measurement, OD measurement started to decrease. This means, wood water concentration upper than %30 changed its behavior from positive to negative affect. In previous cultures, wood water can be assumed as growing factor, both *Chlorella minutissima* and *Chlorella vulgaris* culture growing were inhibited in KM40 samples. This results can be explain with higher concentration of glucose and neutral acids in wood water can effect in negative ways to cell growing. [48]



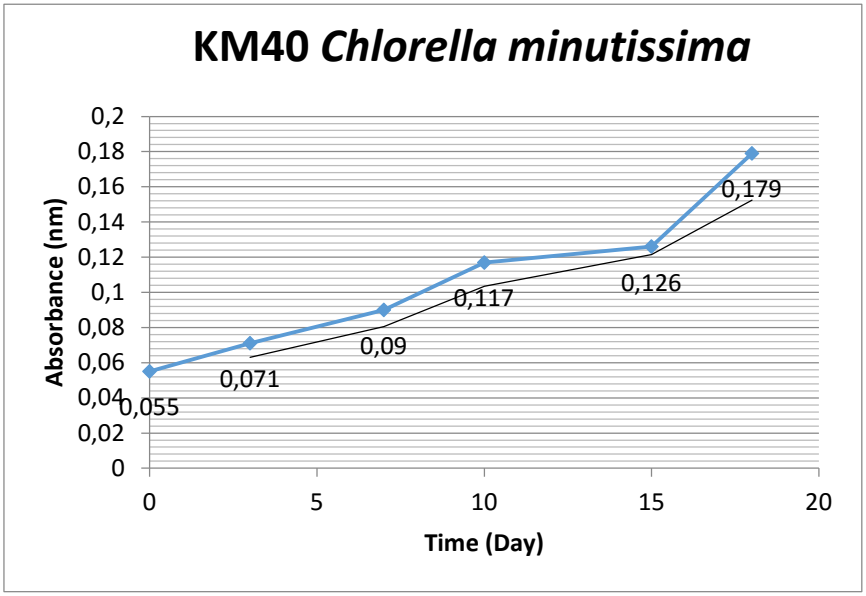


Figure 6.6 Absorbance of KM40 sample according to time.

In KM50 samples, the same results with KM40 were obtained. The growing of both two cultures started irregular and other than expected behavior. At this point, glucose and neutral acid in wood water acted as an inhibitor for cellular growing after the %30 concentration and both two culture get stress. That's why, optical measurements were lower than other concentrated samples.[48]

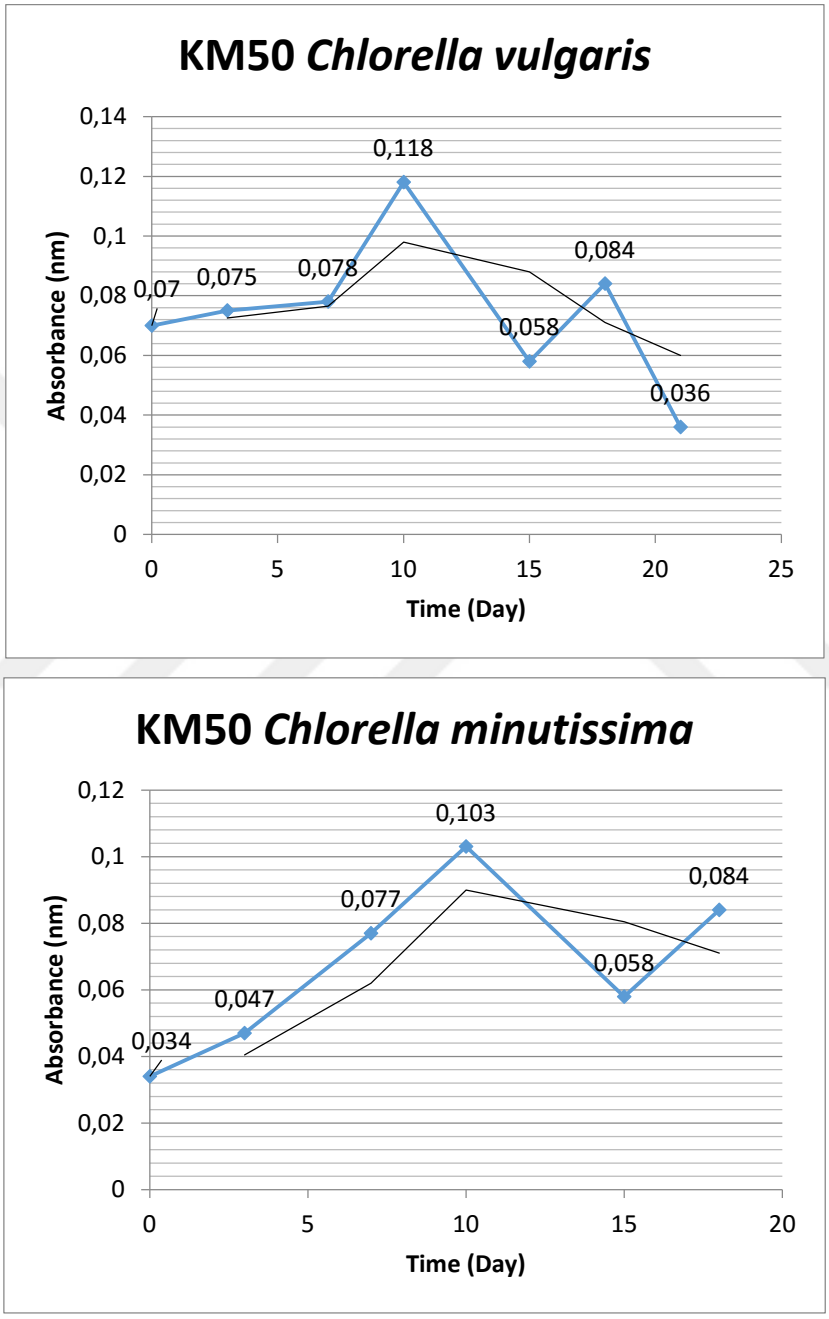


Figure 6.7 Absorbance of KM50 sample according to time.

6.1.2 Iroko Wood Extract Water Samples OD Results

In the period of 21 days, Iroko wood extract water mixture (I) samples were incubated in shaker. Optical density measurement was performed during process regularly. The logarithmic phases were observed in clearly in *C. vulgaris* culture but OD results were higher in *C. minutissima* culture. In both cultures, iroko wood had a higher results when compare beechoak wood samples. This can be Salicylic acid containing in iroko wood. Salicylic acid is used for production of aspirin and this acid type has a toxic effects on bacteria and fungus family. This can inhibit the bacterial and fungal griwing in medium so contamination can be prevented. Results are shown below;

