ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

MSc THESIS

Gökhan TÜCCAR

BIODIESEL PRODUCTION FROM MICROALGAE

DEPARTMENT OF MECHANICAL ENGINEERING

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INSTITUTE OF NATURAL AND APPLIED SCIENCES

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DEPARTMENT OF MECHANICAL ENGINEERING

We certify that the thesis titled above was reviewed and approved for the award of degree of the Master of Science by the board of jury on 04/01/2011.

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ABSTRACT

MSc THESIS

BIODIESEL PRODUCTION FROM MICROALGAE

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The objective of this study is to observe cultural characteristics of *Chlorella vulgaris* and to identify availability of microalgae biodiesel in diesel engines as alternative fuel. Microalgae biodiesel was blended with diesel fuel with the volumetric ratio of 5%, 10%, 20%, and 50%. Fuel properties of blends were identified and the performance characteristics and exhaust emissions of the engine fuelled with blends were analyzed. The fuel properties determination experiment showed that except its low Cetane number, microalgae biodiesel satisfies European Biodiesel Standards (EN 14214). According to experimental data obtained from engine test rig it was found that, although microalgae biodiesel caused a slight reduction in torque and brake power values, the emission values of the engine using microalgae biodiesel were improved.

Key Words: Microalgae, Biodiesel, Fuel Properties, Engine Performance, Exhaust Emissions.

ÖZ

YÜKSEK LİSANS TEZİ

MİKROALGLERDEN BİYODİZEL ÜRETİMİ

Gökhan TÜCCAR

ÇUKUROVA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ
MAKİNE MÜHENDİSLİĞİ ANABİLİM DALI

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: Doç. Dr. Hüseyin AKILLI
: Doç. Dr. Olcayto KESKİNKAN

Bu çalışmanın amacı *Chlorella vulgaris*'in kültürel özelliklerini incelemek ve mikroalg biyodizelinin motorda alternatif yakıt olarak kullanılabilirliğini araştırmaktır. Mikroalg biyodizeli dizel yakıtıyla 5%, 10%, 20% ve 50% hacimsel oranlarında karıştırılmıştır. Hazırlanan karışımların ve saf biyodizelin yakıt özellikleri belirlenmiş ve mikroalg biyodizeli kullanan bir motorun performans özellikleri ve egzoz emisyonları analiz edilmiştir. Yakıt analiz deneyleri, düşük Setan sayısı dışında Mikroalg biyodizelinin Avrupa Biyodizel Standartlarını (EN 14214) karşıladığını göstermiştir. Motor deneylerinden alının veriler ışığında mikroalg biyodizelinin motorun tork ve fren gücünü az da olsa düşürdüğü ancak motorun emisyon değerlerini iyileştirdiği belirlenmiştir.

Anahtar Kelimeler: Mikroalg, Biyodizel, Yakıt Özellikleri, Motor Performansı Egzoz Emisyonları

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LIST OF ABBREVIATIONS AND NOMENCLATURE

GHG	: Greenhouse Gas
CO_2	: Carbon Dioxide
PBR	: Photobioreactors
OD	: Optical Density
ATP	: Adenosine Triphosphate
DOE	: Department of energy
ASP	: Aquatic Species Program
PP	: Pour Point
N	: Nitrogen
CO	: Carbon Monoxide
NO_X	: Nitrogen Oxide
H_2CO_3	: Carbonic Acid
MB	: Microalgae Biodiesel
ME	: Methyl Ester

1. INTRODUCTION

Energy is essential input for economics growth, social development, human welfare and improving the quality of life. Since their exploration, the fossil fuels continued as the major conventional energy source. With increasing trend of modernization and industrialization, the world energy demand is also growing at faster rate. Apart from their production, majority of developing countries import crude oil to cope up their increasing energy demand. Thus, a majority of their hard earned export earnings is spent for purchase of petroleum products. Besides the energy crisis, the other problem of concern is the pollution of environment due to fossil fuel combustion. Internal combustion engines have fallen victim to the crises (Sahoo et. al., 2005). The transportation and energy sectors are the major sources, responsible in European Union (EU) for more than 20% and 60% of greenhouse gas (GHG) emissions, respectively (European Environmental Agency, 2004). GHG contributes not only to global warming but also to other impacts on the environment and human life. Oceans absorb approximately one-third of the CO_2 emitted each year by human activities and as its levels increase in the atmosphere, the amount dissolved in oceans will also increase turning the water pH gradually to more acidic. This pH decrease may cause the quick loss of coral reefs and of marine ecosystem biodiversity with huge implications in ocean life and consequently in earth life (Ormerod et. al., 2010). To find clean and renewable energy sources ranks as one of the most challenging problems facing mankind in the medium to long term. The associated issues are closely connected with economic development and prosperity, quality of life, global stability, and require from all countries tough decisions and long term strategies. For example, many countries and regions around the world established targets for CO_2 reduction in order to meet the sustainability goals agreed under the Kyoto Protocol (Teresa et. al., 2010).

