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OPTIMIZATION STUDY OF NOVEL BIODIESEL PRODUCTION METHODS FROM CHLORELLA PROTOTHECOIDES OIL

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LIST OF SYMBOLS

rpm	Round per minute
KOH	Potassium Hydroxide
NaOH	Sodium Hydroxide
KHz	KiloHertz
MHz	MegaHertz
GHz	GigaHertz

LIST OF ABBREVIATIONS

FAME	Fatty Acid Methyl Ester
GC	Gas Chromatography
YTU	Yıldız Technical University

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ABSTRACT

OPTIMIZATION STUDY OF NOVEL BIODIESEL PRODUCTION METHODS FROM *CHLORELLA PROTOTHECOIDES* OIL

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Department of Bioengineering

MSc. Thesis

Adviser: Assoc.Prof. Dr. Didem ÖZÇIMEN

There is a growing interest in biodiesel as an alternative fuel for diesel engines because of the high oil prices and environmental issues related to massive greenhouse gas emissions. Due to its easy cultivation and productivity, and its high lipid content, microalgal biomass has become a promising biodiesel feedstock. Currently, microalgal biodiesel production is performed with traditional methods which consume a lot of energy and solvents. In order to achieve high yield of biodiesel product without causing more environmental problems, it is necessary to use an alternative method which can reduce the energy and alcohol consumption and shorten the reaction time. There are some researches uses various methods such as supercritical, microwave and ultrasonic assisted methods. In this study, transesterification step of biodiesel production is focused by using novel methods which are microwave assisted and ultrasonic assisted to produce fatty acid methyl esters. The transesterification reactions were performed by using *Chlorella protothecoides* oil as feedstock, methanol, and potassium hydroxide as the catalyst. Effects of reaction parameters such as methanol:oil ratio, catalyst/oil ratio and reaction time on conversion yields of biodiesel product were also studied.The highest methyl ester production by microwave assisted method was obtained with 1.5% KOH catalyst/oil ratio under the conditions of 9:1 methanol/oil molar ratio and 10 min reaction time. The analysis results show that reaction time is the most effective parameter among other parameters. The experimental results showed that high yield of methyl ester was obtained with microwave-assisted transesterification in a short reaction time, efficiently, and this method may be an alternative solution to decrease cost of algal biodiesel production. Similarly, the highest methyl ester production with ultrasonic assisted method was obtained under the conditions of 9:1 methanol/oil molar ratio, 1.5% KOH catalyst/oil ratio, and for reaction time of 40 minutes. It was also found that catalyst/oil molar ratio was the most effective parameter on methyl ester yield according

to statistical data. The results showed that ultrasonic assisted transesterification may be an alternative way to produce biodiesel efficiently and cost effectively.

Key words: Algal biodiesel, *Chlorella protothecoides*, Methyl ester, Transesterification, Ultrasound, Ultrasonic, Microwave

ÖZET

YENİ METOTLAR İLE *CHLORELLA PROTOTHECOIDES* YAĞINDAN BİYODİZEL ÜRETİMİ OPTIMIZASYONU ÇALIŞMASI

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Dizel fiyatlarının ve çevre problemlerine yol açan sera gazı emisyonlarının giderek artmasından dolayı dizel motorlarda kullanılmak üzere alternatif yakıt olan biyodizele olan ilgi gün geçtikçe artmaktadır. Mikroalgler, kolay yetiştirilebilmesi, üretkenliği ve yüksek yağ içeriğinden dolayı gelecek vadeden yeni nesil biyodizel hammaddesi olmuştur. Son zamanlarda mikroalglerden biyodizel eldesi çok fazla enerji ve çözücü kullanımı gerektiren geleneksel yöntemlerle yapılmıştır. Daha kısa sürede daha yüksek verimli, daha az enerji ve alkol gerektiren biyodizel eldesi için yeni metotların kullanılması gerekmektedir. Son yıllarda süperkritik, mikrodalga ve ultrasonik metotları gibi farklı alternatif yöntemler literatürde sunulmuştur. Bu çalışmada yeni metotlardan mikrodalga ve ultrasonik metotları ile yağ asidi metil ester üretimi yapılmıştır. Transesterifikasyon reaksiyonlarında hammadde olarak *Chlorella protothecoides* yağı, katalizör olarak potasyum hidroksit ve alkol olarak metanol kullanılmıştır. Reaksiyon parametrelerinde metanol:yağ oranı, katalizör:yağ oranı ve reaksiyon süresinin ürün verimliliği üzerindeki etkisi de incelenmiştir. Mikrodalga yöntemi ile yapılan transesterleşme çalışmalarında en yüksek verim %1.5 KOH katalizör:yağ oranının 10 dakika reaksiyon süresi içerisinde 9:1 metanol:yağ oranı koşullarında elde edilmiştir. İstatistiksel analiz çalışmaları sonucunda reaksiyon süresi parametresinin diğerlerine göre en etkili parametre olduğu görülmüştür. Bu çalışma sonucunda yüksek verimli metil ester eldesinin kısa reaksiyon süresinde, verimli bir şekilde mikrodalga yardımı ile yapılabildiği ve bu metodun alglerden biyodizel üretimi maliyetlerinin azaltılmasında alternatif bir çözüm olabileceği görülmüştür.

Benzer şekilde, ultrasonik metot ile en yüksek metil ester üretimi 9:1 metanol:yağ, %1.5 KOH katalizör:yağ oranı ve 40 dakika reaksiyon süresi koşullarında elde edilmiştir. İstatistiksel verilere göre katalizör:yağ oranın metil ester üretiminde diğer parametrelere göre en etkili parametre olduğu bulunmuştur. Bu sonuçlara göre ultrasonik yöntemle transesterifikasyonun daha ekonomik ve verimli olmasından dolayı biyodizel üretimine alternatif olabileceği gösterilmiştir.

Anahtar Kelimeler: Alg, Biyodizel, *Chlorella protothecoides*, Metil Ester, Tranesterifikasyon, Ultrasonik, Mikrodalga.

CHAPTER 1

INTRODUCTION

1.1 Literature Review

Recently, microalgae are not economically viable. The main part, which makes the process expensive due to operation and capital costs, are algae growth, harvesting, dewatering, and fuel conversion. Because of this, novel methods like microwave assisted biodiesel production from microalgae are studied to investigate the efficiency. In this study, the novel methods which are microwave method and ultrasonic method used recently as new technology on transesterification are researched. Studies carried out with algae for biodiesel production via microwave assisted process are summarized in Table 1.1. Most of the studies which were used algae as feedstock for biodiesel production via ultrasonic assisted process, were carried out with in-situ transesterification and these studies were summarized in Table 1.2.

Table 1. 1 Studies of microalgal biodiesel production via microwave assisted process

Algal species	Method	Alcohol	Algal biomass ratio:alcohol (wt/vol)	Results (FAME yield %)	Ref.
Nannochloropsis	Direct transesterification	Methanol: Chloroform	1: (1:2)	37.1	[1]
Chlorella vulgaris	In-situ transesterification	Methanol	1:8	58.12 ± 2.84	[2]

Table 1.2 Microalgal biodiesel production studies via ultrasonic assisted transesterification

Algal species	Method	Alcohol	Algal biomass ratio:alcohol (wt/vol)	Results (FAME yield %)	Ref.
Enteromorpha compressa	In-situ transesterification	Methanol	5.5: 1	66.78	[6]
Scenedesmus sp.	In-situ transesterification	Methanol	60:1	71.37	[7]
Nannochloropsis	Direct transesterification	Methanol: Chloroform	1: (1:2)	37.1	[1]
Chlorella vulgaris	In-situ transesterification	Methanol	1:8	58.12 ± 2.84	[2]
Chlorella, sp.	In-situ transesterification	Ethanol	6-9:1	96.2	[5]
Caulerpa peltata	In-situ transesterification	Methanol	12:1	98.11	[8]

Patil *et al.* (2012) made a study on biodiesel production from dry microalgae (*Nannochloropsis*) by using microwave-assisted process. KOH was used as catalyst in the study and microwave condition is set to 800 W and the performance of the study is around 80% [4]. Koberg *et al.* (2011) used *Nannochloropsis* for algal biodiesel production with microwave-assisted method. The higher biodiesel yield which was around 37.1%, was achieved in comparison with sonication technique [1].

Although biodiesel production from algal sources have been reported before, there is not any study on investigation of microwave assisted biodiesel production from *C. protothecoides* and the transesterification parameters on biodiesel yield. In this study, transesterification of *C. protothecoides* oil was studied by using microwave assisted method to examine reaction factors, which affect the purity of the ester product, including the molar ratio of methanol to microalgal oil, the amount of alkaline catalyst, and the reaction time, by using factorial design technique and methyl ester properties of algal product was characterized in order to investigate the potential as a fuel.

In the study of Ehimen et al., in situ transesterification of microalgae by ultrasound technique was investigated. The reaction was carried out in 1 h with the use of methanol/oil ratio to 315:1. The result was 0.295 ± 0.003 g biodiesel/g dry Chlorella which shows that this is higher than mechanically stirred in situ technique [9].

1.2 Objective of the Thesis

This research aims transesterification of *C. protothecoides* oil by using ultrasonic and microwave assisted method and also investigates reaction parameters which affect the purity of the ester product, including the molar ratio of methanol to microalgal oil, alkaline catalyst/oil ratio, and the reaction time by using factorial design technique. Methyl ester properties of algal product were also characterized in order to evaluate its potential as a fuel.

1.3 Original Contribution

Although there are studies reported before about biodiesel production from algal sources, there is not any study on investigation of ultrasonic and microwave assisted biodiesel production from *C. protothecoides* by using statistical evaluation and the transesterification parameters on biodiesel yield. Due to rapid growth rate and high oil content, *C. protothecoides* has been shown that it can be used as biodiesel feedstock. The production with microwave and ultrasonic assisted methods will be helpful for time saving, less beside products and higher amount of yields.

CHAPTER 2

Algae and Biodiesel

Petroleum diesel can be defined as low-sulfur diesel made from crude oil. Biodiesel can be defined firstly as any diesel produced with biomass. Recently, the definition of biodiesel is changed to the diesel made from vegetable oils, animal fats, and alcohols [10].

Biodiesel is a fuel including methyl or ethyl esters fatty acids. The name of biodiesel comes from two concepts. “Bio” stands for the usage of renewable and biological sources instead of fossil sources. Diesel stands for to show availability of using in diesel engines [11].

As it is known fossil fuel stocks for Turkey and all other countries decreasing and getting scarcer. All countries mostly focus on alternative energy sources. Turkey is one of the countries who imports fossil fuels and their energy economy is dependent on others. It is stated by the government that Turkey’s trade deficit is around $100 billion in 2011 and more than half of this amount is caused by energy imports. Turkey is imported petroleum for $25 billion and natural gas for $20 billion in 2011. Government of Turkey has planned to decrease importing of energy sources they put renewable energy in their Vision 2023 agenda. Turkey will be willing to pay huge amounts for renewable energy plants.[12].

Not only the stocks decrease but also demand for the energy is increasing. Global demand for energy is expected to grow by 49% until 2035 [13]. The fossil fuel economy is also unstable because there are environmental concerns, economic dependence and security issues [14].

Since these changes are observed in the world, the trends are shifted through renewable energy. Biodiesel productions from other plant sources haven’t met with the

expectations since they are not feasible and economic to be able to compete with fossil diesel fuel. Thus different sources were looked for [15]. Algae has higher production rate and smaller area is need for their growth compared to oil crops and animal fat. Microalgae use sunlight and CO_2 for biomass production. Then they are used as biofuel feedstocks, fertilizers, foods, and other products. Algae can be used for different types of biofuels production and since the prices for diesel and economic concerns are increased they are preferred to use as biodiesel production. [16].

Three major products can be extracted from algal biomass which are carbohydrates, lipids and proteins. Carbohydrates and lipids mostly preferred for biofuel production and proteins are mostly used as co-product as animal feed [17].

Algae are 4.7 times more productive than the next best option, oil palm, with an areal productivity of 3000 gal per acre. Historically, algal biodiesel has been too costly to produce to make it competitive with biodiesel. This is still a current challenge, however with improving technology the gap is shrinking.