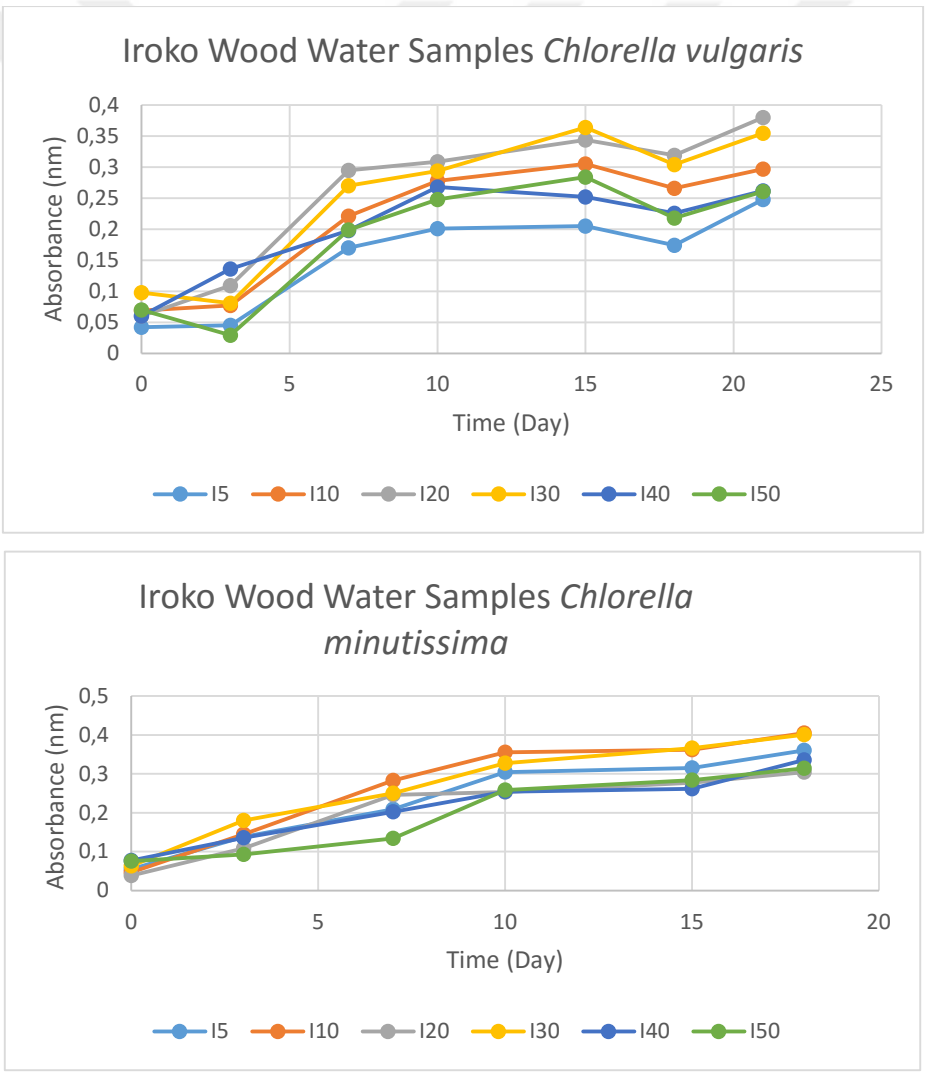


Figure 6.8 Absorbance of all I samples according to time.

In I5 samples, the optical measurement results likely same with control group. Cell concentration and growing rate were very similar because of the wood water concentration level. %5 percent concentration is not enough for affective and visible changing in growing Figure. On the other hand, *Chlorella minutissima* culture growing Figure was More sustainably and linear than *Chlorella vulgaris*. This can be the differences between growing times between two species. *Chlorella vulgaris* grow takes shorter time than *Chlorella minutissima.* So OD Figure and microorganism growing phases were observed in different production day and absorbance level.

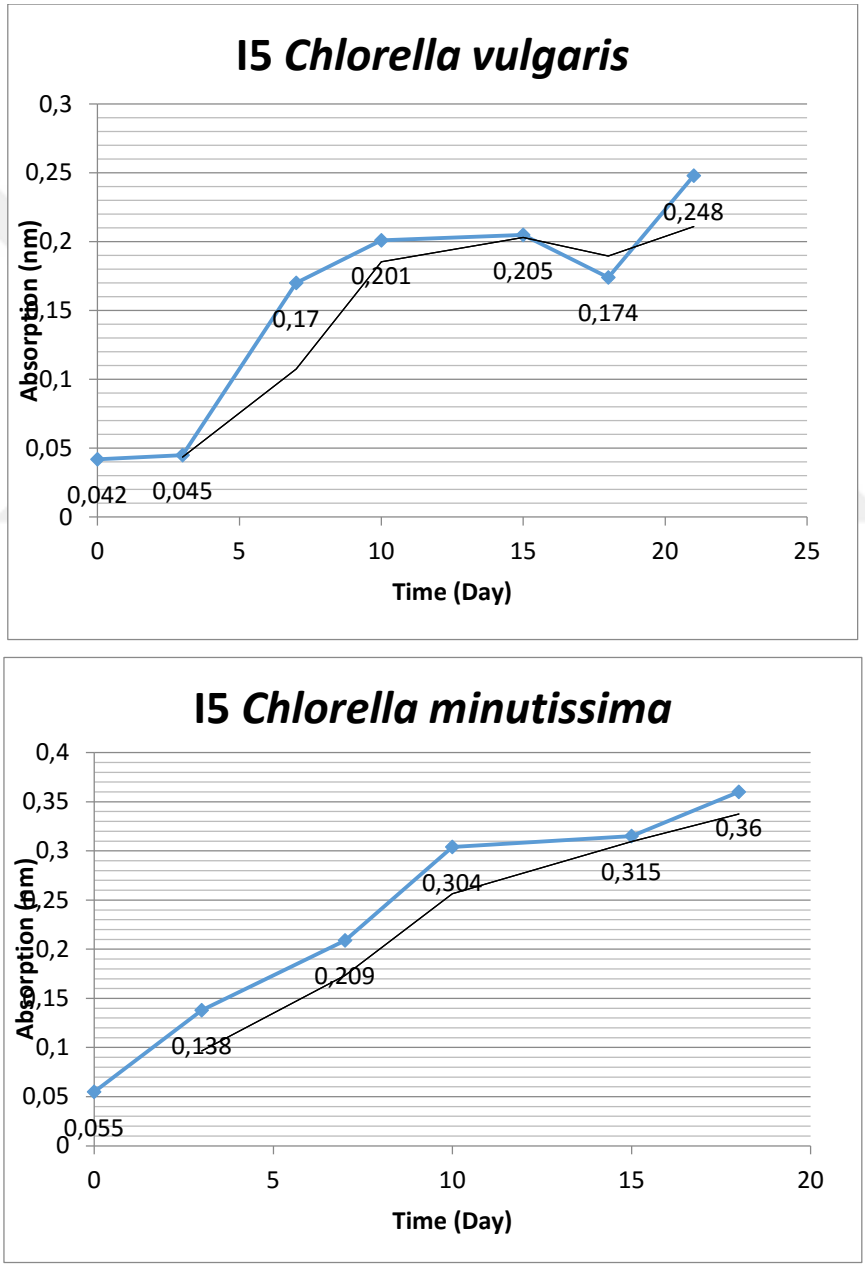


Figure 6.9 Absorbance of I5 samples according to time.

In I10 samples, all samples were growth continuously. Microorganism growing phases were observed more clearly in *Chlorella minutissima* culture when compare *Chlorella vulgaris* culture. This means, the adaptation of *C. minutissima* culture was more prolific than *C. Vulgaris* culture. Also the concentration of wood water was lower level, adaptation of culture on environment was easier than other concentrated samples.