Biodiesel is defined as the monoalkyl esters of vegetable oils or animal fats. Biodiesel is the best candidate for diesel fuels in diesel engines. The biggest advantage that biodiesel has over gasoline and petroleum diesel is its environmental friendliness. Biodiesel burns similar to petroleum diesel as it concerns regulated

pollutants (Demirbas, 2007). The release of sulfur content and carbon monoxide would be cut down by 30% and 10%, respectively, by using biodiesel as energy source. Using biodiesel as energy source, the gas generated during combustion could be reduced, and the decrease in carbon monoxide is owing to the relatively high oxygen content in biodiesel. Moreover, biodiesel contains no aromatic compounds and other chemical substances which are harmful to the environment. Recent investigations have indicated that the use of biodiesel can decrease 90% of air toxicity and 95% of cancers compared to common diesel source (Sharp, 1996) Currently, biodiesel is made from a variety of feedstocks, including pure vegetable oils, waste cooking oils, and animal fat; however, the limited supply of these feedstocks impedes the further expansion of biodiesel production. Microalgae have long been recognized as potentially good sources for biofuel production because of their high oil content and rapid biomass production. In recent years, use of microalgae as an alternative biodiesel feedstock has gained renewed interest from researchers, entrepreneurs, and the general public.

For years, aquatic environments have been a source of food, minerals and natural products. In the water, very different and extreme environments can be found, among aquatic microorganisms microalgae and cyanobacteria are a very interesting source of a wide range of compounds. They do not only have the capacity to produce high-value compounds, but also the ability to do it using only sunlight, carbon dioxide and water. For this reason microalgae and cyanobacteria are called photoautotrophic microorganisms, i.e. they need light as their main energy source (phototropic) and they can grow on a very simple culture medium (autotrophic). This is possible due to the only biological process of importance that can harvest energy from the sun photosynthesis (Barbosa, 2003). Biodiesel from microalgae seems to be the only renewable biofuel that has the potential to completely displace petroleum-derived transport fuels without adversely affecting supply of food and other crop products. Most productive oil crops, such as oil palm, do not come close to microalgae in being able to sustainably provide the necessary amounts of biodiesel (Chisti, 2008).

Algae are recognised as one of the oldest life-forms. They are primitive plants, i.e. lacking roots, stems and leaves, have no sterile covering of cells around the reproductive cells and have “chlorophyll a” as their primary photosynthetic pigment. Algae structures are primarily for energy conversion without any development beyond cells, and their simple development allows them to adapt to prevailing environmental conditions and prosper in the long term. Prokaryotic cells (cyanobacteria) lack membrane-bound organelles and are more similar to bacteria rather than algae. Eukaryotic cells, which comprise of many different types of common algae, do have these organelles that control the functions of the cell, allowing it to survive and reproduce. Eukaryotes are categorised into a variety of classes mainly defined by their pigmentation, life cycle and basic cellular structure. The most important classes are: green algae, red algae and diatoms. Algae can either be autotrophic or heterotrophic; the former require only inorganic compounds such as CO_2, salts and a light energy source for growth; while the latter are nonphotosynthetic therefore require an external source of organic compounds as well as nutrients as an energy source. Some photosynthetic algae are mixotrophic, i.e. they have the ability to both perform photosynthesis and acquire exogenous organic nutrients. For autotrophic algae, photosynthesis is a key component of their survival, whereby they convert solar radiation and CO_2 absorbed by chloroplasts into adenosine triphosphate (ATP) and O_2 the usable energy currency at cellular level, which is then used in respiration to produce energy to support growth (Brennan et. al., 2009).

There are two classifications of algae: macroalgae and microalgae (Figure 1.1). Macroalgae are the large (measured in centimeters), multi-cellular algae often seen growing in ponds. These larger algae can grow in a variety of ways. The largest multi-cellular algae are called seaweed; an example is the giant kelp plant which can be more than 30 meters long. Microalgae, on the other hand, are tiny (measured in micrometers), unicellular algae that normally grow in suspension within a body of water (Wen et. al., 2009).

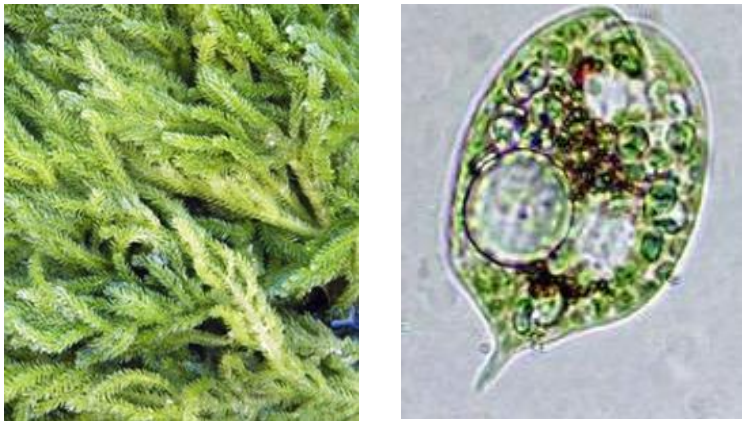


Figure 1.1.Macroalgae & Microalgae

Algal biomass contains three main components: carbohydrates, proteins, and lipids/natural oils. Because the bulk of the natural oil made by microalgae is in the form of tricylglycerol molecule, which is the right kind of oil for producing biodiesel, microalgae are the exclusive focus in the algae-to-biofuel arena. Microalgae grow very quickly compared to terrestrial crops. They commonly double in size every 24 hours. During the peak growth phase, some microalgae can double every 3.5 hours. Oil content of microalgae is usually between 20 percent and 50 percent (dry weight, Table 1.1), while some strains can reach as high as 80 percent (Wen et. al., 2009).