Algal biodiesel can be used in traditional diesel engines, which is why it is a viable alternative to petrol diesel. The process of growing algae naturally captures carbon dioxide from the atmosphere, which is released when the biodiesel is used, preventing new carbon dioxide to be released as is the case with petrol biodiesel. This is a large part if the appeal to this method of producing biodiesel, it is a ‘green’ product. Figure 2.1 shows the proposed process in a simplified form, to emphasize the recycle of materials and carbon dioxide.

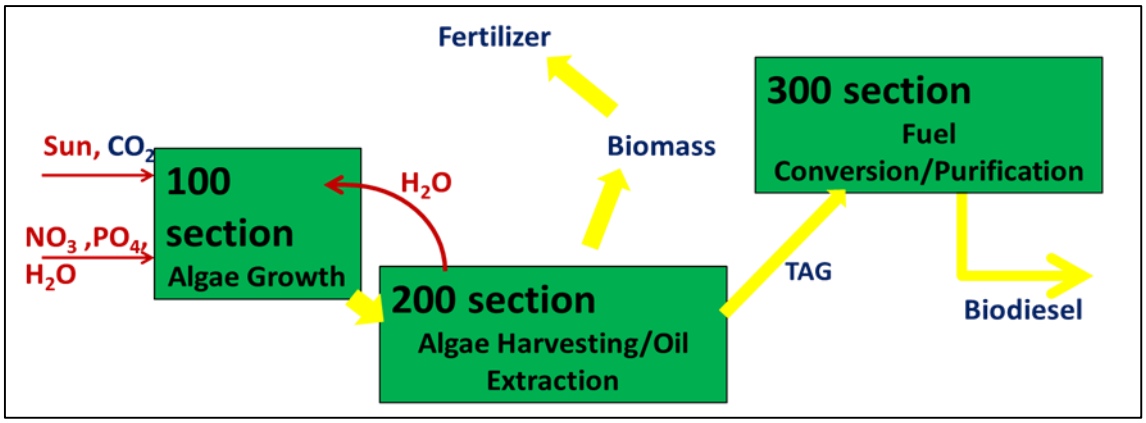


Figure 2. 1 Simple biodiesel production process scheme with CO_2 and nutrition.

A main process for algal biodiesel includes algae growth, cultivation, oil extraction, transesterification and purification steps. This process makes algal biodiesel to be expensive and most of the cost caused by extraction problems. However, most of other steps like algae drying, chemical transesterification, and purification also make process costly. That is why different types of methods for algal biodiesel are studied to make it cost-efficient [14],[16].

2.1 Properties of Biodiesel

Biodiesel is a product produced by reaction of alcohol including short chain (methanol or ethanol mostly) with plant (canola, sunflower, soybean, algae,) or animal fat with catalyst. Biodiesel doesn't include any fossil; however, they can be used as fossil fuel by mixing with fossil fuel in some ratios. Pure biodiesel can be used in diesel engines with some minor modifications in the engine. Moreover, they can be used without any modification with engine if they are mixed with petroleum based diesel.

Some terms are used to define ratio of biodiesel in the fuels. These are listed as:

- B5 : 5% Biodiesel + 95% Diesel
- B20 : 20% Biodiesel + 80% Diesel
- B50 : 50% Biodiesel + 50% Diesel
- B100 : 100% Biodiesel

Biodiesel doesn't have any toxic effect. If biodiesel is incepted, the dosage cause to death is 17.4 g biodiesel/kg body weight. The results of the test done on humans for

touching biodiesel with hand shows that the toxicity is around 4% which is less than soap.

2.2 Advantages and Disadvantages of Biodiesel

Biodiesel is non-toxic environmentally friendly renewable source which is also biodegradable. Biodiesel production helps to improve local economies since the infrastructure of the fuel system changes. Greenhouse gas emissions will be reduced so that CO_2, SO_2, CO and some other gasses will be emitted less compared to petroleum based diesel. There will be less shipping cost if it could be produced locally. It is a good source for countries who depends other countries on energy. The flash point of the biodiesel is higher than petroleum diesel, thus it can be said to be a safer fuel. No modification is required for B_{20} but higher rates require modifications in the engine. Biodiesel damages less compared to diesel since it has feature of lubricator. Furthermore, by using biodiesel noise, smoke and vibration will be less [18], [19], [20], [21].

In contrast, biodiesel also have some flaws. It emits higher rate of NO_x to the atmosphere compared to regular diesel. It may be corrosive for the materials of copper and brass. The viscosity of biodiesel is higher than conventional diesel. It has lower volatility. Moreover the pour and cloud points might be problem in cold weather. [18], [19], [20], [21].

2.3 Comparison of Diesel and Biodiesel

Viscosity is one of the most important parameter for fuels. When the viscosity is increased fluidity of the fuel decreases and it is not preferred for fuel injection systems. The size of particles which is spray on combustor is significant. Smaller particles are easier to mix with air, heat and ignite. Especially at low temperatures, viscosity is increasing which affects the fluidity and that also affects the fuel injection system that damages to spray system. High viscosity requires more powered pump. When the chains of hydrocarbons get longer, viscosity is also increased. More double bonds in the chain decrease the viscosity. The efficiency of burning petroleum diesel depends on the amount of sulfur, and aromatic hydrocarbons, metals, raw petroleum wastes In contrast,

with biodiesel there is none of these issues. Moreover, the density and viscosity of biodiesel is very close to petroleum diesel [22], [23].

2.4 Biodiesel Production Methods from Oil

2.4.1 Traditional Method

Biodiesel is produced by transesterification of oils. Transesterification is accomplished by the chemical reaction where oil and alcohol (ethanol or methanol) mixed in the presence of catalyst to produce fatty acid esters and glycerol. This process called methanolysis when methanol is used and ethanolysis when ethanol is used. No matter which alcohol is used the ratio for the transesterification is same [24].

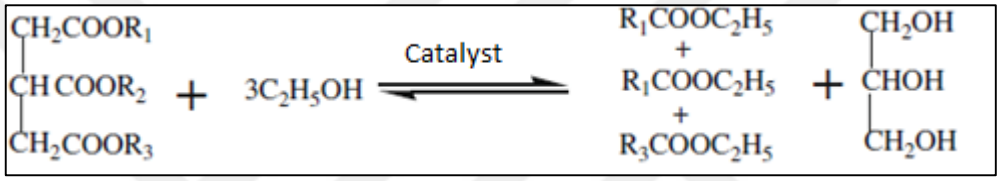


Figure 2. 2 Ethanolysis reaction [24]

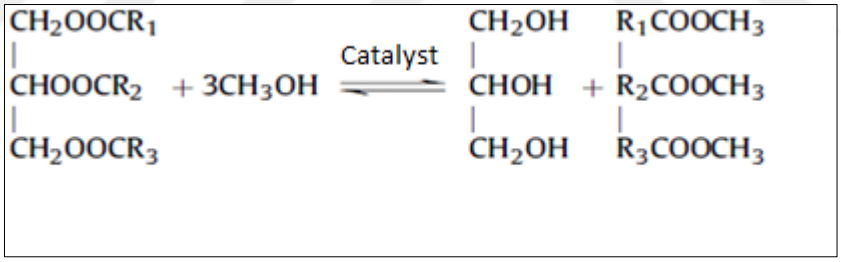


Figure 2. 3 Methanolysis Reaction [24]

In these reactions the lowest ratio of oil: alcohol must be 1:3. To be able to convert all oil to fatty acid esters the alcohol might be more than the ratio. This reaction is a reversible reaction. The mechanism of the reaction follows the chain reactions of converting triglycerides to diglycerides, then diglycerides to monoglycerides and finally monoglycerides to esters (biodiesel) and glycerin (by-product). In all three reactions fatty acid esters are produced. The reactions and production of fatty acid esters can be seen in Figure 2.5 [25] ,[26]

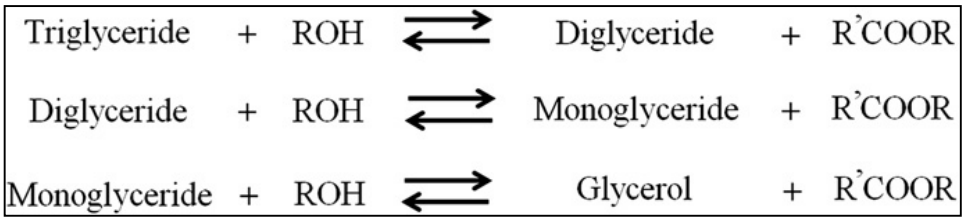


Figure 2. 4 Converison of oil to alcohol and fatty acid esters with alcohol. [25].

When the process is completed the product might have some impurities such that remaining monoglycerides, diglycerides and triglycerides. They cause to crystallization and affect the pour and cloud point of esters [25].

There are some other parameters plays significant roles in efficiency of transesterification reaction. These are: type of oil, humidity and free fatty acid, type and of catalyst, type and amount of alcohol, mixing, heating and reaction time.

2.4.1.1 Type of Oil

Raw material of biodiesel is important since the reaction depends on types of fatty acids and their ratios. Also sometimes oil has some impurities inside which are also affecting the efficiency. For example, waste cooking oil is used as biodiesel raw material and the efficiency of the oil is less than some others since it has some impurities in raw material.

2.4.1.2 Humidity and Free Fatty Acid

The amount of free fatty acid has to be less than 3% in base-catalyst used transesterification reactions. Increase in the acidity of may cause to decrease in the conversion rates. If the amount of the catalyst is much higher than required, it causes to saponification. The water inside the oil causes to saponification during the transesterification reaction which also reduce the activity of catalyst. It is not beneficial also since separation of glycerin gets harder. Moreover it is also caused to gel formation and increase in viscosity [27]. It also requires washing the product several times with warm water. This is an extra expense for the process.

2.4.1.3 Type of Catalyst

There is several catalysts that can be used in transesterification reactions. Catalyst can be alkali, acid, enzyme, or heterogenic. Mostly preferred and most effective catalysts between those are alkali catalysts such as sodium hydroxide, potassium hydroxide, sodium methoxide or potassium methoxide. Alkali catalyzed reactions are faster than acid-catalytic transesterification reactions. Reactions can occur in low temperatures with a small amount for catalyst and Algal Biorefinery for Biodiesel Production with little or no darkening of color of the oil. However they are sensitive to water and free fatty acids [28].

There are some studies on microalgae oil to produce biodiesel by using alkali transesterification. One of the study is done by Velasquez-Orta et al. on biodiesel production from *Chlorella vulgaris*. In that study, alkali transesterification was used for conversion and they achieved 71% of FAME yield [29]. Another study was done by Ferrentino et al. on biodiesel production from *Chlorella sp.* oil and they used alkali catalyst in transesterification. They have obtained high yield from their experiment [30]. In another study, Carvalho et al. used alkali catalyst for transesterification for biodiesel production from algae oil. In their study, *Chlorella emersonii* oil was used and they have obtained 93% conversion yield [30].

If some special oils used for the production like if the oil includes high content of free fatty acids and water, acid catalysts can be preferred. This is because alkali catalysts can give faster saponification reaction with fatty acids. When the free fatty acid content is higher than 3%, acid catalysts are used and esterification between free fatty acids and acid catalysts occurs. This is mostly applied as pre-reaction if animal oil or waste oil is used as source [31], [32].

In the acid-catalytic sulfuric, phosphoric, hydrochloric and organic sulfonic acids can be used as catalysts. Very high yields can be obtained by acid catalysts. These reactions need the use of high alcohol-to-oil molar ratios in order to obtain good product yields in practical reaction times. But ester yields do not proportionally increase with molar ratio, and the reaction time is very long (3–48 h) [28], [33], [34]. Xu et al. studied the acid transesterification of microalgae (Heterotrophic *C. Protothecoides*) oil. They used methanol as alcohol and achieved 80% of FAME yield [35].

Johnson studied on *Schizochytrium limacinum* and used acid catalyst for transesterification and he achieved 82.6% of biodiesel yield.