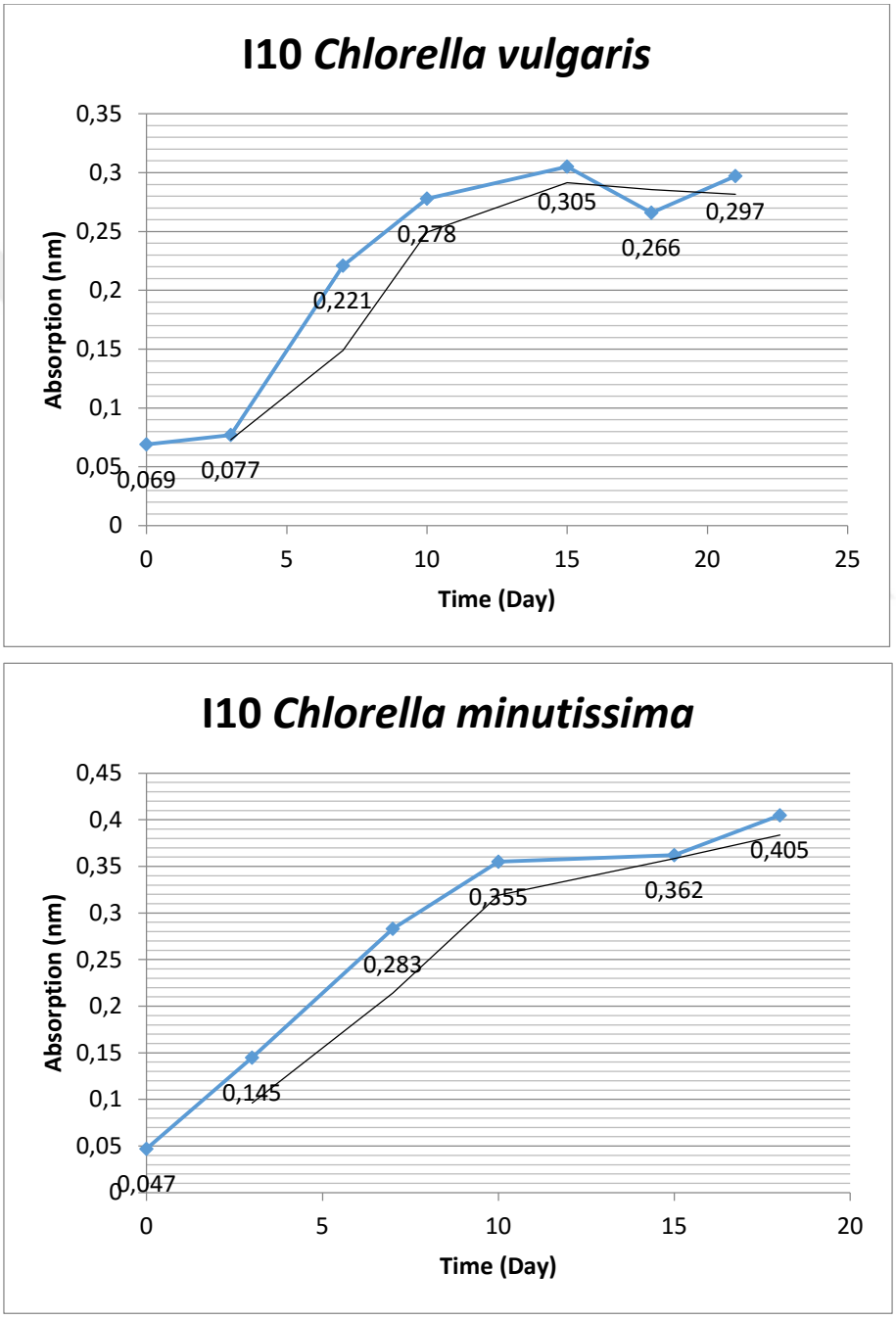


Figure 6.10Absorbance of I10 samples according to time.

In I20 samples, OD results were similar with control group. This can be concentration of wood water was not enough to effect the growing rate. On the other hand, *Chlorella minutissima* culture results were more linear and acceptable when compare *Chlorella vulgaris* culture. So, Iroko wood is more affective medium additives for *Chlorella minutissima* and also for biomass production.

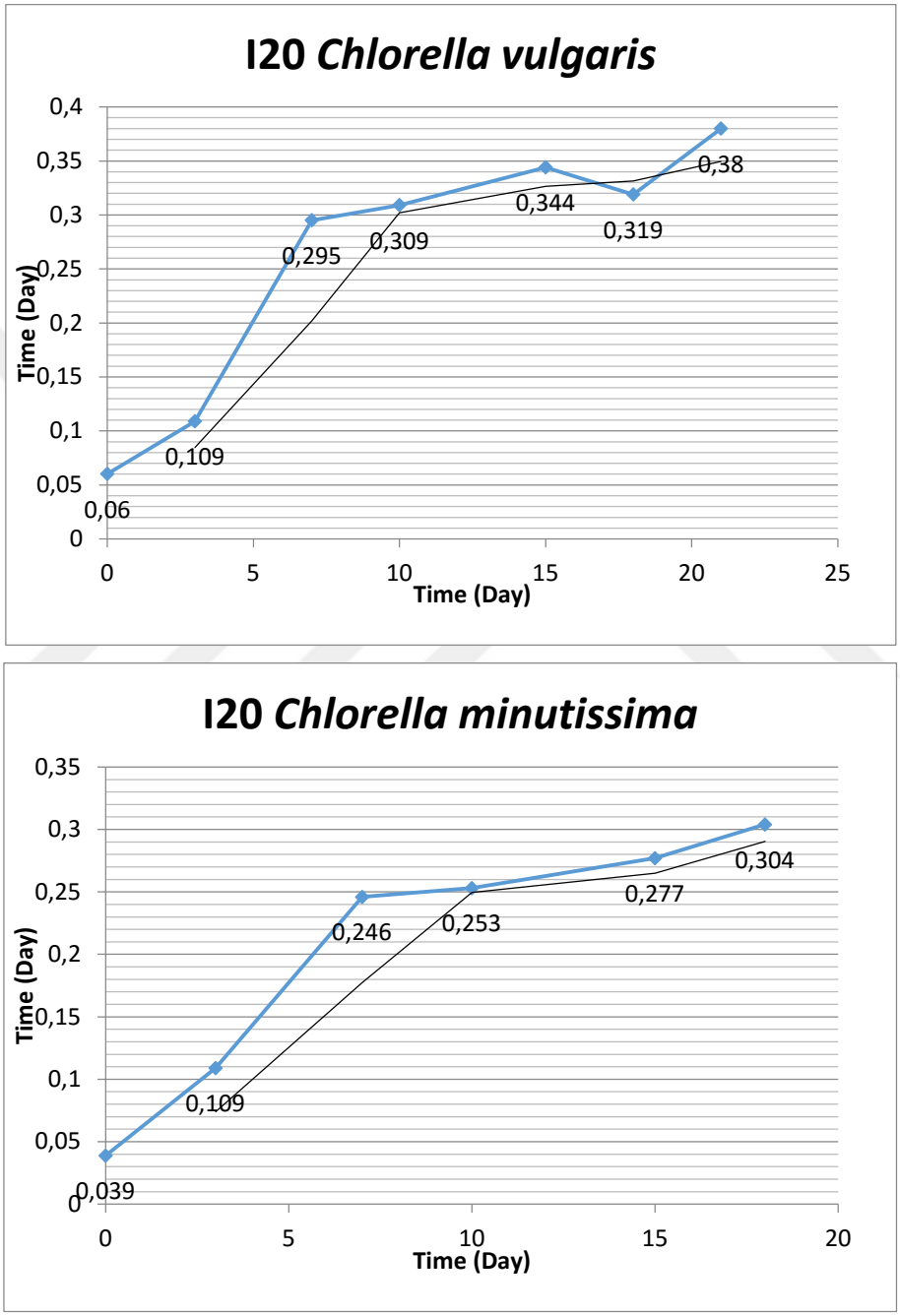


Figure 6.11 Absorbance of I20 samples according to time.

In I30 samples, both *Chlorella* species growth and this growing were observed according to optical density measurement. *Chlorella vulgaris* culture results were more abnormal than other culture. Also death phase were shown when make a growing Figure with using OD data in *Chlorella vulgaris* culture. On the other hand, growing Figure of *Chlorella minutissima* culture was directly increased so there was no death phase observed. This may be occurred for the production time was not enough to observing all growing phase.