Table 1.1. Oil content of microalgae (Adapted form Chisti, 2007)

Microalgae	Oil content (% dry weight)
Botryococus braunii	25–75
Chlorella sp.	28–32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16–37
Nitzschia sp.	45–47
Phaeodactylum tricornutum	20–30
Schizochytrium sp.	50–77
Tetraselmis suecia	15–23

Compared with terrestrial crop, which take a season to grow and only contain a maximum of about 5 percent dry weight of oil, microalgae grow quickly and contain high oil content. This is why microalgae are the focus in the algae-to-biofuel arena (Wen et. al., 2009). Table 1.2 lists the potential yields of oil produced by various crops and compares these values to oil yields from an open pond growing microalgae.

Table 1.2. Comparison of some sources of biodiesel (Adapted form Chisti, 2007)

Crop	Oil yield (L/ha)
Corn	172
Soybean	446
Canola	1190
Jatropha	1892
Coconut	2689
Oil palm	5950
Microalgae	57,800

In general, production of microalgal biomass is a very expensive process compared to growing terrestrial crops. For photosynthetic growth, light, carbon dioxide, water and inorganic salts should be supplied to microalgae and temperature must remain generally within 20 to 30 °C. Under these conditions microalgae convert about 3-8% of solar energy to biomass whereas observed yields for terrestrial plants are about 0.5% (Chisti, 2007). To minimize expense, biodiesel production must rely on freely available sunlight, despite daily and seasonal variations in light levels.

Raceway ponds are made of a closed loop recirculation channel that is typically about 0.3 m deep (Figure 1.2). Mixing and circulation are produced by a paddlewheel (Figure 1.2). Flow is guided around bends by baffles placed in the flow channel. Raceway channels are built in concrete or compacted earth, and may be lined with white plastic. During daylight, the culture is fed continuously in front of the paddlewheel where the flow begins (Figure 1.2). Broth is harvested behind the paddlewheel, on completion of the circulation loop. The paddlewheel operates all the time to prevent sedimentation. Raceway ponds for mass culture of microalgae have been used since the 1950s. Extensive experience exists on operation and engineering of raceways. The largest raceway-based biomass production facility occupies an area of 440,000 m^2. This facility, owned by Earthrise Nutritionals, is used to produce cyanobacterial biomass for food. In raceways, any cooling is achieved only by evaporation. Temperature fluctuates within a diurnal cycle and seasonally. Evaporative water loss can be significant. Because of significant losses to atmosphere, raceways use carbon dioxide much less efficiently than photo bioreactors. Productivity is affected by contamination with unwanted algae and microorganisms that feed on algae. The biomass concentration remains low because raceways are poorly mixed and cannot sustain an optically dark zone. Raceways are perceived to be less expensive than photo bioreactors, because they cost less to build and operate. Although raceways are low-cost, they have a low biomass productivity compared with photo bioreactors (Chisti, 2007).

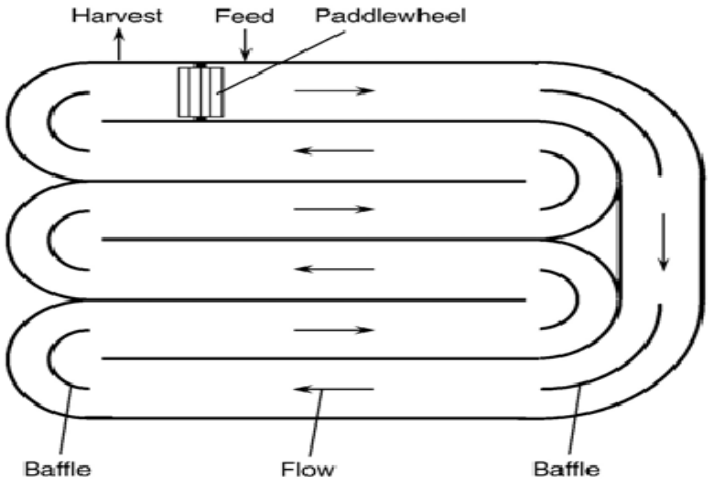


Figure 1.2. Arial view of a raceway pond

Unlike open raceways, photobioreactors (PBR) permit essentially single-species culture of microalgae for prolonged durations. PBRs have been successfully used for producing large quantities of microalgal biomass. A tubular PBR consists of an array of straight transparent tubes that are usually made of plastic or glass. This tubular array, or the solar collector, is where the sunlight is captured (Figure 1.3). The solar collector tubes are generally 0.1 m or less in diameter. Tube diameter is limited because light does not penetrate too deeply in the dense culture broth that is necessary for ensuring a high biomass productivity of the PBR. Microalgal broth is circulated from a reservoir (i.e. the degassing column in Figure 1.3) to the solar collector and back to the reservoir. Continuous culture operation is used, as explained above. The solar collector is oriented to maximize sunlight capture. In a typical arrangement, the solar tubes are placed parallel to each other and flat above the ground (Figure 1.3). Horizontal, parallel straight tubes are sometimes arranged like a fence (Figure 1.4), in attempts to increase the number of tubes that can be accommodated in a given area. The tubes are always oriented North–South (Figure 1.4). The ground beneath the solar collector is often painted white, or covered with white sheets of plastic, to increase reflectance. A high reflectance increases the total light received by the tubes.