There are some problems like recovery of glycerol or removing catalysts from product and need of wastewater treatment in acid or alkali-catalytic transesterification. Enzymatic catalysts like lipases are able to catalyze the transesterification of triglycerides effectively. With this process glycerol can be easily recovered however enzymatic catalysts are often more expensive than chemical catalysts. The high cost of enzyme production is the main problem to the commercialization of enzyme-catalyzed processes. But using solvent-tolerant lipases and immobilized lipases can be a solution for this. Lipase catalyzed transesterification considered to be one of the most effective reactions for production of biodiesel [28]. In another study Tran et al. used microalgae oil (*Chlorella vulgaris ESP-31*) for producing biodiesel. Their method was enzyme-catalyzed transesterification which lipase used in this process. In the result, they reported that they achieved 94.78 % of FAME yield [36].

2.4.1.4 Oil: Alcohol Ratio and Type of Alcohol

Alcoholysis reaction uses three moles of alcohol and one mole of triglycerides to produce three moles of fatty acid esters and one mole of glycerin. This reaction is a reversible and to forward it excess amount of alcohol is needed. On the other hand, excess amount of alcohol increase the dissolubility which causes to problems for the separation of glycerin. If glycerin dissolved in the solution, the efficiency for ester conversion is reduced because the reaction moves toward reactant site [37].

Most preferred alcohols for transesterification methyl and ethyl alcohols even though butanol and isopropanol can be used. The reactions require mixing to increase mass transfer since methanol and ethanol is not mixed with triglycerides in ambient temperature. It is easy to see the emulsions forming during the reaction. In methanolysis reactions these emulsions are destroyed easily and faster to form lower glycerin and higher methyl ester phase. However, in ethanolysis reactions these emulsions are more stable and which cause to problems in separation and purification steps [37].

2.4.1.5 Reaction Time and Heat

Time also affects the reaction efficiency because the boiling temperature of oil and alcohol changes the rate of transesterification. That is why depending on type of alcohol and oil, the temperature should be set for higher efficiency. If the amount of alcohol in the reaction decreases, the efficiency gets lower also. It should be remembered that alcohol is a volatile and has to be kept in the solution during reaction.

Time is another important parameter for reaction efficiency. The aim is to have shortest time hest rates. If the reaction is not completed within the time the efficiency will decreases but if the reaction time is longer than the required time, efficiency may not change however the cost of the reaction will increase and will not be feasible. Thus, it is important to determine time required for each reaction using different alcohols and different oils.

2.4.1.6 Mixing

Oil is not mixed with methanol and sodium hydroxide solution. To be able to run the reaction properly mixing is needed. Since the reaction is started and the two phases are mixed no more mixing is needed [38], [39].

2.4.2 Microwave Assisted Method

Microwave-assisted process or microwave irradiation is the most yield efficient method and it requires the least reaction time. In this method, microwaves activate differences in small degrees of polar molecules and ions, because the molecular friction and chemical reactions start. Molecules have not the enough time to relax and heat generation occurs in a short time because energy interacts with molecules very quickly. Even though this technology have some significant advantages, there are some disadvantages also. For the usage of biodiesel production, higher columns should be available to process. However microwave mostly used in small scale productions like in laboratories, therefore using in large scales in industry is a flaw for this technique. Penetration depth of MW radiation into the absorbing materials, which is only a few centimeters, depending on their dielectric properties, it the most significant limitation of the scale up of this technology. Another disadvantage is the absorption of waves can change up to dielectric properties of materials. Furthermore, safety issues are also

disadvantages for the microwave technology [40].

Microwaves are between infrared and radio waves in spectrums. They are electromagnetic waves occurred with combination of electrical and magnetic field. The ranges for microwaves are between 300 MHz to 30 GHz. In researches, 2.45 GHz is mostly used. There are some ranges for microwave bands. It covers the bands of L (1-2 GHz), S (2-4 GHz), C (4-8 GHz), X (8-12 GHz), Ku (12-18 GHz), K (18-26 GHz), Ka (26-40 GHz), Q (30-50GHz) [41], [42].

Since microwaves heats with irradiation, each material is not suitable to receive the irradiations for heating. There are some materials that available for these applications which are sulphur, teflon, glass, plastic, paper, exc. [42]. There are some materials which doesn't absorb the heat and reflect the microwave energy so that they don't give any reactions. However recently, it is thought that metal particles also give reaction with microwave irradiations [43].

A material including chemical bonds has average bond energy of 84-335 kJ/mol. However microwave photons have energy of 0,123 kJ/mol. Thus it can be said that microwave irradiations is not able to destroy the bonds or ionize them. Even though the researches are made with 2450 MHz power, it only causes to spinning of molecules. However, the irradiations can move easily within the samples. Since the molecules' kinetic energy is increased it is much easier to exceed activation energy which also helps the reaction to be done faster [42].

Microwave is an alternative method for traditional method. It enables to have transesterification reactions in a shorter time with higher efficiency. Recently, since microwave technology is improved and easily controlled, it became a novel method for transesterification reactions. Instead of heating with traditional methods, microwave irradiation is used as a heat source for transesterification also. Microwave heating is more effective than traditional way of heating so that better results can be obtained for this application [41]. Moreover, this technology is also useful for the reactions which requires high activation energy [44].

2.4.2.1 Microwave Heating Mechanism

Microwave includes two parts one is magnetic field and another is electrical field which is perpendicular to magnetic field. Electrical field is used for supplying the heat which microwave irradiations diffuse [45].

The main idea behind microwave is to stimulate polar molecules and ions. This simulation has to be done under an electrical and magnetic field. The transfer of heating in microwaves does not occurred by conduction like traditional heating. In microwave, it depends on dielectric properties of materials. If the material has high dielectric constant, it can be heated up faster by the mechanisms of polarization and ionic conduction [45].

Dipolar polarization is the process of generation of heat in polar molecules. If there is a minor difference in charges in atoms, it causes to polarization in the molecules and make it dipole. For instance, the difference on charges of hydrogen and oxygen molecules makes the water molecule dipole. Slight charge difference between two atoms causes to dipole. Net electricity dipole is the sum of dipole vectors. In benzene, the sum of dipole vectors is equal to zero so that benzene doesn't have a polar infrastructure which also results in that it doesn't get heated under microwaves. However, water molecules are heated under microwaves because it has oxygen and two hydrogen molecules which the sum of dipole vectors is not zero [46].

Dipoles are sensitive to external electric field and they rotate to get in a straight line [45]. The amount of heat is related to frequency of waves. The other factor also affects the amount of heat is the capability of dipoles to get in line and the polarity of dipole. If the wave's frequency is low than the heat received will be less also because dipoles have the time get in line. For more frequent waves, magnetic field dipoles don't heated because they don't have enough time to rotate so that they are not able to transfer the heat. 2450 MHz is the wave which gives time for magnetic field change and the response of dipoles. In other words, polarization goes slower than the change in electric field. Thus, the movements of molecules cause to collision and friction with other molecules and generate heat [47]. Polar molecules and polar solvents make the reactions more reactive so that dipolar reactions run much faster under microwave conditions.

2.4.3 Ultrasonic Assisted Method

Ultrasonic assisted process is a method that meets green chemistry principals and has a wide application area in chemical processes including biodiesel production. Mixing is a significant parameter which affects biodiesel yield in transesterification reactions. Powerful mixing creates smaller droplets than the conventional mixing and increases the contact areas between the oil phases. Required activation energy is also provided by this effective mixing for initiating transesterification reactions. Ultrasonication is an effective mixing method which provides needed activation energy and better liquid-liquid mass transfer [48]. Ultrasonic bath and ultrasonic probe are usually operated at a fixed frequency and mainly used as ultrasonic apparatus. The frequency varies according to the device of transducer which is 20 kHz for probes and 40 kHz for bath. Ultrasonic assisted transesterification of oil has significant advantages in comparison with conventional stirring methods such as; reducing the reaction time, increasing the rate of chemical reaction and decreasing the molar ratio of alcohol requirement, increasing the yield and conversion. Production of biodiesel via ultrasound process is effective and economically functional method due to its time and energy savings [49].

2.4.3.1 Ultrasonic Probe Mechanism

Power Generator convert the 120/20 V and 50/60 Hz electricity to 35000 Hz high frequency direct current. This current is converted to a mechanical vibration by a converter. Thus, the electrical energy is converted to mechanical energy (sound-waves). These sound-waves increases the pressure in non-flow liquids causes to press and tension between molecules. Therefore, high pressure and low pressure fields occurs in the solution and causes to pressure on molecules and bubbles appears on the liquid. Interaction of small bubbles with ultrasonic affect absorbs the energy from sound-waves. The resonance frequency of the small bubbles absorbs stream and gas from the environment and keeps growing until it reaches the same frequency of sound-waves. These bubbles created by ultrasonic effect are not stable because of the other occurred bubbles around. As a result of this, some bubbles gets bigger and bigger and blast because of unstable form. This blast may call as a cavitation activity which results with a heat effect (5000 °C) on a point and propagation of shock waves in all direction. The blast of bubbles creates a chemical and mechanical energy effect [50].

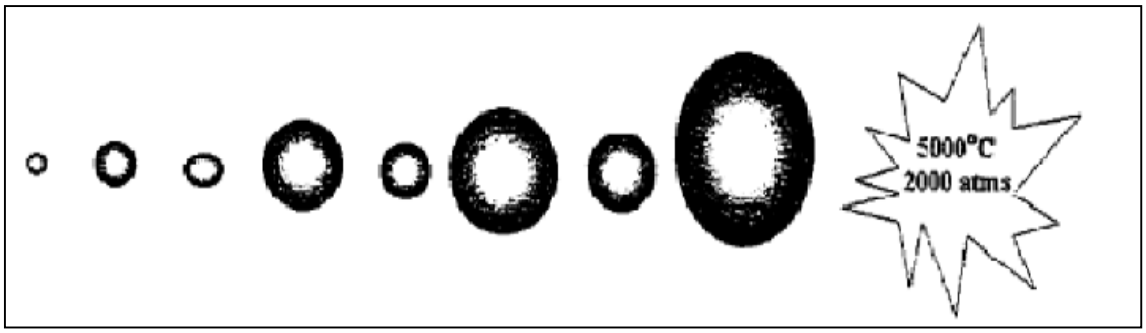


Figure 2. 5 Scheme of bubble creation and blast [50]

Each bubble is a reactor which creates more than 1000 atm pressure and a few thousands °C temperature in the liquid at the moment it blast. Since the temperature created is instant, the cold liquid around absorbs the heat. The temperature of the liquid is not increased suddenly while the lifetime of the hot point is too short. The temperature and pressure of the point where blast occurred is approximately 5000 °C, 1000 atm and the life time is less than a microsecond [51].

The advantages of transesterification reaction with ultrasonic method:

- Less reaction time
- Less energy consumption [52]
- Less usage of methanol required
- Simpler method compared to others [53]

2.5 Algae Strains and Properties

Algae are simple organism which includes chlorophyll. They can be divided into two groups mainly as micro and macroalgae. Macroalgae also divided into groups of green, brown, and red. Microalgae are phytoplankton and divided into groups of diatoms, dynoflagellates, green and brownish flagellate and blue-green algae. Algae are mostly seen in oceans, seas, and lakes and soils [54].

There are millions of different algae in the world. However, there is no standard for categorizing them. One of the classification is Archaeplastida, Chlorophyta (green algae),Rhodophyta (red algae), Glaucophyta, Chlorarachniophytes, Euglenids, Heterokonts, Bacillariophyceae (diatoms), Axodine, Bolidomonas, Eustigmatophyceae, Phaeophyceae (brown algae), Chrysophyceae (golden algae), Raphidophyceae,

Synurophyceae, Xanthophyceae (yellow-green algae), Cryptophyta, Dinoflagellates, Haptophyta [55].

2.5.1 Macroalgae

Macroalgae are mostly observed in coastal areas of seas and oceans. Types of macroalgae are determined based on their pigments such as brown, red, and green macroalgae. Between those some brown and green algae types used for biodiesel production [56].