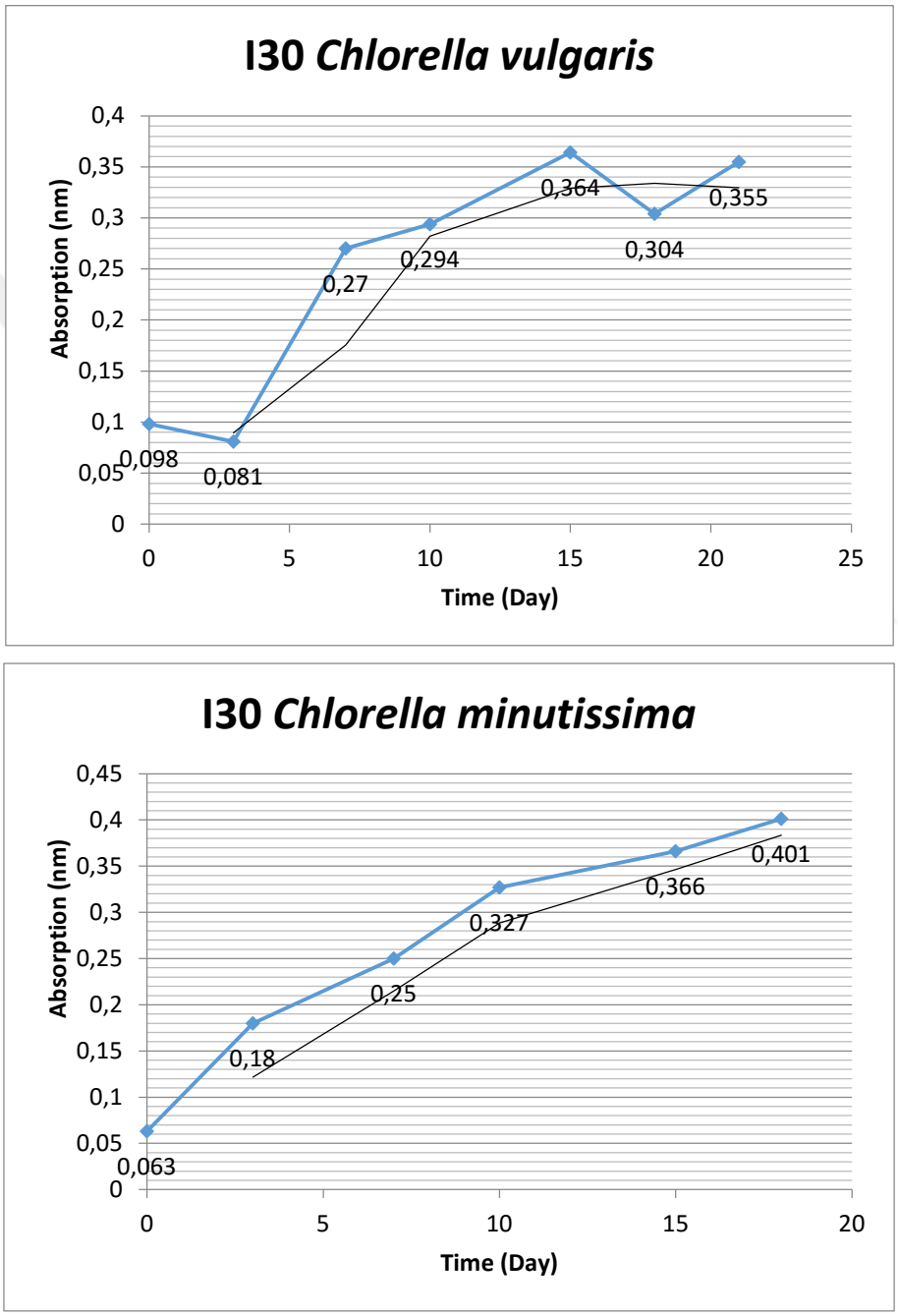


Figure 6.12 Absorbance of I30 samples according to time.

In I40 samples, *Chlorella minutissima* culture results were more linear and acceptable when compare *Chlorella vulgaris* culture. That means, Iroko wood is more affective medium additives for *Chlorella minutissima* and also for biomass production. In addition, this sample gave the best result for *Chlorella minutissima* culture in all I samples.

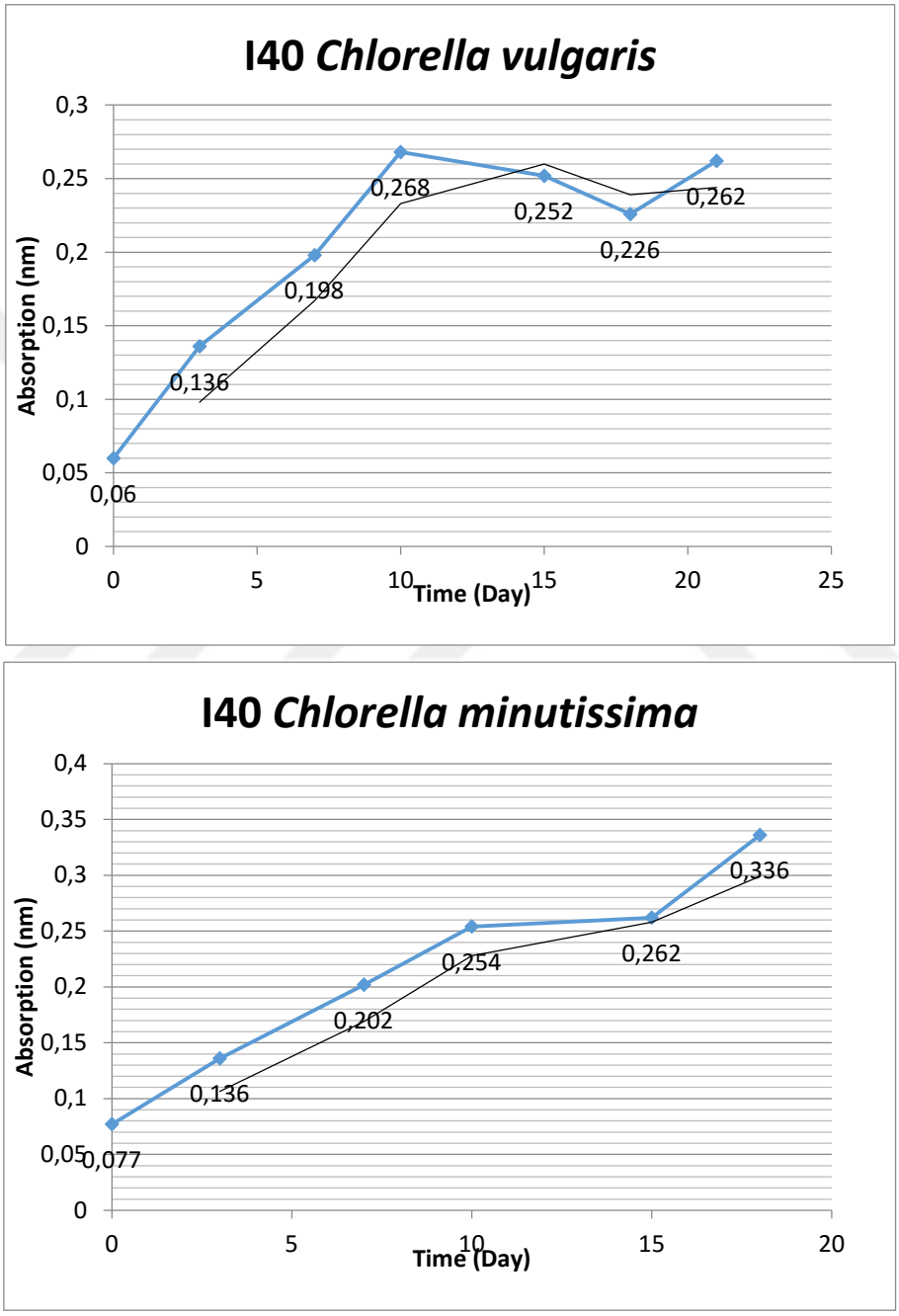


Figure 6.13 Absorbance of I40 samples according to time.