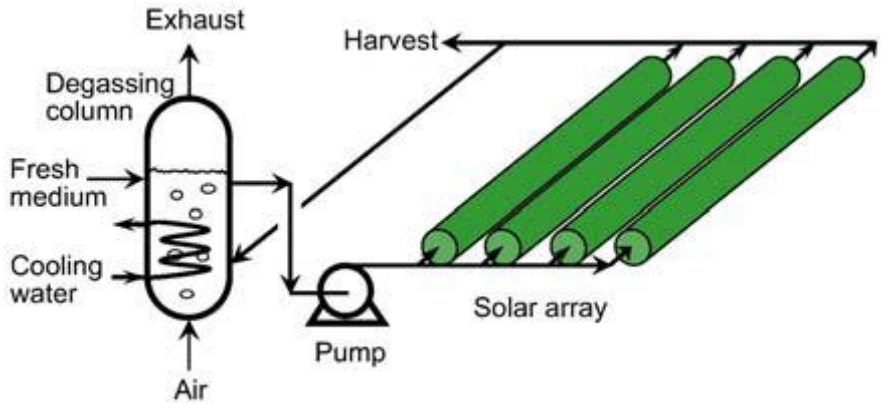


Figure 1.3. A tubular PBR with parallel run horizontal tubes

Instead of being laid horizontally on the ground, the tubes may be made of flexible plastic and coiled around a supporting frame to form a helical coil tubular PBRs (Fig. 1.5). PBRs such as the one shown in Fig. 4 are potentially useful for

growing a small volume of microalgal broth, for example, for inoculating the larger tubular PBRs (Fig. 1.3) that are needed for producing biodiesel. Other variants of tubular PBRs exist, but are not widely used. Artificial illumination of tubular PBRs is technically feasible, but expensive compared with natural illumination. Nonetheless, artificial illumination has been used in large-scale biomass production particularly for high-value products.

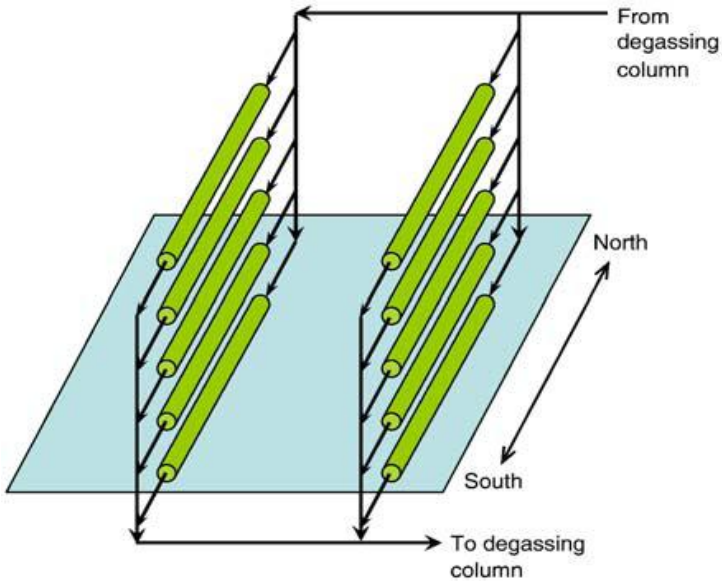
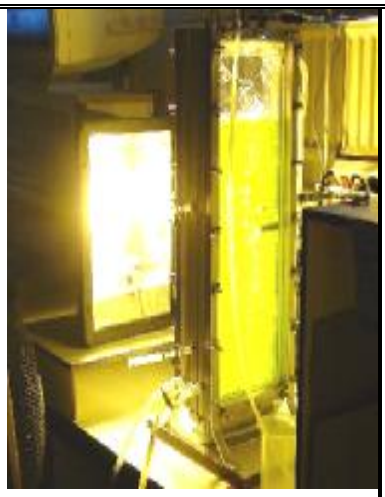


Figure 1.4. A fence-like solar collector

Biomass sedimentation in tubes is prevented by maintaining highly turbulent flow. Flow is produced using either a mechanical pump (Fig. 1.3), or a gentler airlift pump. Mechanical pumps can damage the biomass, but are easy to design, install and operate. Airlift pumps have been used quite successfully. PBRs are designed using the same methods that were originally developed for designing conventional airlift reactors. Airlift pumps are less flexible than mechanical pumps and require a supply of air to operate. Periodically, PBRs must be cleaned and sanitized. This is easily achieved using automated clean-inplace operations. Photosynthesis generates oxygen. Under high irradiance, the maximum rate of oxygen generation in a typical tubular PBR may be as high as 10 g O_2 m^3 min^{-1}. Dissolved oxygen levels much greater than the air saturation values inhibit photosynthesis. Furthermore, a high concentration of dissolved oxygen in combination with intense sunlight produces