Brown algae have two pigments, xanthophyll and fucoxanthin, which dominates others and gives brown color to the algae [57]. As it is mentioned, algae include carbohydrates, proteins and lipids. Brown algae mostly stores polysaccharides and higher alcohols in their biomasses and the main carbohydrate reserve in them is laminarin. Brown algae can serve different benefits. They have several features such as cytotoxic and antitumor, antioxidant, anti-Inflammatory, antiviral and antifungal activity [58], [59]. Their storages can change according to type of species, location, salinity and seasons [60].

Green macroalgae (figure 2.6) include the pigments of chlorophyll A and B which gives green color [54]. Green macroalgae can store higher amounts of carbohydrates and this makes them more efficient for bioenergy production. Moreover, they also have higher cellulose content [56]. They also have a lot of features like anti-inflammatory substances, cytotoxic and immunosuppressive activities, antibacterial activity, antiviral activity, antifungal activity [61].



Figure 2. 6 An example for gren algae image. [62]

Red algae (Figure 2.7) include phycoerythrin and phycothcyanin pigments which give red color to organism by dominating other pigments. The cell walls of these algae made of cellulose, agar and carrageenan [57]. These type of macroalgae is considered as the most important active metabolite resource and there is almost 8000 red algae species. They also have similar features to green and brown algae such as: Cytotoxic activities, antiviral activity, anti-inflammatory activity, antimicrobial activity, free radical scavenger activity [54], [63].



Figure 2. 7 An example for red algae image [62]

2.5.2 Microalgae

They are unicellular photosynthetic cells but because of some complexes they create larger colonies. They are neither prokaryotic organisms like bacteria nor eukaryotic cells. They grow in salty and fresh water. There is more than 3000 microalgae species in the world [60], [64].

Diatoms and green algae (Figure 2.8) are mostly used as biofuel source. Green algae are *Chlorella, Spirulina, Dunaliella and Haematococcus. Dunaliella* is the dominant one in the sea and they are mostly cultivated for high value protein production [60]. Blue-green algae which are called cyanobacteria have a lot of common structure with bacteria. They are called as algae since they contain chlorophyll and other components. They have also nitrogenic components because all of the prokaryote species convert atmospheric nitrogen to ammonium [54]. Blue green algae can have three different shapes that are conical, unicellular or filamentous. Similar to other microalgae they also

have some futures like anticancer and cytotoxic activities, antibacterial activity, antifungal activity, immunosuppressive activity [54], [66], [67].



Figure 2. 8 An image for green microalgae under microscope [68].

Dinoflagellates are classified as primitive algae and unicellular. If they reach to high concentrations in the surface of oceans, they are caused to death of fishes. They give brown to red color in the sea. [65], [69]. If these microalgae is consumed by some species like shellfish and humans consume the shellfish, it may end up with serious health problems in humans [54].

Diatoms are the most versatile and mostly seen frequently. They are feasible for large-scale biodiesel applications since their doubling time is short and they are easy to grow. Moreover they produce less secondary metabolites compared to dinoflagellates [70].

Microalgae are one of the most preferred biodiesel stocks since they can produce lipids and have high photosynthetic efficiency. However, Macroalgae are not able to store high content of lipids. They mostly used for natural sugars and other carbohydrates that they contain. These carbohydrates may be used for alcohol-based bioenergy.

2.5.3 Lipid content of Microalgae Species

Algae have capability of accumulating high content of lipids. The average lipid content can reach to 77% of dry mass under certain conditions. However if the conditions are not set well for lipid production the average lipid can be around 10-15 %

[71]. Table 2-1 shows lipid contents and of some mostly selected algae strands.

Table 2. 1 Lipid content of some microalgae species

Microalgae	Oil content (dry weight %)
Botryococcus braunii	25-75
Chlorella protothecoides	14-57
Crypthecodinium cohnii	20-51
Dunaliella tertiolecta	16-71
Nannochloris sp.	20-56
Neochloris oleoabundans	29-65
Phaeodactylum tricornutum	18-57
Schizochytrium sp.	50-77
Skeletonema coastatum	13-51

Lipid content and fatty acid composition of microalgae can change depending on several parameters. These parameters can be available nutrition, environment, and growth conditions. The structure of fatty acid affects the process of production. For example in biodiesel production microalgae is needed to include high concentration of unsaturated fatty acid because others reduces the efficiency of esterification of biodiesel

[71]. Note that the goal of this step is to produce as many triglycerides as possible, not as much algae as possible. It is found that under ‘stress’, microalgae will store energy in the form of triglyceride liquid droplets inside the cytoplasm [72]. Because of this, control of the algae’s metabolism is necessary also. There are many difficulties involved in growing algae. Due to the nutrient-rich nature of the media, contamination with competing species and predators is unavoidable at the scale required for the process. Peak cell densities are limited by carbon dioxide transport into the media and optical density [73].

That is why selecting algae is one of the significant processes. Selected algae type affects each steps of biodiesel production process. For example the site that algae growth is important for the environment and conditions. Sizes of microalgae may cause difficulties for the steps of harvesting, dewatering, filtration and oil extraction. For the esterification step, the structure of algae affects the efficiency.

CHAPTER 3

Material and Methods

3.1 Materials

Algae oil is used as raw material during the experiments which are stored in 1L plastic bottles. Algae oil is obtained from a company. The characteristics of *Chlorella Protothecoides* oil is given in Table 3.1. 99.5 % pure Methyl alcohol (Merck), and KOH (Merck) are used for transesterification process.

Table 3. 1 Characteristics of *C.protothecoides* oil

Property	Unit	Result
Density at 15ºC	kg / m³	867
Viscosity at 40ºC	mm² / s	3.8
Flash point	º C	124
Carbon residue (on 10% distillation residue)	% (m/m)	0.2
Total contamination	mg / kg	2
Oxidation stability, 110ºC	Hours	12
Calorific Value	MJ/kg	37.49
Acid value	mg KOH / g	0.3
Iodine value	mg KOH / g	47
Sulfated ash content	% (m/m)	0.01
Water content	mg / kg	80
Methanol content	% (m/m)	0.08
Sulfur content	mg / kg	2
Phosphorus content	mg / kg	3
Triglyceride content	% (m/m)	0.02
Free glycerol	% (m/m)	0.008
Total glycerol	% (m/m)	0.02

3.2 Devices

For ultrasonic biodiesel assisted transesterification reactions, Bandelin Sonoplus (Germany) (Figure 3.1), for Microwave assisted transesterification reactions, Milestone Microsynth microwave (USA) (Figure 3.2) is used. For methyl ester analysis, gas chromatography is used (Figure 3.3).



Figure 3. 1 Bandelin Sonoplus Ultrasonic Homogenizator

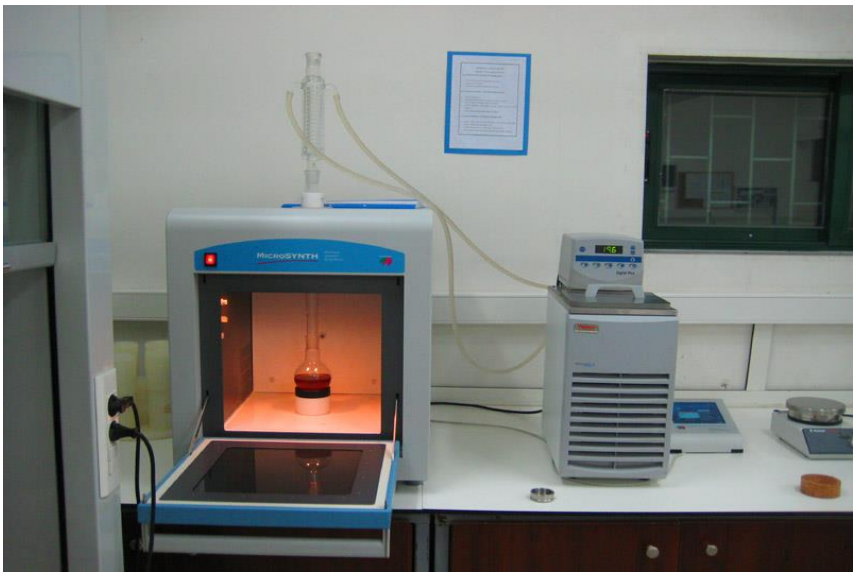


Figure 3. 2 Milestone Microsynth microwave



Figure 3. 3 Gas Chromatography

3.3 Biodiesel Production Methods

The experiments are performed with two different methods which are ultrasonic assisted biodiesel production and microwave assisted biodiesel production. In ultrasonic biodiesel assisted transesterification reactions, Bandelin Sonoplus (Germany) (Figure

3.1) ultrasonic homogenizer is run with 20 kHz frequency and 200 W power. For Microwave assisted transesterfication reactions, Milestone Microsynth microwave (USA) is run with single mode operating system at 2.45 GHz with adjustable power between 1-1000. To be able to have a constant temperature in solution, magnetic nucleus is used and stirred at 400 rpm.

3.4 Experimental Procedure

Transesterification reactions for both microwave assisted and ultrasonic assisted methods are conducted with different methanol:microalgae oil ratios and different amount of catalyst to be able to determine the most efficient biodiesel production conditions. Three parameters are mainly varied and compared which are methanol:microalgae oil ratio, catalyst ratio and time.

Transesterification reactions are performed with 3:1, 6:1 and 9:1 methanol microalgae oil ratios, 0.5 wt. %, 1 wt. % and 1.5 wt. % catalyst(KOH):microalgae oil ratios during the reaction times 20, 30 and 40 minutes for ultrasonic assisted method and 1, 5 and 10 minutes for microwave assisted method.

3.4.1 Ultrasonic Transesterification

Ultrasonic transesterifications are conducted with 250 mL of Erlenmeyer. In each experiments 50 g *Chlorella prothetotocoides* oil is taken into the flask by weighing with precise scale. Then catalyst and alcohol is added into flask in required amounts. The Ultrasonic Homogenizator probe is placed into the solution while in contact with the solution and without touching to bottom. The reactions performed for the variables methanol:oil ratios, catalyst:oil ratios and time as it is mentioned above.

3.4.2 Microwave Transesterification

The reactions are performed as it is shown in Figure 3.4. In experiments 50 g of microalgae is used to be able to have the oil at the same level of thermal sensor of the device. Methyl alcohol and catalyst solution is prepared in required amounts and then added to 50 g weighted microalgae oil into round bottom flask. Then the round bottom flask is placed into microwave. Reaction temperature is set 64 °C. The reactions performed for the variables methanol:oil ratios, catalyst:oil ratios and time as it is

mentioned above. The product in the round bottom flask which is obtained after microwave procedure is placed into ice bath.

To be able to mix the solution magnetic stirrer is dropped into flask. Reaction is started on heated magnetic stirrer while stirring at 500 rpm and 64 °C constantly. 64 °C is constantly provided by using a water bath on heated magnetic stirrer. Cooling mechanism is placed at the top of flask just in case of alcohol escape.