In I50 samples, *Chlorella minutissima* culture results were more linear and acceptable when compare *Chlorella vulgaris* culture. This can caused with homogenization of culture. After a while, *Chlorella vulgaris* become be caking because of this OD measurement result were affected in a negative way. The reason of caking may be content of neutral acids that makes clumping. This means, Iroko wood is more affective medium additives for *Chlorella minutissima* and also for biomass production.

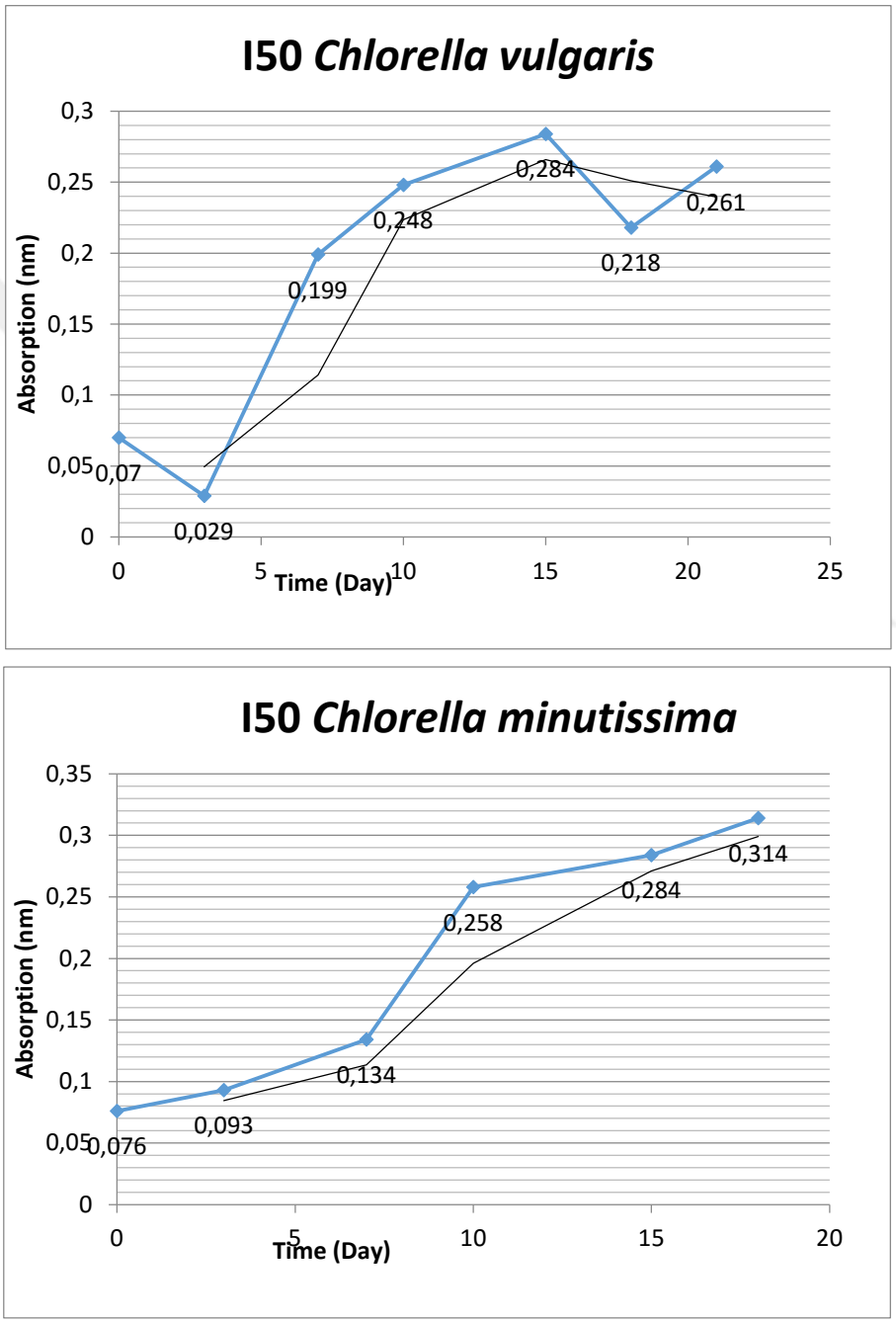


Figure 6.14 Absorbance of I50 samples according to time.

6.1.3 Bold Basal Medium Samples (Control Group) OD Results

In the period of 21 days, Control group samples were incubated in shaker because comparison of all samples for growing rate. Optical density measurement was performed during process regularly. In control group, *Chlorella minutissima* culture results were increased linearly and still continuous at the end of production. On the other hand, optical measurement data of *Chlorella vulgaris* culture were irregular and unusual. Results are shown below;

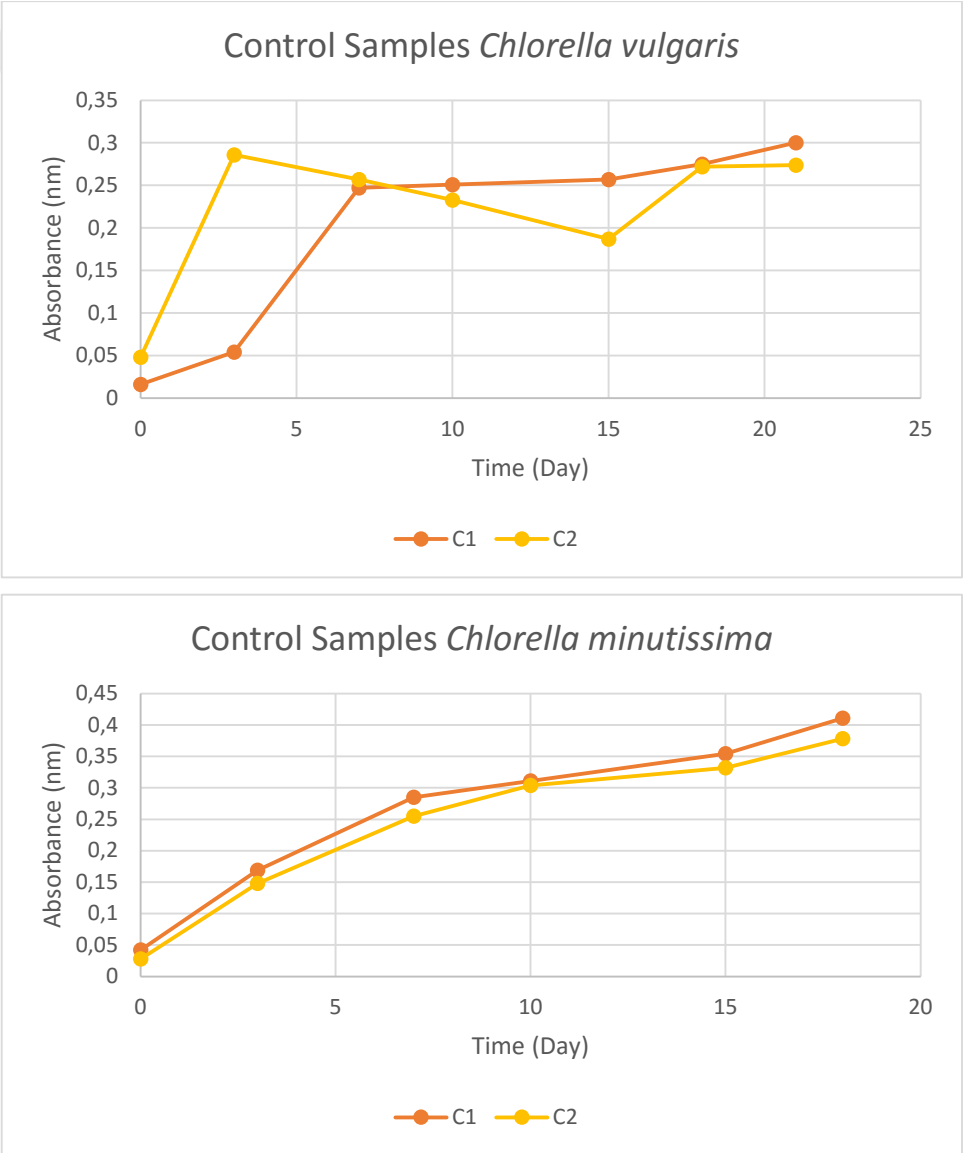
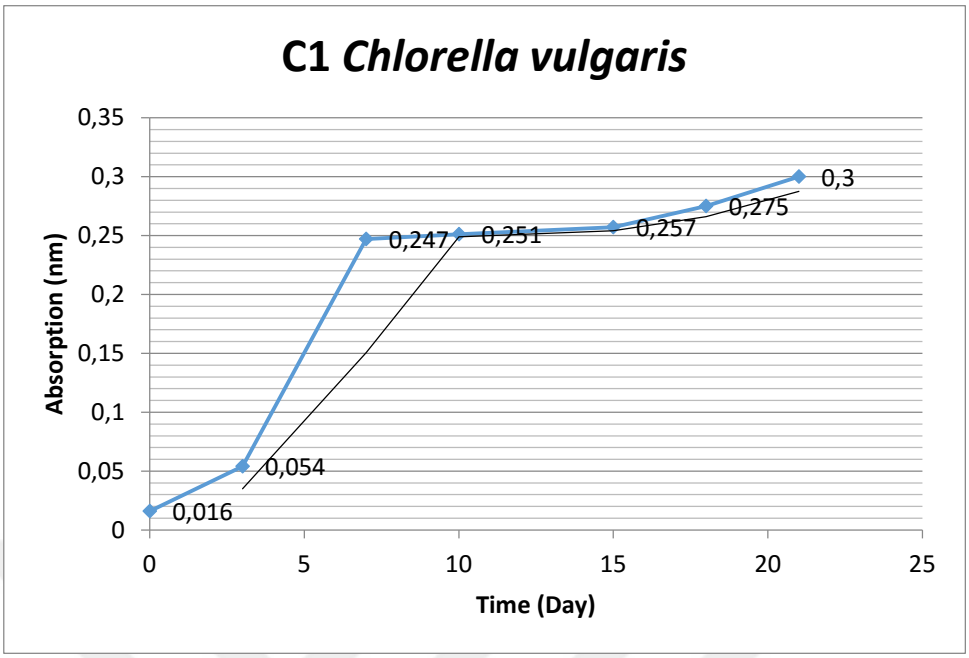