photooxidative damage to algal cells. To prevent inhibition and damage, the maximum tolerable dissolved oxygen level should not generally exceed about 400% of air saturation value. Oxygen cannot be removed within a PBR tube. This limits the maximum length of a continuous run tube before oxygen removal becomes necessary. The culture must periodically return to a degassing zone (Fig. 1.3) that is bubbled with air to strip out the accumulated oxygen. Typically, a continuous tube run should not exceed 80 m, but the exact length depends on several factors including the concentration of the biomass, the light intensity, the flow rate, and the concentration of oxygen at the entrance of tube. In addition to removing the accumulated dissolved oxygen, the degassing zone (Fig. 1.3) must disengage all the gas bubbles from the broth so that essentially bubblefree broth returns to the solar collector tubes. Gas–liquid separator design for achieving complete disengagement of bubbles has been discussed. Because a degassing zone is generally optically deep compared with the solar collector tubes, it is poorly illuminated and, therefore, its volume needs to be kept small relative to the volume of the solar collector. As the broth moves along a PBR tube, pH increases because of consumption of carbon dioxide. Carbon dioxide is fed in the degassing zone in response to a pH controller. Additional carbon dioxide injection points may be necessary at intervals along the tubes, to prevent carbon limitation and an excessive rise in pH. PBRs require cooling during daylight hours. Furthermore, temperature control at night is also useful. For example, the nightly loss of biomass due to respiration can be reduced by lowering the temperature at night. Outdoor tubular PBRs are effectively and inexpensively cooled using heat exchangers. A heat exchange coil may be located in the degassing column (Fig. 1.3). Alternatively, heat exchangers may be placed in the tubular loop. Evaporative cooling by water sprayed on tubes, can also be used and has proven successful in dry climates. Large tubular PBRs have been placed within temperature controlled greenhouses, but doing so is prohibitively expensive for producing biodiesel (Chisti, 2007).

		
Figure 1.5. Helical tubular photobioreactor	Figure 1.6. Flat panel photobioreactor	Figure 1.7. Spinning tube-in-tube photobioreactor

In addition to producing biofuel, algae can also be explored for a variety of other uses, such as fertilizer, pollution control, and human nutrition. Certain species of algae can be land-applied for use as an organic fertilizer, either in its raw or semi-decomposed form. Algae can be grown in ponds to collect fertilizer runoff from farms; the nutrient-rich algae can then be collected and reapplied as fertilizer, potentially reducing crop-production costs. In wastewater-treatment facilities, microalgae can be used to reduce the amount of toxic chemicals needed to clean and purify water. In addition, algae can also be used for reducing the emissions of CO_2 from power plants (Wen et. al., 2009).

Although, non-fossil energy sources have a high share of energy supply in Turkey and renewable energy sources have made great contributions in Turkey's energy supply, there is still a need for more research and development on renewable energies to increase their efficient utilization (Demirbas, 2008). Turkey's crude petrol consumption was reached 31 million ton in 2005 and due to lack of fossil resources, Turkey's dependency level is around 70%, which may rise over 80% by 2030. Currently Turkey satisfies 40% of its energy needs by oil. 90% of its oil supplies are imported from the Middle East (Saudi Arabia, Iran, Iraq and Syria) and the Russian Federation. When it is compared to the European Union, Turkey depends on import more and the volatilities in the Middle East may affect Turkey more

(Unler, 2008). As a result, for Turkey to search renewable biofuels which can displace petroleum derived transportation fuels, which contribute to global warming and are of limited availability appear as an important priority. Therefore, the aim of this thesis is to investigate growth characteristics of microalgae and to determine the affect of microalgae biodiesel to engine performance parameters and exhaust emissions.

2. PRELIMINARY WORK

Biodiesel, obtained from methyl/ethyl esterification of fatty acids from vegetable oils, has received significant attention since the last decade. Since biodiesel can be produced from renewable resources in local industries, it has become a fuel of growing interest. Hence, the production and use of biodiesel implies social and environmental benefits as compared to fossil fuels. The use of vegetable oil in internal combustion engines is not a recent innovation. Rudolf Diesel (1858–1913), creator of the diesel cycle engines, used peanut vegetable oil to demonstrate his invention in Paris in 1900. In 1912, Diesel said, ‘‘The use of vegetable oils as engine fuel may seem negligible today. Nevertheless, such oils may become, in the passing years, as important as oil and coal tar presently.” Nowadays, it is known that oil is a finite resource and that its price tends to increase exponentially, as its reserves are fast depleting.

Although during the World War II, some German scientists attempted to extract lipids from diatom in order to resolve energy crisis, the studies on liquid fuel from microalgae had begun in mid-1980s (Huang et. al., 2010). The U.S. Department of Energy (DOE) initiated a research program called the Aquatic Species Program (ASP) in 1979. The program was closed in 1995 due to a budget reduction. Over the 16-year project period, ASP pursued research in three major areas. The first area was the study of the biological aspect of microalgae. It included screening and collecting a variety of algal species to access their potential for high oil production, investigating the physiology and biochemistry of the algae, and using molecular-biology and genetic engineering techniques to enhance the oil yield. The second research area was the development of algal mass-production systems. Several demonstration culture systems located in California, Hawaii, and New Mexico were conducted during the project period. However, in these outdoor systems, it was difficult to maintain the algal-oil production capacity originally obtained in the laboratory scale, and researchers encountered a severe contamination of undesirable native species. It should be noted that DOE suggested open ponds as the major system for algal-biofuel production because of their relative low cost. The cost of

enclosed photobioreactors was still prohibitive due to capital and maintenance costs, particularly for production of biofuels. The third research area was analysis of the resource availability, including land, water, and CO_2 resources. DOE concluded that there were significant amounts of land, water, and CO_2 to support the algal-biofuel technology. In summary, after 16 years of research, DOE concluded that algal-biofuel production was still too expensive to be commercialized in the near future. In its research, three factors limited commercial algal production: the difficulty of maintaining desirable species in the culture system, the low yield of algal oil, and the high cost of harvesting the algal biomass. In recent years, with energy prices reaching historic highs, algal-biofuel production has gained renewed interest. Both university research groups and start-up businesses are researching and developing new methods to improve algal-process efficiency, with a final goal of commercial algal-biofuel production. The research and development efforts can be categorized into several areas:

1. Increasing oil content of existing strains or selecting new strains with high oil content;

2. Increasing the growth rate of algae;

3. Developing robust algal-growing systems in either open-air or enclosed environments;

4. Developing co-products other than oil;

5. Using algae in bioremediation; and

6. Developing an efficient oil-extraction method (Wen et. al., 2009).

Yue et. al. (2005) isolated fresh water microalgae, which has high CO_2 tolerance, and its cultural characteristics were investigated. The ZY-1 strain was identified as genus *Chlorella.* It showed maximum growth at 10% (v/v) CO_2 enriched air flowing condition, and a good growth rate in a broad range of physically controllable conditions, including CO_2 concentration up to 70% (v/v), CO_2 enriched air flow rate, temperature and pH value.

Bulut (2009) treated *Chlorella vulgaris* (Chlorophyceae) to , % 50 N, % 100 N, % 50 P + % 50 N , % 50 P deficiencies and nitrit addition were treated to with the aim of to determine the effects of the defficient nutrient and different nitrogen

source on lipid content and protein was carried out in laboratory conditions. Protein and lipid values of the last day were found as % 50.794 , % 13.287 for control group, % 20.339, % 17.497 for % 50 N(-),% 13.013, % 35.599 % 100 N(-),% 21.373, % 20.518 for % 50 P(-) + % 50 N(-),% 21.373, % 20.518 for % 50 P(-) and % 38.158, % 16.727 for the group nitrit added. The highest lipid content was recorded with the culture to which treated % 100 N(-) with 0,18 g L-1 dry-weight and 9,252 mg L-1 chlorophyll *a* content.

Rodolfi et. al. (2009) screened thirty microalgal strains in the laboratory for their biomass productivity and lipid content. Four strains (two marine and two freshwater), selected because robust, highly productive and with a relatively high lipid content, were cultivated under nitrogen deprivation in 0.6-L bubbled tubes. Only the two marine microalgae accumulated lipid under such conditions. One of them, the eustigmatophyte Nannochloropsis sp. F&M-M24, which attained 60% lipid content after nitrogen starvation, was grown in a 20-L Flat Alveolar Panel photobioreactor to study the influence of irradiance and nutrient (nitrogen or phosphorus) deprivation on fatty acid accumulation. Fatty acid content increased with high irradiances (up to 32.5% of dry biomass) and following both nitrogen and phosphorus deprivation (up to about 50%). To evaluate its lipid production potential under natural sunlight, the strain was grown outdoors in 110-L Green Wall Panel photobioreactors under nutrient sufficient and deficient conditions. Lipid productivity increased from 117 mg/L/day in nutrient sufficient media (with an average biomass productivity of 0.36 g/ L/day and 32% lipid content) to 204 mg/L/day (with an average biomass productivity of 0.30 g/L/day and more than 60% final lipid content) in nitrogen deprived media. The experiments showed that this marine eustigmatophyte has the potential for an annual production of 20 tons of lipid per hectare in the Mediterranean climate and of more than 30 tons of lipid per hectare in sunny tropical areas.

Umdu et. al. (2009) presented the activities of Al_2O_3 supported CaO and MgO catalysts in the transesterification of lipid of yellow green microalgae, Nannochloropsis oculata, as a function of methanol amount and the CaO and MgO loadings at 50 ^{o}C. They found that pure CaO and MgO were not active and

CaO/Al_2O_3 catalyst among all the mixed oxide catalysts showed the highest activity. Not only the basic site density but also the basic strength is important to achieve the high biodiesel yield. Biodiesel yield over 80 wt.% CaO/Al_2O_3 catalyst increased to 97.5% from 23% when methanol/lipid molar ratio was 30.

Vijayaraghavan et. al. (2009) conducted a novel approach in the production of biodiesel; using algal biomass as a raw material. The effect of drying algal biomass and its role on lipid content during extraction process was investigated. Transesterification of algal oil was conducted, using ethanol in the presence of potassium hydroxide as a catalyst. A gas chromatography-mass spectroscopy (GC-MS) chromatogram was used to analyze the organic compounds present in the crude biodiesel sample after the transesterification process. The lipid content in the algal biomass was determined to be 45%. Biodiesel derived from algae had a fuel value with the following characteristics: density, 0.801 kg/L; ash content, 0.21%; flash point, 98^oC; pour point, -14 ^oC; cetane number, 52; minimum gross calorific value, 40 MJ/kg; and water content, 0.02 vol%.