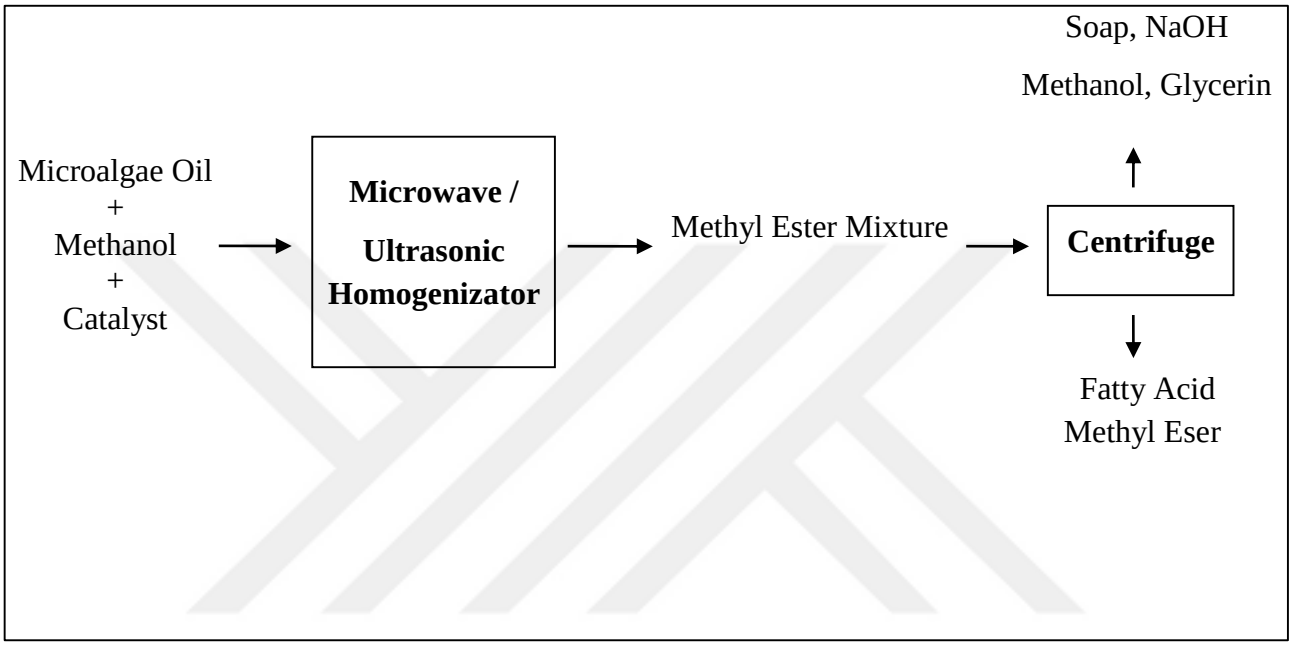


Figure 3. 4 Process Diagram

3.4.3 Purification

The product of transesterification reaction contains fatty acid methyl ester, glycerin, and methanol and catalyst which is not react during transesterification. Since the transesterification is completed the mixture directly taken into tubes to be centrifuged to be able to separate methyl ester from others. Centrifuge is run for 5- 10 minutes with 5000 rpm. The mixture will have two different phases after centrifugation and the bottom phase contains pure methyl ester and top phase called supernatants contains other chemicals, by products and other impurities. To be able to have more purified fatty acid methyl ester the washing and drying processes is achieved a couple times. However the degree of purity of methyl ester after washing and drying process was almost same as the results obtained before. This shows that the obtained methyl ester is pure enough to be analyzed. Thus washing and drying processes are not required.

3.5 Analyzing FAME

Pure Fatty acid methyl esters are analyzed with FID Gas chromatography (YL Instruments 6100 GC). The GC column used in the analysis is ZB-FFAP (30 m x 0.32 mm x 0.25 μm). From each experiment 1mL sample is taken and pour in to column. To be able to identify the peaks, methyl margarate in the amount calculated based on the weight as internal standard and 250 mL n-heptane is added into column. Column is placed in to GC since it is ready. Hydrogen gas is used as carrier gas and the flow rate is set to 2 mL/min. The temperature is set to start with 75 °C and heated to 140 °C with the rate of 16 °C/min and later on heated to 300 °C with the rate of 15 °C/min. The peaks are differentiated by the retention times of the isomers of standard methyl esters.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 GC results of Microwave Assisted Biodiesel Production

Gas chromatography is used to analyze the content of the biodiesel produced by microwave. Table 4.1 gives information about the total biodiesel peak area obtained. As the results are compared between each other it's seen that the time, methanol: oil molar ratio and catalyst oil ratio affect biodiesel content in direct proportion.

Table 4.1 Total area of GC Peaks for each sample

Methanol:oil ratio	Catalyst	Time (min)	Total area (uV. Min.)
3.1	0.5	1	241.72
		10	317.7
	1.5	1	278.71
		10	350.26
9.1	0.5	1	303.15
		10	321.72
	1.5	1	331.57
		10	363.45
6.1	1	5	320.68

There are several lipids in biodiesel samples. The most important and known was selected and their ratios for each sample are determined. Comparing with others the 4 peak which has the highest area is selected. Table 4.2 shows the area of C=16 (palmitic) peaks from GC analysis.

Table 4.2 Palmitic Acid ratios in Biodiesel Samples

Methanol:oil ratio	**Catalyst**	**Time (min)**	**C16=0**
3.1	0.5	1	3.82
		10	3.997
	1.5	1	4.142
		10	4.24
9.1	0.5	1	3.998
		10	4.08
	1.5	1	4.179
		10	4.25
6.1	1	5	4.143

The highest amount of fatty acid methyl ester determined in algal biodiesel is Oleic acid (C18=1) in all samples. The ratios of oleic acid in each sample are given in Table 4.3. Oleic acid is almost covering 50 % of the FAME.

Table 4.3 Oleic Acid Content of Biodiesel Samples

Methanol:oil ratio	**Catalyst**	**Time (min)**	**C18=1**
3.1	0.5	1	48.243
		10	49.827
	1.5	1	50.75
		10	50.833

Table 4.3 (cont'd)

9.1	0.5	1	50.53
		10	51.11
	1.5	1	52.29
		10	53.424
6.1	1	5	50.833

The peak right after oleic acid's peak is determined as linoleic acid. This is the second biggest peak in FAME which is around 17 % of total peak area. The area of oleic acid in each sample is given in Table 4.4.

Table 4.4 Linoleic Acid Content of Biodiesel Samples

Methanol:oil ratio	**Catalyst**	**Time (min)**	**C18=2**
3.1	0.5	1	16.45
		10	17.36
	1.5	1	17.51
		10	18.33
9.1	0.5	1	17.47
		10	17.67
	1.5	1	17.82
		10	18.85
6.1	1	5	17.77

The last significant peak seen in GC was determined as Linolenic Acid (C18=3). Linolenic acid peaks are mostly determined around 5% of total. The ratios for each sample are given in Table 4.5. There are some other peaks that have less area and not significant for biodiesel.

Table 4.5 Linolenic Acid Content of Biodiesel Samples

Methanol:oil ratio	**Catalyst**	**Time (min)**	**C18=3**
3.1	0.5	1	4.79
		10	5.065
	1.5	1	5.131
		10	5.33
9.1	0.5	1	4.7
		10	5.09
	1.5	1	5.155
		10	5.38
6.1	1	5	5.235

4.2 GC Results of Ultrasonic Assisted Biodiesel Production

Biodiesel production was also performed by using ultrasonic method. Fatty acid methyl ester content of each sample is analyzed by gas chromatography for each sample. The results gave us 4 significant peaks. These peaks are determined as palmitic acid, oleic acid, linoleic acid and linolenic acid. It was expected that algal biodiesel includes oleic acid more than others. Our results show the similar conclusion. The total area of the peaks for each sample is shown in Table 4.6. When the methanol:oil ratio increased the total of the peaks also increased. Biodiesel yield is also directly proportional to catalyst ratio and time.

Table 4.6 Total area of GC Peaks for Each Sample

Methanol:oil ratio	Catalyst %	Time (min)	Total (uV. Min.)
3:1	0.5	20	217.41
		40	265.82
	1.5	20	349.30
		40	381.23

Table 4.6 (cont'd)

9:1	0.5	20	246.02
		40	317.36
	1.5	20	349.73
		40	457.26
6:1	1	30	345.05

The first significant peak was determined as palmitic acid and the average area of palmitic acid was around 4%. The rates for palmitic acid in biodiesel samples are shown in table 4.7.

Table 4.7 Palmitic Acid ratios in Biodiesel Samples

Methanol:oil ratio	**Catalyst**	**Time (min)**	**C16=0**
3:1	0.5	20	3.99
		40	4.14
	1.5	20	4.19
		40	4.31
9:1	0.5	20	4.22
		40	4.13
	1.5	20	4.14
		40	4.27
6:1	1	30	4.16

The largest peak area was found as second significant peak which is determined as oleic acid. In this method ratios of oleic acid which is almost 50 % are also close to results of

microwave assisted method. The effect of time, catalyst and methanol: oil ratio can be seen from the data given in Table 4.8.

Table 4.8 Oleic Acid ratios in Biodiesel Samples

Methanol:oil ratio	**Catalyst**	**Time (min)**	**C18=1**
3:1	0.5	20	48.41
		40	48.889
	1.5	20	51.33
		40	51.79
9:1	0.5	20	49.92
		40	49.02
	1.5	20	51.68
		40	52.96
6:1	1	30	49.96

Other significant peak was determined as linoleic acid (C18=2) and it covers almost 17 percent of total area. This is the second highest fatty acid content found in FAME samples. The percentages of linoleic acid for each sample are given in Table 4.9.

Table 4.9 Linoleic Acid ratios in Biodiesel Samples

Methanol:oil ratio	**Catalyst**	**Time (min)**	**C18=2**
3:1	0.5	20	16.73
		40	16.9
	1.5	20	17.92
		40	18.08
9:1	0.5	20	17.21
		40	17.03
	1.5	20	17.95
		40	18.82
6:1	1	30	17.41

The last significant peak was interpreted as linolenic acid. The average area for linolenic acid is almost 5 percent in each FAME sample. The results for Linolenic acid are shown below in Table 4.10.

Table 4.10 Linolenic Acid ratios in Biodiesel Samples

Methanol:oil ratio	**Catalyst**	**Time (min)**	**C18=3**
3:1	0.5	20	5.030
		40	4.85
	1.5	20	5.08
		40	5.22
9:1	0.5	20	5.02
		40	4.90
	1.5	20	5.05
		40	5.32
6:1	1	30	4.97

4.3 Statistical Evaluation

2^3 Factorial design technique was used to determine the each effects and interactions of methanol and KOH, reaction time on the yield of FAME. FAME produced by microwave and ultrasonic assisted transesterification reactions and the design matrix is given in Table 4.11 and 4.12. The effects of methanol/oil molar ratio (X_1), catalyst/oil ratio (X_2), Reaction time (X_3) on the methyl ester yield (y(FAME) was evaluated. The experimental variables and their levels for microwave assisted FAME production were given as below:

X_1: Methanol/oil molar ratio: 9:1 (Superior level), 6:1 (Central level), 3:1 (Inferior level)

X_2: Catalyst/oil ratio (wt%): 1.5 (Superior level), 1 (Central level), 0.5 (Inferior level)

X_3: Reaction time: 10 (Superior level), 5 (Central level), 1 (Inferior level).

The experimental variables and their levels for ultrasonic assisted FAME production were given as below:

X_1: Methanol/oil molar ratio: 9:1 (Superior level), 6:1 (Central level), 3:1 (Inferior level)

X_2: Catalyst/oil ratio (wt%): 1.5 (Superior level), 1 (Central level), 0.5 (Inferior level)

X_3: Reaction time (min): 20 (Superior level), 30 (Central level), 40 (Inferior level).

Estimating the methyl ester yield and understanding the effects of experimental variables on the yield requires a regression equation.. X_1, X_2 and X_3 are the normalized values used in regression equations. Estimating the microwave assisted methyl ester yield values of microalgal oil, a regression Eq. (4.1) is derived. To be able to determine the effects of each variable in ultrasonic assisted methyl ester yield Eq. (4.2) is derived.

$$y(\text{FAME }(\%)) = 0.860 + 0.0551\ X_1 + 0.0377\ X_2 + 0.0576\ X_3 \quad (4.1)$$

The coefficient of determination for regression Eq. (4.1) was determined as 0.91.

$$y(\text{FAME }(\%)) = 0.709 + 0.0427\ X_1 + 0.134\ X_2 + 0.0705\ X_3 \quad (4.2)$$

The coefficient of determination for regression Eq. (4.2) was determined as 0.95.