Figure 6.15 Absorbance of C samples according to time.



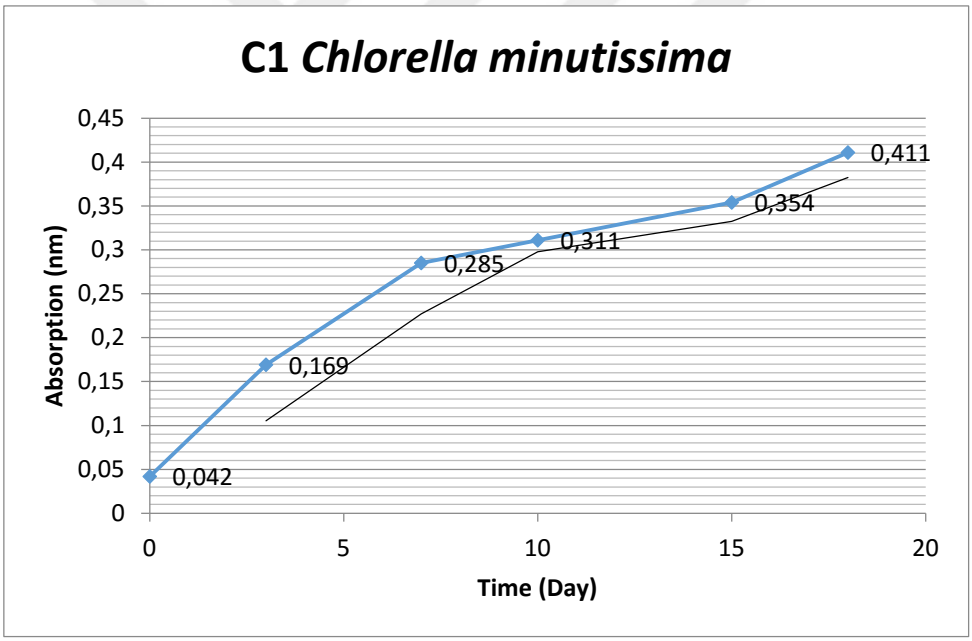


Figure 6.16 Absorbance of C1 samples according to time.

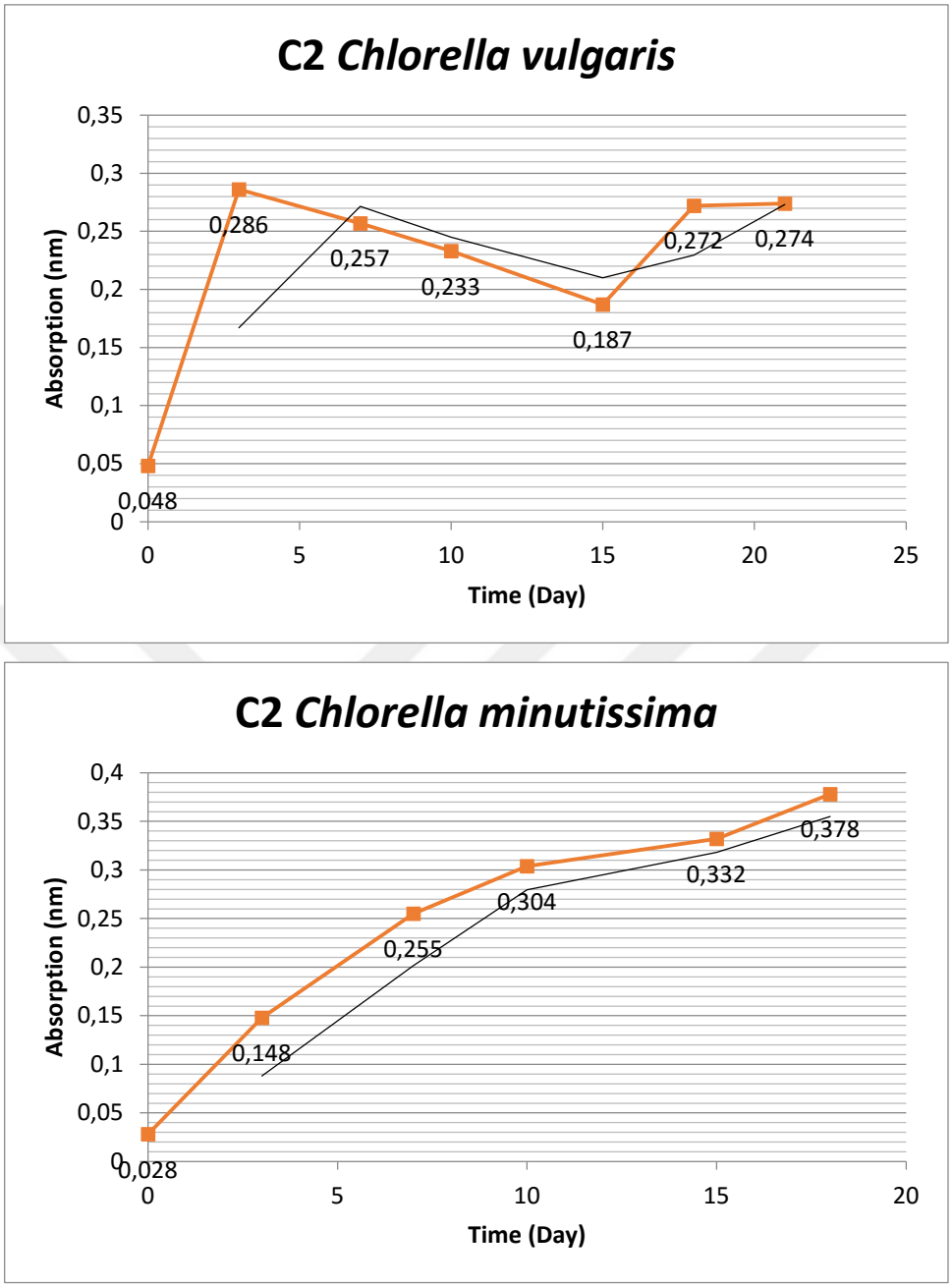


Figure 6.17 Absorbance of C2 samples according to time.