Converti et. al. (2009) studied the effects of temperature and nitrogen concentration on the lipid content of Nannochloropsis oculata and Chlorella vulgaris in view of their possible utilization as novel raw materials for biodiesel production and also investigated various lipid extraction methods. They found that, the lipid content of microalgae was strongly influenced by the variation of tested parameters; indeed, an increase in temperature from 20 to 25 ^oC practically doubled the lipid content of N. oculata (from 7.90 to 14.92%), while an increase from 25 to 30 ^oC brought about a decrease of the lipid content of C. vulgaris from 14.71 to 5.90%. On the other hand, a 75% decrease of the nitrogen concentration in the medium, with respect to the optimal values for growth, increased the lipid fractions of N. oculata from 7.90 to 15.31% and of C. vulgaris from 5.90 to 16.41%, respectively.

Cheng et. al. (2009) used sugar cane juice as alternative carbon supply for oil production. As a result, the highest oil content of 53.0% by cell dry weight was achieved. Fermentation in a 5 L bioreactor showed that algae using sugar cane juice hydrolysate (SCH) grow faster than that using glucose. Conversion ratios of sugar/biomass and sugar/oil using SCH were 15.2 and 8.8% higher than that using

glucose, respectively. Biodiesel prepared from algal oil by transesterification is mainly composed of 9-octadecenoic acid methyl ester, 9,12-octadecadienoic acid methyl ester, and hexadecenoic acid methyl ester. Their results suggested that sugar cane is a good feedstock for biodiesel production. Response surface methodology upon exploring the effect of C/N and concentration of yeast extraction (YE) on the yield of biomass and oil was performed. The optimal production with the highest output-cost coefficient of 0.061 (0.004 was achieved when C/N was 26.9 and YE was 0.60 g L^{-1}).

Krohn et. al. (2010) demonstrated the production of algal biodiesel from Dunaliella tertiolecta, Nannochloropsis oculata, wild freshwater microalgae, and macroalgae lipids using a highly efficient continuous catalytic process. The heterogeneous catalytic process uses supercritical methanol and porous titania microspheres in a fixed bed reactor to catalyze the simultaneous transesterification and esterification of triacylglycerides and free fatty acids, respectively, to fatty acid methyl esters (biodiesel). Triacylglycerides and free fatty acids were converted to alkyl esters with up to 85% efficiency as measured by 300 MHz 1H NMR spectroscopy. The lipid composition of the different algae was studied gravimetrically and by gas chromatography. The analysis showed that even though total lipids comprised upwards of 19% of algal dry weight the saponifiable lipids, and resulting biodiesel, comprised only 1% of dry weight.

Damiani et. al. (2010) analyzed the lipid content and composition of Haematococcus pluvialis exposed to stress conditions to assess the potential of this microalga as a biodiesel feedstock. The total lipid content of control cells was 15.61% dry weight, whereas that of cells exposed to continuous high light intensity with nitrogen-sufficient medium (A-stress condition) or under continuous high light intensity with nitrogen-deprivation medium (B-stress condition) was 34.85% dry weight and 32.99% dry weight, respectively. The fatty acid profile was similar under all conditions and indicated that the main components were palmitic, stearic, oleic, linoleic, linolenic and linolelaidic acids. The neutral lipid fraction increased about 2-fold under both stress conditions. The percentage of saturated fatty acids in the neutral lipid fraction was 30.36% and 29.62% in cultures grown under A-stress and

B-stress, respectively, and 27.81% under control conditions. The monounsaturated fatty acid content was not significantly different in control and A-stress cultures (20.07% and 19.91%, respectively), but was 18.96% under B-stress. The content of polyunsaturated fatty acids was 47.23% under B-stress and 43.15% under A-stress. Growth-rate was higher under A-stress compared to B-stress. They found that culture conditions still have to be improved in order to achieve an adequate energy balance in mass culture.

Yoo et. al. (2010) cultivated Botryococcus braunii, Chlorella vulgaris, and Scenedesmus sp. with ambient air containing 10% CO_2 and flue gas. The biomass and lipid productivity for Scenedesmus sp. with 10% CO_2 were 217.50 and 20.65 mg L^{-1} d^{-1} (9% of biomass),while those for B. braunii were 26.55 and 5.51 mg L^{-1} d^{-1} (21% of biomass). With flue gas, the lipid productivity for Scenedesmus sp. and B. braunii was increased 1.9-fold (39.44 mg L^{-1} d^{-1}) and 3.7-fold (20.65 mg L^{-1} d^{-1}), respectively. Oleic acid, a main component of biodiesel, occupied 55% among the fatty acids in B. braunii. They suggested that Scenedesmus sp. is appropriate for mitigating CO_2, due to its high biomass productivity and C-fixation ability, whereas B. braunii is appropriate for producing biodiesel, due to its high lipid content and oleic acid proportion.

Johnson et. al. (2010). explored a novel attached culture system for growing the alga Chlorella sp. as biodiesel feedstock, with dairy manure wastewater being used as growth medium. Among supporting materials tested for algal attachment, polystyrene foam led to a firm attachment, high biomass yield (25.65 g/m^2, dry basis), and high fatty acid yield (2.31 g/m^2). The biomass attached on the supporting material surface was harvested by scraping; the residual colonies left on the surface served as inoculum for regrowth. The algae regrowth on the colony-established surface resulted in a higher biomass yield than that from the initial growth on fresh surface due to the downtime saved for initial algal attachment. The 10-day regrowth culture resulted in a high biodiesel production potential with a fatty acid methyl esters yield of 2.59 g/m^2 and a productivity of 0.26 g/m^{-2} day^{-1}. The attached algal culture also removed 61–79% total nitrogen and 62–93% total phosphorus from dairy manure wastewater, depending on different culture conditions. The biomass

harvested from the attached growth system (through scraping) had a water content of 93.75%, similar to that harvested from suspended culture system (through centrifugation). Collectively, the attached algal culture system with polystyrene foam as a supporting material demonstrated a good performance in terms of biomass yield, biodiesel production potential, ease to harvest biomass, and physical robustness for reuse.