Table 4.11 The microwave assisted methyl ester yields and design matrix obtained at different reaction conditions

Experimental number	Methanol: oil molar ratio	Catalyst oil ratio (wt.%)	Time (min)	X_1	X_2	X_3	Total area (uV.Min)	Total area (%)*
1	3:1	0.5	1	-1	-1	-1	241.72	66.15
2			10	-1	-1	1	278.71	76.27
3		1.5	1	-1	1	-1	317.7	86.94
4			10	-1	1	1	350.26	95.86
5	6:1	1	5	0	0	0	320.68	87.76
6	9:1	0.5	1	1	-1	-1	303.15	82.96
7			10	1	-1	1	321.72	88.04
8		1.5	1	1	1	-1	331.57	90.74
9			10	1	1	1	363.45	99.47

*Total area data were converted percent (%)

Table 4.12 The ultrasonic assisted methyl ester yields and design matrix obtained at different reaction conditions

Experimental number	Methanol: oil molar ratio	Catalyst oil ratio (wt.%)	Time (min)	X_1	X_2	X_3	Total area (uV.Min)	Total area (%)*
1	3:1	0.5	20	-1	-1	-1	217.41	47.34
2			40	-1	-1	1	265.82	57.88
3		1.5	20	-1	1	-1	349.30	76.06
4			40	-1	1	1	381.23	83.01
5	9:1	0.5	20	1	-1	-1	246.02	53.57
6			40	1	-1	1	317.36	69.10
7		1.5	20	1	1	-1	349.73	76.15
8			40	1	1	1	457.26	99.56
9	6:1	1	30	0	0	0	345.05	75.13

*Total area data were converted percent (%).

It can be seen from the regression equation (4.1) derived for microwave assisted FAME which is given above, X_3 (coefficient of reaction time) is the highest among all other variables and it can also be indicated that its effect on methyl ester yield is the strongest. Moreover, it can be seen that the alcohol/oil molar ratio and the catalyst/oil ratio also have positive influence on the methyl ester yield. Interactive effects of X_1 - X_2 (methanol/oil molar ratio and catalyst/oil ratio), X_2X_3 (catalyst/oil ratio and time) and X_1 - X_3 (methanol/oil molar ratio and time) are minor. Therefore, these interactional effects are not included in regression model equation.

It can be seen from the regression equation (4.2) derived for ultrasonic assisted FAME which is given above, the coefficient of catalyst/oil molar ratio was the highest among all other variables. This shows that effect of catalyst/oil molar ratio on the methyl ester yield is the strongest. It can also be commented that the reaction time and the methanol/oil ratio has a positive effect on the methyl ester yield. Interactive effect of X_1 - X_2 (methanol/oil molar ratio and catalyst/oil ratio), X_2 – X3 (catalyst/oil molar ratio and time) and X_1 – X_3 (methanol/oil ratio and time) have minor effect on equation that is why these interactional effects were not presented in equation of regression model.

A statistical technique is required to control the, reliability and accuracy of the fitted models and analyze the data. The model reliability was checked by ANOVA technique.

Minitab statistical software (Minitab 14) was used to compute the ANOVA parameters for the model equation which is given in Table 4.13 and Table 4.14.

F value shows that if it is greater than unity, it indicates more certain and adequately explained coefficients. Whereas, low probability in regression model is demonstrate high significance. The suitability of the model was controlled by the determination coefficient (R^2). In order to have a better correlation between experimental and predicted values, R value should be close to 1.

As it is seen in Table 4.13 showing the results for microwave assisted FAME data, F value is determined 18.55 with a very low probability (p-value < 0.04%) shows that the model is significant. The value of the determination coefficient $R^2 = 0.91$ means that only about 9% is not explained by the model. The value of the adjusted determination coefficient R^2 (adj) is found as 0.868 which is also high and shows importance of the model. In addition to this analyze, the real regression equation (4.3) was obtained by converting the normalized values of experimental variables to real values. Although the equation obtained with normalized values (−1, 0, 1) of experimental variables indicates that the positive or negative effect of parameters on the yield, the equation obtained with real values helps determining methyl ester yield value without doing experiments in the investigated parameter range.

$$y_{real} = 0.572 + 0.0150\ X_1 + 0.149\ X_2 + 0.00906\ X_3 \quad (4.3)$$

By considering these result, the optimum conditions for maximum FAME yield with microwave assisted method are determined at catalyst/oil ratio 1.5 wt%, methanol/oil molar ratio 9:1 and reaction time of 10 min.

Table 4. 13 ANOVA results of statistical evaluation for the microwave assisted methyl ester yield.

Sources of variations	Degree of freedom	Sum of squares	Mean square	F-value	Probability
Regression model	3	0.074067	0.024689	18.55	0.004
Error	5	0.006653	0.001331		
Corrected total	8	0.080720			

$R^2 = 0.91$, R^2(adj) = 0.868

As it is seen in Table 4.14 showing the results for ultrasonic assisted FAME data, the *F*-value of 34.14 with a very low probability value (p-value < 0.01%) implies that the model is significant. The value of the determination coefficient was calculated as R^2 = 0.95 which represents that only about 5% of the total variation is not explained by the respective model. In addition to this, the value of the adjusted determination coefficient adj. R^2= 0.928 was also very high and provide greater importance of the model. When the normalized values of experimental variables were converted to real values, the real regression equation (4.4) was derived. The equation obtained with normalized values (−1, 0, 1) of experimental variables shows that the positive or negative effect of parameters on the yield. On the other hand, the equation obtained with real values helps determining methyl ester yield value without doing any experiments in the investigated parameter range.

$$y_{real} = 14.5 + 1.42\ X_1 + 26.7\ X_2 + 0.705\ X_3 \quad (4.4)$$

By taking these result into consideration, the optimum conditions for maximum FAME yield with ultrasonic assisted method are determined at catalyst/oil ratio 1.5 wt%, methanol/oil molar ratio 9:1 and reaction time 40 min.

Table 4. 14 ANOVA results of statistical evaluation for the ultrasonic assisted methyl ester yield

Sources of variations	Degree of freedom	Sum of squares	Mean square	*F*-value	Probability
Regression model	3	0.197495	0.065832	34.14	0.001
Error	5	0.009641	0.001928		
Corrected total	8	0.207136			

R^2 = 0.95, R^2(adj) = 0.926

It was observed that there were four main fatty acids which are palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and linolenic acid (C18:3) in noticeable quantities. Methyl ester yields of these fatty acids and design matrix which is obtained at different reaction conditions were given in Table 5. As can be seen in Table 4.15 and Table 4.16, FAME yields of oleic and linoleic acids were the highest among them.

Table 4. 15 Methyl ester yields of microwave assisted fatty acids and design matrix obtained at different reaction conditions

Experimental number	Methanol: oil molar ratio	Catalyst oil ratio (wt)	Time (min)	X1	X2	X3	C16=0	C18=1	C18=2	C18=3
1	3:1	0.5	1	-1	-1	-1	3.82	48.243	16.45	4.79
2			10	-1	-1	1	3.997	49.827	17.36	5.065
3		1.5	1	-1	1	-1	4.142	50.75	17.51	5.131
4			10	-1	1	1	4.24	52.42	18.33	5.33
5	6:1	1	5	0	0	0	4.143	50.833	17.77	5.235
6	9:1	0.5	1	1	-1	-1	3.998	50.53	17.47	4.7
7			10	1	-1	1	4.08	51.11	17.67	5.09
8		1.5	1	1	1	-1	4.179	52.29	17.82	5.155
9			10	1	1	1	4.25	53.424	18.85	5.38

Table 4. 16 Methyl ester yields of ultrasonic assisted transesterification fatty acids and design matrix obtained at different reaction conditions

Experimental number	Methanol:oil molar ratio	Catalyst oil ratio (wt)	Time (min)	X_1	X_2	X_3	**C16=0**	**C18=1**	**C18=2**	**C18=3**
1	3:1	0.5	20	-1	-1	-1	3.99	48.41	16.73	5.03
2			40	-1	-1	1	4.14	48.89	16.9	4.85
3		1.5	20	-1	1	-1	4.19	51.33	17.92	5.08
4			40	-1	1	1	4.31	51.79	18.08	5.22
5	9:1	0.5	20	1	-1	-1	4.22	49.92	17.21	5.02
6			40	1	-1	1	4.13	49.02	17.03	4.90
7		1.5	20	1	1	-1	4.14	51.68	17.95	5.05
8			40	1	1	1	4.27	52.96	18.82	5.32
9	6:1	1	30	0	0	0	4.16	49.96	17.41	4.97

Since oleic and linoleic acids have the highest yield, they are selected to derive regression equations and determine the ANOVA results. Thus the effect of these acids on the yield was determined determine the effects of these parameters on FAME yield.

The produce oleic and linoleic acids with the method of microwave assisted transesterification was analyzed and the results and regression equation is given in equation (4.5), (4.6) and Table 4.17.

$$y_{(C18:1)} = 0.510 + 0.00764\ X_1 + 0.0115\ X_2 + 0.00621\ X_3 \qquad R^2=0.97 \qquad (4.5)$$

$$y_{(C18:2)} = 0.177 + 0.00270\ X_1 + 0.00445\ X_2 + 0.00370\ X_3 \qquad R^2=0.931 \qquad (4.6)$$

Table 4. 17 ANOVA results of statistical evaluation for the C18:1 and C18:2 microwave assisted transesterification methyl ester yield.

	Sources of variations	Degree of freedom	Sum of squares	Mean square	*F*-value	Probability
C18:1	Regression model	3	0.00182780	0.00060927	54.56	0.001
	Error	5	0.00005583	0.00001117		
	Corrected total	8	0.00188364			
C18:2	Regression model	3	0.00032626	0.00010875	22.33	0.003
	Error	5	0.00002436	0.00000487		
	Corrected total	8	0.00035062			

R^2 = 0.97, R=0.984, R^2(adj) = 0.953 for C18:1

R^2 = 0.931, R=0.964, R^2(adj) = 0.889 for C18:2

Unlike Eq. (4.1) results, Eq. (4.5) and Eq. (4.6) show that the coefficient of catalyst/oil molar ratio is the highest among all the variables, so it can be reported that its effect on the C18:1 and C18:2 methyl ester yields is the strongest. It can also be said that the reaction time and the methanol/oil ratio also positively influence the C18:1 and C18:2 methyl ester yield. The value of the determination coefficients $R^2_{C18:1}$ = 0.97 and $R^2_{C18:2}$ 0.93 represents that there is only about 3% and 7% probability of failing in this model respectively.

Regression equation and ANOVA results were obtained also for ultrasonic assisted transesterification of oleic (C18:1) and linoleic (C18:2) as Eq.(4.3) and given in Eq. (4.7), Eq.(4.8) and table 4.18.

$$y_{(C18:1)} = 0.504 + 0.00395\ X_1 + 0.0144\ X_2 + 0.00165\ X_3 \qquad R^2=0.924 \qquad (4.7)$$

$y_{(C18:2)} = 0.176 + 0.00173\ X_1 + 0.00612\ X_2 + 0.00128\ X_3$ $R^2=0.913$ (4.8)

Table 4. 18 ANOVA results of statistical evaluation for the C18:1 and C18:2 ultrasonic assisted methyl ester yield

	Sources of variations	Degree of freedom	Sum of squares	Mean square	*F*-value	Probability
C18:1	Regression model	3	0.00180548	0.00060183	20.22	0.003
	Error	5	0.00014884	0.00002977		
	Corrected total	8	0.00195432			
C18:2	Regression model	3	0.00033693	0.0001123	17.51	0.004
	Error	5	0.00003207	0.00000641		
	Corrected total	8	0.00036901			

R^2 = 0.92, R=0.959, R^2(adj) = 0.878 for C18:1

R^2 = 0.91, R=0.955, R^2(adj) = 0.861 for C18:2

In the same way, it can be seen from the Eq. (4.7) and Eq. (4.8), the highest coefficient was belonged to the catalyst/oil molar ratio among all the variables. Therefore, it can be commented that effect of catalyst/oil molar ratio on the C18:1 and C18:2 methyl ester yields was the strongest. It can also be reported that C18:1 and C18:2 methyl ester yields were influenced positively from the reaction time and the methanol/oil ratio. The value of the determination coefficients were found as $R^2_{C18:1}$ = 0.92 and $R^2_{C18:2}$ = 0.91, which showed that only about 8% and 9% of the total variation was not explained by the respective model. Similarly, the optimum conditions for maximum FAME yield of C18:1 and C18:2 were determined at catalyst/oil ratio 1.5 wt%, methanol/oil molar ratio 9:1 and time 40 min.