6.2 Total Carbohydrate Analysis

Production process was performed with different medium contents and different production parameters depend on the scale-up process strategies. After production, from the supernatant, samples were taken and total glucose analysis (determination of including glucose amount) was performed. Spectrophotometric results were translated to gram per liter unit using reference data.

These data were prepared with measurement of known concentrated glucose samples and concentration percentage was increase lineally. Reference results were drawn as a figure and linear chart was prepared. Then glucose amount for production results were calculated with slope of reference chart. This measurement was performed 2 times which are during the first and the last OD measurement at 490 nm of process.

In KM samples, the highest glucose production was in %30 concentrated sample in both *C.vulgaris* specie. On the other hand, glucose production rate of *Chlorella minutissima* had the highest level in %40 concentrated culture. Also all KM and I samples gave greater than the control group.

In I samples, the highest glucose production was in %30 concentrated sample in both *C.vulgaris* specie. On the other hand, glucose production rate of *Chlorella minutissima* had the highest level in %40 concentrated culture. Also all KM and I samples gave greater than the control group.

Table 6.1 Total glucose analysis of KM group samples *Chlorella vulgaris*.

Time (Day)	KM5	KM10	KM20	KM30	KM40	KM50
0	0,156	0,135	0,194	0,205	0,244	0,196
21	0,337	0,532	0,592	0,729	0,426	0,655

Table 6.2 Total glucose analysis of I group samples *Chlorella vulgaris*.

Time (Day)	I5	I10	I20	I30	I40	I50
0	0,088	0,125	0,174	0,255	0,247	0,247
21	0,459	0,542	0,843	0,883	0,736	0,829

Table 6.3 Total glucose analysis of C group samples *Chlorella vulgaris*.

Time (Day)	C1	C2
0	0,115	0,139
21	0,444	0,467

Table 6.4 Total glucose analysis of KM group samples *Chlorella minutissima*.

Time (Day)	I5	I10	I20	I30	I40	I50
0	0,091	0,076	0,067	0,112	0,127	0,131
18	0,761	0,755	0,751	0,882	1,009	1,093

Table 6.5 Total glucose analysis of I group samples *Chlorella minutissima*.

Time (Day)	KM5	KM10	KM20	KM30	KM40	KM50
0	0,124	0,138	0,179	0,172	0,186	0,208
18	0,558	0,585	0,604	0,662	0,839	0,917

Table 6.6 Total glucose analysis of C group samples *Chlorella minutissima*.

Time (Day)	C1	C2
0	0,088	0,116
18	0,826	0,883

6.3 Kinetic Modeling with Specific Grow Rate and Doubling Time Calculation

During production, optical density measurement was performed periodically. Specific grow rate and doubling time of cultures were calculated from these optical density data. In this point, initial concentration which was measured at zero time, and maximum concentration of production were used. Logarithmic division of max. concentration and min. concentration was divided by the time which production reached max. concentration. This equation gives specific grow rate and helped to understand cellular growing rare depending on the time. This method was performed on all of KM, I and C samples in both *C.vulgaris* and *C.minutissima* species.

After calculation of SGR, cellular doubling time was measured for each sample. DT can be calculated as Natural logarithm of 2 is divided by specific grow rate. In *C.vulgaris* samples, both KM and I samples had higher SGR and DT data than control group.

This means, wood water can increase growing rate. The best result of DT for *C.vulgaris* was on KM30 sample. Beech, Oak wood mixture is more effective than Iroko wood and also the optimum concentration for microalgae production is determined as %30 concentration in both KM and I samples.

On the other hand, Iroko wood was more affective for growing in *C.minutissima* culture. The highest DT result was in I group instead of KM. Also both KM and I samples gave higher DT and SGR value when compare with control group. The maximum DT value was in %40 concentrated medium in both KM and I samples. This also means that wood water can increase the growing rate of *C.minutissima* species. But later time of production, wood water become inhibitor and growing rate start to decrease. This can be according to increasing glucose level. After a point, glucose amount of medium made a stress to cells and growing had a negative effect. The results of doubling time and specific grow rate were calculated as bellow:

Table 6.7 Doubling time and specific grow rate of KM sample for *C.vulgaris*.

Sample	Doubling Time T_D ($ln2/d^{-1}$)	Specific Grow Rate (d^{-1})
KM5	**10,37134703**	**0,066529449**
KM10	**4,565136225**	**0,151145544**
KM20	**5,643682467**	**0,122260599**
KM30	**13,398579674**	**0,107836432**
KM40	**8,375050728**	**0,082387561**
KM50	**8,21359689**	**0,052218938**

Table 6.8 Doubling time and specific grow rate of I sample for *C. vulgaris*.

Sample	Doubling Time T_D ($ln2/d^{-1}$)	Specific Grow Rate (d^{-1})
I5	**6,528566518**	**0,105689357**
I10	**6,964044735**	**0,099080351**
I20	**5,926826557**	**0,116419806**
I30	**9,887598962**	**0,087479093**
I40	**6,610319683**	**0,149664242**
I50	**7,390328615**	**0,093365266**

Table 6.9 Doubling time and specific grow rate of Control group for *C. vulgaris*.

Sample	Doubling Time T_D ($ln2/d^{-1}$)	Specific Grow Rate (d^{-1})
C1	**4,943378**	**0,139580655**
C2	**4,318373**	**0,082948909**

Table 6.10 Doubling time and specific grow rate of KM sample for *C.minutissima*.

Sample	Doubling Time T_D ($ln2/d^{-1}$)	Specific Grow Rate (d^{-1})
KM5	**5,185166186**	**0,133071916**
KM10	**5,949527696**	**0,115975593**
KM20	**7,770928486**	**0,088792478**
KM30	**7,662361018**	**0,090050573**
KM40	**8,52495438**	**0,065558479**
KM50	**6,225366587**	**0,110836846**

Table 6.11 Doubling time and specific grow rate of I sample for *C.minutissima*.

Sample	Doubling Time T_D ($ln2/d^{-1}$)	Specific Grow Rate (d^{-1})
I5	**6,048310352**	**0,114081448**
I10	**5,069810035**	**0,136099774**
I20	**5,930418304**	**0,116349297**
I30	**6,710514817**	**0,102823706**
I40	**10,781175736**	**0,119352885**
I50	**7,851372247**	**0,087882727**

Table 6.12 Doubling time and specific grow rate of Control sample for *C.minutissima*.

Sample	Doubling Time T_D ($ln2/d^{-1}$)	Specific Grow Rate (d^{-1})
C1	**4,87759175**	**0,142108486**
C2	**3,894609302**	**0,17797605**

6.4 Scale-Up Modeling

In 100 ml small scale production, media optimization and kinetic modeling were performed for the optimum environment on microalgae growth. After determining conditions, large scale production was performed. For this case, 5 L and 20 L photobioreactor were used. In the first scale-up step which was from 100 ml to 5 L, calculated Reynold's number was set a constant for determination of other parameters for 5 L microalgae production.

Table 6.13 Scale-up model parameters for shaken flask and photobioreactors.

Parameter	Shaker flask (100mL)	Photobioreactor (5L)	Photobioreactor (20L)
N (rpm)	150	216	144
Re	295,08	295,08	441,901
v_{tip} (m/s)	-	0,5652	0,5652
N_p	2	2	2,1

Then, impeller tip velocity, shaking frequency (rpm) and power number of stirred tank values were calculated using scale-up related formulas. In this case, Re number of shaker flask set as a constant. Thus, N (rpm) number was rised from 150 to 216 according to calculations. This increase was directly proportional with production volume. When the working volume is increased in process, rpm value must be increased to keep the same conditions in both production such as Re number and stirring speed. After determination of conditions and parameters in theoretically for 5 L photobioreactor, second step of scale-up calculations were performed.

For this reason, some parameters were set as a constant in 5 L production and applied to calculations for 20 L production. In this process, impeller tip velocity (V_{tip}) was determined as a constant which was calculated in the first step. So, N (rpm), Re and N_P values were changed for keep the same production conditions. N (rpm) value was decrease because of the formula which is impeller tip velocity (V_{tip}) can be found with multiplying N number and diameter of tank. Therefore, impeller tip velocity (V_{tip}) set as a constant and diameter of tank was increased from 5L to 20L, N (rpm) value must be decreased to keep production condition.

On the other hand, Re number is increased according to calculations. This is related with photobioreactor diameter, N (rpm) and medium viscosity. According to Re equations, if the diameter of photobioreactor rised and fluid viscosity is nearly not changeable, Re number is

increased directly. So, Re number of 20L photobioreactor has bigger value than 5L one. At the end, all parameters were calculated for determination of 20 L production conditions with using the same formulas in 5L photobioreactor calculations.



Figure 6.18 Photobioreactors for 5L and 20L production.

Scale-up process contains many considerable experimental issues and complex theoretical calculations. Although there were lots of researches and production trials performed, calculated theoretical values, rates and estimations couldn't become a reality for large scale production. To increase the production stability and the percentage of success, literature should be considered and pilot plant production number should be increased. Causing problems and deficiencies can be solved and realized more clearly when more intermediate scale productions are performed. Also theoretical calculations and scale-up strategies should be worked in more sensitive and accuracy. In the future, scale-up process will be more important for industrial biological production and this leads improving of quantification and qualification of products, stabilizing of microorganism growing and media component determination and also concretizing of economical calculations and projects accessibility.

Calculated parameters were determined according to production purpose and working volume. These parameters can increase with purpose, medium and microorganism type. Also theoretically calculations on production parameter and conditions were performed in order to

optimization of process and achieve the maximum efficiency of production in practice. Scale-up modeling calculations and parameters were performed as bellow;

Table 6.14 Photobioreactors dimensions and configurations.

Dimensions and Configurations	Photobioreactor (5L)	Photobioreactor (20L)
***VL* (L)**	**5.00**	**10.00**
Number of impellers	**2.00**	**2.00**
Number of blades per impeller	**4.00**	**4.00**
***Dt* (cm)**	**19.5**	**20.0**
***Dint* (cm)**	**18.5**	**19.0**
***Di* (cm)**	**5.0**	**5.0**
***Ds* (cm)**	**8.00**	**12.00**
***Ht* (cm)**	**27.0**	**70.0**
***Hl* (cm)**	**19.5**	**31.8**
***Hi* (cm)**	**5.5**	**8.00**
***Hi-i* (cm)**	**9.0**	**9.0**
***Hs-i* (cm)**	**3.5**	**5.70**
***Hs* (cm)**	**2.0**	**2.30**

Where;

V_L	: Working volume
D_t	: Diameter of vessel
D_{int}	: Internal diameter of vessel
D_i	: Diameter of impeller
D_s	: Diameter of ring sparger
H_t	: Height of vessel
H_l	: Height of liquid
H_i	: Distance of the low impeller to the bottom of vessel
H_{i-I}	: Distance of two impellers
H_{s-I}	: Distance of the sparger to the impeller
H_s	: Distance of the sparger to the bottom of vessel

CHAPTER 7

CONCLUSION

- Microalgae are a promising source of biofuels and other valuable chemicals. The low cell density and slow growth rate that have traditionally characterized microalgae cultures, however, have resulted in a reduced economic feasibility. To develop a sustainable microalgae process it is required to increase culture productivity, maximize production yield, and reduce production costs. To achieve these goals it is necessary to improve the current understanding of the dynamic behavior of microalgal cultures.
- In this thesis, investigation of different medium additives for microalgae growth was performed. For this case, *C.Vulgaris* and *C.Minutissima* microalgae species were used with separately culture. The temperature, light source, mixing rate and pH values were reached in literature and fixed in each strains. Also the medium was preferred Bold Basal Medium according to literature. On the other hand, different type of three extract water were used for medium additives which are Beech, Oak wood extract water mixture and Iroko wood extract water. These three extract waters were added in with BBM solution with different concentration.
- The aim of this project was understanding the effects of wood extract water on microalgae production. For this case, biomass growing and glycose production were observing during process. All optical density data were measured and evaluated after finishing the process. As a result, experimentally perspective on different kind of medium additives is developed.

- In KM samples, both *C.Vulgaris* and *C.Minutissima* were growth smooth and growing phases such as lag, stationary, log and death can be easily observed on their OD Figures. The most production potential rate was on KM30 group in both two species. After %30 ratio of wood water, caking of microalgae colonies was observed and this could be cause an incorrect result in both OD and glycose analysis. Also cell color were turn into yellowish instead of green in these sample during production.
- This situation explains that, wood water concentration that more than %30 percentage makes a stress on *C.Vulgaris* and *C.Minutissima* species and cells tried to survive and start to produce lipid. That's why, glycose production decreased when compare the control group. Also, *C.Vulgaris* and *C.Minutissima* cell growth were compared each other and it was understood that *C.Minutissima* can better adapted and accommodate oneself than *C.Vulgaris* with Beech, Oak wood extract water mixture medium that can be observed on OD Figures. In both KM samples, there were no contamination observed and it was assumed that production data were reliable.
- In I samples, data were measured likely with KM group. The growing OD Figure of I group was more smooth and linear than KM ones. This was shown that Iroko wood water is more suitable additive for microalgae growth than Beech, Oak wood water. Also, after the %30 percentage of wood water, algal growth could affect negatively and production ration was decreased beside other concentrated wood water medium and control group. On the other hand *C.Vulgaris* could grow faster and fluent than *C.Minutissima*. This case reverberated on OD Figure and glycose analysis data. *C.Minutissima* growing phases were intermix each other on Figure and it was almost linear rising. On *C.Vulgaris* samples, each phases were observed separately and starting and end point of growing phases could be determined distinctly. Also in both I samples, there were no contamination observed and it was assumed that production data were reliable.
- According to production results and measurements, wood water can be used as a medium additives because of including some growing factors, hormones, acids and vitamins. These substances can play a role on cellular growing pathways and reactions. As usual, lower concentration of wood water has no effects on production. On the other hand, higher concentration of wood water has a negative effect on microalgae production. It can be caused by glycose stress or some substance become an inhibitor for growing. The optimum rate was detected as %30-40 percentage. For two type of

wood water, Iroko wood water had better result than Beech, Oak wood mixture. For evaluation the effects on microalgae species, *C.Minutissima* growing has better growing results and adapted quickly than *C.Vulgaris* for both wood water.

- After this experiment, the content of wood water can be researched and substances effects on growing can be observed by separately. This thesis will illuminate the investigation of medium additives and understanding the effects on cell growing.

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PUBLISHMENTS

Conference Papers

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