4.4 The Effect of Methanol:Oil Ratio on FAME Yield

The ratio of alcohol to triglyceride has a major role in the yield of FAME. Three moles of alcohol and one mole of glyceride is required to obtain three moles of fatty acid ester and one mole of glycerol by transesterification reactions, stoichiometrically. As alcohol

in biodiesel productions, mainly methanol or ethanol is used. However, because of its low cost and the amounts required in the reactions, methanol is mostly preferred. Molar ratio of alcohol can be varied according to catalyst (acid/alkali) used in transesterification reactions. While acid-catalyzed reactions need higher molar ratio, similar results can be achieved by using low alcohol/oil ratio in alkali catalyzed reactions.

Transesterification reaction is suggested to be an equilibrium reaction, lager amounts of alcohol can enhance the reaction to result in the equilibrium favorable to the FAME formation. Even though methyl ester production of any oil requires three moles of methanol for each mole of oil in theory, stoichiometrically, an excess amount of methanol is desirable for the completion of the reaction in practice [74].

In the literature, various molar ratios of alcohol:oil were tried in transesterification of vegetable and microbial oils. Huang et al. (2014) studied biodiesel production from microalgae oil catalyzed by a recombinant lipase and they obtained maximal conversion rates with methanol: oil ratio 3:1 to 5:1 and ethanol: oil ratio 5:1 as 86.6 and 87.5%, respectively [75]. Patil et al. (2011) investigated the effect of microwave irradiation on the simultaneous extraction and transesterification (in situ transesterifcation) of dry algal biomass to biodiesel. Biodiesel experiments were performed under a matrix of conditions: reaction times of 3, 6, and 9 min; catalyst concentrations in the range of 1 – 3 wt.% of dry biomass; and dry algae to methanol (wt/vol) ratios of 1:9 – 1:15 to find optimal conditions. They determined the optimal conditions for this process as: dry algae to methanol (wt/vol) ratio of around 1:12, catalyst concentration about 2 wt.%, and reaction time of 4 min [3]. Macías-Sánchez et al. studied biodiesel production from *Nannochloropsis gaditana* wet microalgal biomass by direct transesterification. Their results showed that increasing methanol/biomass ratio cause an increase in FAME yield because the transesterification equilibrium is more displaced toward the formation of product as the methanol concentration increases. However in some cases, high alcohol/oil ratio may cause a slight decrease in ester yields due to higher alcohol molar ratio interferes with the separation of glycerol because there is an increase in solubility. In addition to that, an excess of alcohol seems to favor conversion of di-to monoglycerides, but there also is a slight recombination of esters and glycerol to monoglycerides because their concentration keeps increasing during the course of the

reaction, in contrast to reactions performed with low molar ratios. In experiments that base catalyst is used, this effect can be observed because the reaction is fast, whereas in acid catalysis the effect is not apparent, probably due to the low rate of the reaction [76]. Encinar et al., mentioned that a slight decrease of methyl esters content from 94,66 to 91,60 was occurred with increasing methanol/oil molar ratio from 9:1 to 12:1, respectively [77]. Huang et al. (2014) also concluded that higher methanol or ethanol proportions reduced conversion efficiency in their experiments [75]. Choedkiatsakul et al. (2015a) investigated effect of different reaction parameters on biodiesel yield and their results showed that biodiesel yield reached the steady state more rapidly when high methanol/oil molar ratios were used [78]. In another study, Choedkiatsakul et al. (2015b) found 9:1 methanol/oil ratio as optimal molar ratio due to transesterification is a reversible reaction and hence requires a higher amount of methanol to shift the reaction. However, they also reported that an excessive amount of methanol will affect the reaction negatively, because it would increase the solubility of the by-product glycerol for the reverse reaction [79]. Lin et al. (2014) also obtained similar results when biodiesel production was carried out from Jatropha oil with 11:1 methanol/oil molar ratio. It was found that the molar ratios of 8:1 and 9:1 were the most suitable alcohol/oil molar ratio for biodiesel production from Jatropha oil assisted by microwave system [80].

In this study, 3:1, 6:1 and 9:1 methanol/oil molar ratio was used to investigate the effect of methanol/oil ratio on microwave assisted transesterification of microalgal oil in the presence of 0.5%, 1% and 1.5% KOH catalyst/oil ratios (wt.%) at different reaction times (1, 5 and 10 minutes). As it can be seen in the results, when methanol/oil ratio was increased from 3:1 to 9:1 methyl ester yields were also increased in agreement with those previously reported in the literature studies. The highest methyl ester production was obtained with 9:1 methanol/oil molar ratio under 10 min reaction time and 1.5% KOH catalyst/oil ratio (wt.%). The results obtained from our study are in agreement with these studies for methyl ester production.

In this study, 3:1, 6:1 and 9:1 methanol/oil molar ratios were used to investigate the effect of methanol/oil ratio on ultrasound assisted transesterification of microalgal oil in the presence of 0.5%, 1% and 1.5 wt. % KOH catalyst/oil ratios at different reaction times (20, 30 and 40 minutes). As can be seen in the results, when methanol/oil ratio

increased from 3:1 to 9:1, methyl ester yield increased. The highest methyl ester production was obtained with 9:1 methanol/oil molar ratio at the reaction time of 40 min and 1.5 wt % KOH/oil ratio. Similar results have been reported from various transesterification studies. Tamilarasan and Sahadevan have investigated ultrasonic assisted acid base transesterification of *Caulerpa peltata* oil. It was observed that, an increase in the methanol to oil molar ratio from 3:1 to 15:1, concluded with an increase in methyl ester yield. Also the maximum conversion was reached at 15:1 methanol to oil molar ratio [8]. Tamilarasan et al. have studied ultrasound-enhanced rapid in situ transesterification of *Enteromorpha compressa*, marine macroalgae. Various quantity of methanol to algal biomass as 4:1, 4.5:1, 5:1, 5.5:1, 6:1 and 6.5:1 (vol/wt) were applied to observe the effect of the methanol quantity on in situ transesterification process. It was reported that, 5.5:1 methanol to biomass ratio was suggested to be a suitable ratio for in situ transesterification process. For further increase of methanol ratio up to 6:1, no significant difference on the biodiesel yield was observed, and excess usage of methanol to biomass ratio above 6:1 decreased methyl ester yields [8]. Thanh et al. also carried out transesterification of canola oil by use of ultrasonic probe in the presence of KOH at the reaction time of 50 min. The highest methyl ester yield was obtained at molar ratio of 5:1 methanol:oil in their study [81]. The results obtained from our study are in agreement with these studies for methyl ester production.

4.5 The Effect of Catalyst/Oil Ratio

The catalysts used in transesterification process can be divided in two main groups as homogeneous (alkaline and acids) and heterogeneous catalysts. While homogeneous catalysts form a single phase mixture when added to oil and alcohol, heterogeneous catalysts do not mix in the reaction medium. Choosing a catalyst for transesterification process depends on the quality of raw material, the type of alcohol used, the costs of the catalysts and technological route to be used for transesterification. In this study KOH was used as catalyst for transesterification with catalysts ratios of 0.5%, 1% and 1.5% to investigate the effect of catalyst concentration on methyl ester yields. KOH was chosen due to its effectiveness on methyl ester production in comparison with sodium-based catalysts and methoxide catalysts.

The highest methyl ester yield was achieved with 1.5% KOH catalyst/oil ratio under the conditions of 9:1 methanol/oil molar ratio and 10 min reaction time by using microwave assisted transesterification. It was seen in the results that high yields of methyl ester was obtained with increasing catalyst/oil ratio when methanol/oil ratio was kept constant. The study of Kumar *et al*. (2011) also shows that in presence of KOH, the biodiesel yield was found to be 89%, 97% and 96% at 0.5%, 1.0% and 1.5% concentrations, respectively. However, in some cases using higher amounts of catalyst may lead to saponification [82]. Hsiao *et al.* (2011) found that increase in nanopowder calcium oxide quantity over 3% causes a decrease in yield of methyl ester production [83]. Choedkiatsakul *et al.* analyze the effects of three catalyst loading values, 0.5, 1 and 1.5%wt of oil, respectively. Results showed that biodiesel yield increases with respect to increase in catalyst loading from 0.5 to 1%wt of oil. The reason is mentioned as low amount of catalyst used implies a low number of active sites which cause to decrease the transesterification rate [78]. In the study of Lin *et al.* (2014) shows that biodiesel yields increase when the $NaNH_2$ catalyst amount increase 0.75% wt. to 1.50% wt. However it is also indicated that catalyst amounts higher than 1.50% wt., results a slight decrease in biodiesel yield [80].

The effect of KOH/microalgal oil ratio was also studied by using ultrasonic assisted transesterification, with various concentrations of 0.5 wt. %, 1 wt. % and 1.5 wt. %. The highest methyl ester yield was obtained with 1.5 wt % KOH catalyst/oil ratio under the conditions of 9:1 methanol/oil molar ratio and reaction time of 40 min. It is observed that high yields of methyl ester were obtained with increasing catalyst/oil ratio when methanol/oil ratio is kept constant. Thanh et al. have a study on the ultrasonic assisted transesterification of triglycerides with methanol in the presence of KOH catalyst. It was stated that, the highest yield of FAME was achieved more than 98% after 20 and 25 min reaction time at the concentration of 1.5 and 1.0 wt.% KOH, respectively[81]. Tamilarasan and Sahadevan has worked on the effect of catalyst concentration on methyl ester conversion with different catalyst concentrations of 0.25%, 0.5%, 0.75%, 1%, 1.25% and 1.5% NaOH (wt%). It was mentioned that, increase in NaOH catalyst concentration leads to an increase in methyl ester conversion [8].

4.6 The Effect of Reaction Time on Methyl Ester Conversion

Reaction time is one of the parameters affecting conversion rate of oil to biodiesel [6]. Reaction time is a crucial factor that doesn't only determine the efficiency of biodiesel formation but also the quality of final products [2]. For this reason, choosing an optimum reaction time is necessary to the completion of the reaction and producing high yields of methyl esters.

Generally, optimum reaction times provide adequate interaction of the microwave effect with the reaction mixture. The reason microwave assisted method mostly preferred is because of the time required for the transesterification reaction is less. In this study, microwave assisted transesterification reactions were conducted at 1 min, 5 min and 10 min at different methanol:microalgal oil molar ratios and KOH catalyst/oil ratios. It is found that increase in reaction time results with higher methyl ester yields. The highest methyl ester yield was obtained at 10 min reaction time under the conditions of 1.5% KOH catalyst/oil ratio and 9:1 methanol/oil molar ratio. Findings in this investigation of the effect of reaction time on methyl ester yield are well adjusted with the results of other studies in the literature. Hsiao *et al.* (2011) concluded that the use of CaO nanopowder ensures greater efficiency with the lower reaction time than that of CaO powder under the microwave conditions [83]. Kim *et al.* (2011) explains that for a conversion rate higher than 90% by microwave heating, reaction time decreases nearly to 1/7 of the conventional heating [84]. Lin *et al.* (2014) observed that biodiesel yields which is obtained by using the microwave assisted system were 88.3%, 92.7%, 96.2%, 93.5%, 90.4%, and 89.1% for reaction times of 5, 6, 7, 8, 9, and 10 min, respectively. It is mentioned that an increase in reaction time from 5 to 7 min results in a substantial increase of the yield, and oppositely increase from 7 to 10 min causes to decrease in the amount of yield because of increase in solubility of glycerin. Reaction time can be longer in some cases such as simultaneous transesterification process [80]. Cheng *et al.* (2014) reported that a reaction time of 30 min is suitable for the microwave-assisted transesterification of lipids in wet microalgae. A marginal increase in yield of FAME was observed when the reaction time increased from 10 to 30 min. Thus the transesterification reaction is not completed up to 30 min [85].

In order to determine the effect of reaction time on methyl ester yield, ultrasonic assisted transesterification reactions were conducted at 20 min, 30 min and 40 min with

different methanol:microalgal oil molar ratios and KOH catalyst/oil ratios. It is concluded that increase in reaction time results with higher methyl ester yields. The highest methyl ester yield is obtained with the reaction time of 40 min under the conditions of 1.5% KOH catalyst/oil ratio and 9:1 methanol/oil molar ratio. The obtained results show that the effect of reaction time on methyl ester yield is similar with the results of other studies in the literature. The study of Kesgin et al. shows that the highest formation of methyl ester was observed within 20 min at 4:1 and 5:1 molar ratios of alcohol:oil and states that the ultrasonic assisted transesterification reduces the process time to 10-20 min with respect to traditional method [86]. Suganya et al. have studied ultrasound-enhanced rapid in situ transesterification of *Enteromorpha compressa* and analyze the effect of time on the process. In the study, the reaction time is analyzed between 10 min to 120 min which was maintained with the optimized parameters of 10% H_2SO_4 concentration, 30 vol. % of tetra hydro furan and 5.5:1 methanol to biomass ratio. As a result, they have experienced the highest yield of 98.89% in 90 min of reaction time [6]. In the study of Hindryawati and Maniam, transesterification reactions were carried out with different reaction times varying 0 min to 80 min. It was found that the highest methyl ester yield is obtained at the reaction time of 30 min under the conditions of 3 wt.% catalyst and methanol to oil molar ratio of 9:1 at 55°C. It is stated that any further time increase caused no remarkable variations in the content due to equilibrium [87].

4.7 Fatty acid profile in FAME samples

Not only high lipid content, suitable fatty acid composition is also necessary for high quality biodiesel. So that, the quality of the biodiesel depends on fatty acid profile. Transesterification reaction is one of the most significant steps in biodiesel process since it affects the fatty acid profile [88].

Studies shows that that the most favorable biodiesel should have rather low levels of polyunsaturated and low levels of saturated FAs in order to decrease oxidative stability and flow problems in cold weather. Appropriate balance between oxidative stability and cold flow can be provided by monounsaturated fatty acids of palmitoleic acid (16:1) and oleic acid (18:1). It can be seen that the most common feedstocks suitable for biodiesel production were enriched in the five most common C16–C18 fatty acids, as palmitic

(16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids and it has been reported that C16:1 and C18:1 fatty acids (palmitoleic acid and oleic acid, respectively) are the most favorable fatty acids for biodiesel production [89].

In our results, fatty acid profile was also determined to investigate the quality of produced biodiesel. Fatty acid composition of *C.prototheciides* was determined by GC analysis. Results of GC analysis showed that there are four main fatty acids as palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and linolenic acid (C18:3) which are shown in Fig. 4.1 and Fig. 4.2. Other fatty acids such as C16:1, C18:0, C20:0 and C20:1 were found as trace amounts and could not be determined with the results. The highest amount of fatty acid methyl ester determined in algal biodiesel was oleic acid (C18=1) in all samples and it constituted more than 50 % of the FAME. Linolenic acid content which has known as having negative effects on oxidative stability and cold flow properties of biodiesel, was found around 5% of total FAME.

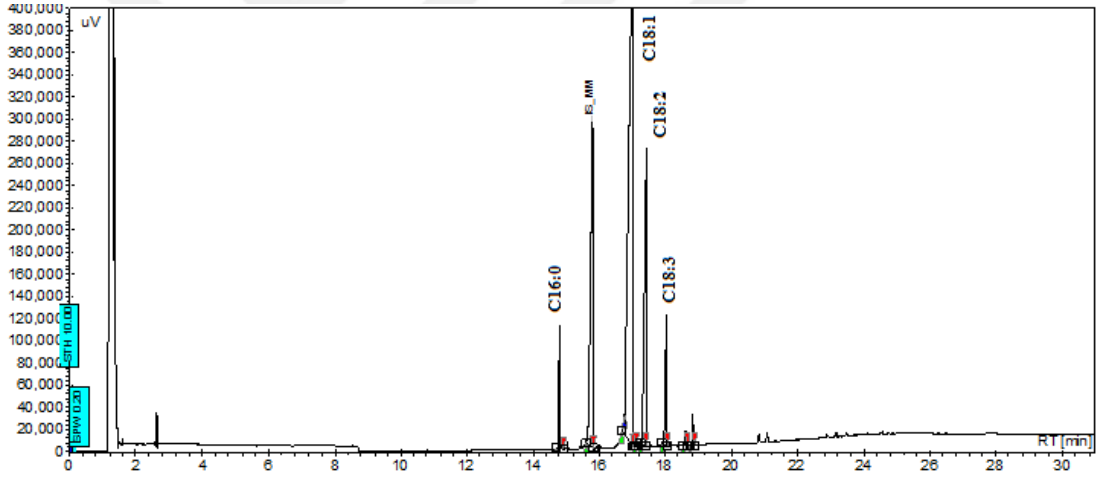


Figure 4.1 GC chromatogram of FAME sample (Microwave Assisted Transesterification) produced under the conditions of 9:1 methanol/oil molar ratio with 10 min reaction time and 1.5 wt % KOH

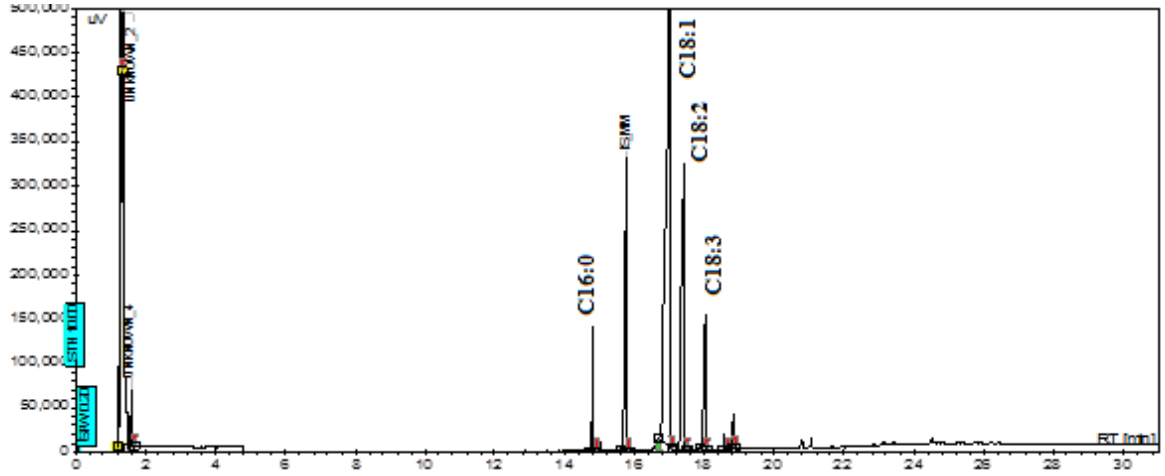


Figure 4.2 GC chromatogram of FAME sample (Ultrasonic Assisted Transesterification) produced under the conditions of 9:1 methanol/oil molar ratio with 40 min reaction time and 1.5 wt % KOH

4.8 Properties of Methyl Esters Product

Important properties that affect biodiesel usage as a fuel is needed to be determined in order to evaluate its potential as a substitute for diesel fuel. Determination of these properties was carried out with characterizing the best obtained products for each method of microwave assisted and ultrasonic assisted with their acid values, iodine values, densities, methyl ester contents, water contents and viscosities. Experimental procedures were applied in according to biodiesel standards.

Methyl ester obtained under the conditions of methanol-oil molar ratio: 9:1; 1.5% KOH; and t = 10 min with microwave assisted method was used to perform characterization experiments and the determined properties were given in Table 4.19 As it can be seen from Table 4.19, acid value, iodine value and density of algal biodiesel are 0.34 mg KOH/g, 88.50 g I_2/100 g and 0.862 g/cm^3, respectively. Water content and viscosity of biodiesel are 0.013% and 4.8 mm^2 /s, respectively. According to these data, it can be said that all the properties obtained from algal biodiesel is in the range of acceptance criteria as compared with commercial biodiesel standards. Therefore, this product can be used as fuel and for other application areas.

Table 4. 19 Biodiesel obtained by microwave assisted transesterification (Methanol-oil molar ratio: 9:1; 1.5% KOH; t = 10 min).

Properties	**Value**	**Min–max value**	**Experimental procedure**
Acid value, mg KOH/g	0.34	-0.500	EN 14104
Iodine value, g I_2/l00g	88.50	-120	EN 14111
Density, g/cm^3 (15°C)	0.862	0.860-0.900	EN ISO 3679
Water content, %	0.013	-0.050	EN ISO 12937
Viscosity, mm^2/s (40°C)	4.8	3.5-5.00	EN ISO 3104

Methyl ester obtained with ultrasonic assisted transesterification under the conditions of methanol-oil molar ratio: 9:1; 1.5% KOH; and t = 40 min was used to perform characterization experiments and the determined properties were given in Table 4.20 As it can be seen from Table 4.20, acid value, iodine value and density of algal biodiesel were found as 0.36 mg KOH/g, 86.40 g I_2/100 g and 0.868 g/cm^3, respectively. Water content and viscosity of biodiesel were measured as 0.014% and 4.7 mm^2 /s, respectively. According to the commercial biodiesel standards, values of the properties of algal biodiesel were in the range of acceptance criteria as compared with commercial biodiesel standards. As a result, this product can be evaluated as a fuel.

Table 4. 20 Biodiesel obtained by ultrasonic assisted transesterification (Methanol-oil molar ratio: 9:1; 1.5% KOH; t = 40 min).

Properties	**Value**	**Min–max value**	**Experimental procedure**
Acid value, mg KOH/g	0.36	-0.500	EN 14104
Iodine value, g I_2/l00g	86.40	-120	EN 14111
Density, g/cm^3 (15°C)	0.868	0.860-0.900	EN ISO 3679
Water content, %	0.014	-0.050	EN ISO 12937

CHAPTER 5

CONCLUSIONS

In this study, microwave assisted and ultrasonic assisted biodiesel productions from microalgal oil of heterotrophically cultivated *C. protothecoides* were performed and parameters affect the transesterification processes were investigated. It is concluded that high yields of methyl ester can be achieved with microwave and ultrasonic assisted transesterification of *C. protothecoides* oil. Effects of various parameters on biodiesel yield were also presented and assessed by comparing with other studies reported in the literature. Results from experiments and characterization tests are in agreement with the studies for biodiesel production.

1. According to our experimental results, the highest methyl ester yield was obtained by microwave and ultrasonic assisted transesterification under the conditions of 9:1 methanol/oil molar ratio with 1.5% KOH catalyst/oil ratio and 10 min reaction time.
2. Both the experimental and statistical results of microwave assisted transesterification show that the reaction time is the most effective parameter on methyl ester yield. It can be seen from the regression equation the coefficient of reaction time is the highest among all the variables and its effect on the methyl ester yield is the strongest.
3. According to experimental and statistical results of ultrasonic assisted transesterification, the catalyst/oil ratio is the most effective parameter on methyl ester yield. It can be seen from the regression equation, the coefficient of catalyst/oil ratio is the highest among all the variables and it can be said that its effect on the methyl ester yield is the strongest.

4. When it was investigated on only oleic and linoleic acid, statistical evaluation shows that effect of catalyst/oil ratio on methyl ester yield of oleic and linoleic acid is the highest in comparison with methanol/oil ratio and reaction time.
5. GC analysis showed that there are four main fatty acids as palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and linolenic acid (C18:3). The highest amount of fatty acid methyl ester determined in algal biodiesel was found as oleic acid (C18=1) in all samples and it constituted more than 50 % of the FAME.
6. Although microwave and ultrasonic assisted transesterification is a very promising way for biodiesel production, there is just a few studies about biodiesel production from microalgal oils by using this method. Besides that, currently ultrasonic assisted transesterification is not seem economically viable for large scale production of algal biodiesel. Therefore, there is still need for innovations for both high productivity and green productions.

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EDUCATION

Degree	Department	University	Date of Graduation
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High School	Math Science	Ahmet Ulusoy College	2006

WORK EXPERIENCE

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2012	Üsküdar University	Research Assistant

PUBLISHMENTS

Books

1.Didem Özçimen, M. Ömer Gülyurt and Benan İnan (2012). Algal Biorefinery for Biodiesel Production, Biodiesel - Feedstocks, Production and Applications. ISBN: 978-953-51-0910-5, InTech, DOI: 10.5772/52679. Available from:

http://www.intechopen.com/books/biodiesel-feedstocks-production-and-applications/algae-as-natural-biorefinery

AWARDS

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