3. MATERIAL AND METHOD

All of the experiments were conducted in Çukurova University Automotive Engineering Department Laboratories. The whole biodiesel production from microalgae process contains five main steps.

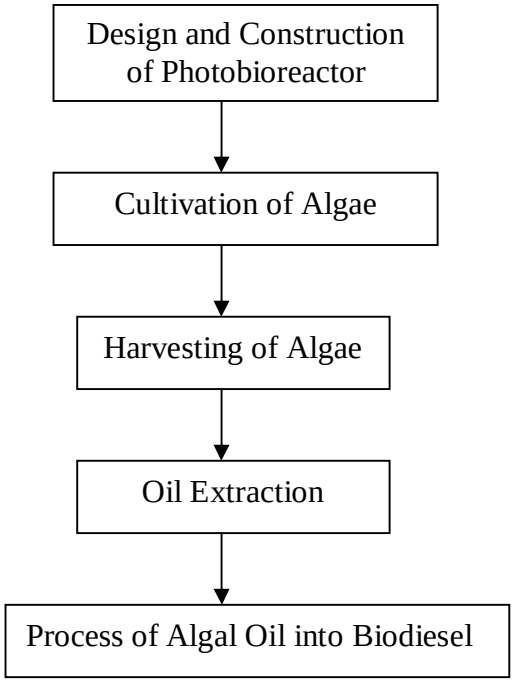


Figure 3.1. Entire process of producing biodiesel from algae

3.1. Design and Construction of Photobioreactor

There are basically two methods of growing algae, namely; open ponds or PBRs, however; in order to have efficient and controlled production, PBRs should be used since open ponds do not provide conditions necessary for high density algal biomass production because variation in light intensity and temperature. A PBR can be described as an enclosed, lighted algal culture vessel designed for controlled production of algae. Enclosed PBRs have the following advantages over open pond production.

1. Better control of algal culture

2. Large surface-to-volume ratio

3. Better control of gas transfer

4. Reduction in evaporation of growth medium

5. More uniform temperature

6. Better protection from outside contamination

7. Higher algal cell densities are possible.

In a well designed photobioreactor, sunlight should be used as energy source as much as possible, however; to save time; algae should be grown even during night hours. In order to make this possible another source of light should be used. Due to their low energy consumption, fluorescent lights were used for this purpose. Another specification of a reactor is to expose the algae to light as much as possible. The most efficient way of utilizing from fluorescent lights is positioning them vertically; therefore this configuration was used in reactor design.

Different geometries and operating methods developed depend on the local conditions, the product to be produced, and economic constraints. Indeed, commercially available closed PBRs still do not represent an optimal solution in many different regards. Even if areal and volumetric productivity is higher than in open ponds, the performance does not come close to theoretical maxima and can not even reach values obtained at lab scale. Besides, the lack of performance, investment, and operation costs are still estimated as being far too high (Posten 2009). Since flat plate PBR is the most robust design among all; this type of PBR of 250 liter was designed for algae production. The thickness of the reactor was limited to 300 mm since light can not penetrate too deeply.

One point of energy loss is the aeration of the reactors. Enriched air has to be produced in a compressor, pumped through tubes, cleaned by membranes and finally has to be sparged against the hydrodynamic pressure. On top a small residual over pressure is needed for removal of the off-gas (Posten, 2009).Mixing and CO_2 supply was accomplished by sparging with CO_2-enriched air. Since mixing only by bubbles is the most gentle way with respect to shear stress for the algae, mixing was performed with a bubbling power supply. Throughout the experiments the

temperature of the water was kept at 25 ^{o}C by the help of an electric heater (Figure 3.2).



Figure 3.2. PBR that is used for algae production

3.2. Cultivation of Algae

Microalgae are remarkably efficient factories capable of taking a waste form of energy of carbon (CO_2) and converting it into a high density liquid form of energy. For the algae to biodiesel cycle to be successful a species of algae that has high grow rates and oil content must be used. *Chlorella vulgaris* which was selected as microalgae culture was obtained from Çukurova University Faculty of Aquaculture. *Chlorella vulgaris* belongs to a genus of single-celled green algae, and is known as one of the fastest growing microalgae, and typically includes 14%-22% of lipid, 51%-58% of protein, 12%-17% of carbohydrates,and 4%-5% of nucleic acid (Kim et. al. 2010).

A Jaworsky media was prepared for growing *Chlorella vulgaris.* This nutrition media was composed of from various inorganic elements (Table 3.1).The inoculation rate was arranged to 20% (20% culture, 80% nutrition media).

Table 3.1. Jaworsky *Chlorella vulgaris* nutrition media (Bulut, 2009)

Jaworsky media (for 200ml)	
$Ca(NO_3)_2.4H_2O$	4.0 g
KH_2PO_4	2.48 g
$MgSO_4.7H_2O$	10.0 g
$NaHCO_3$	3.18 g
EDTA FeNa	0.45 g
EDTA Na_2	0.45 g
H_3BO_3	0.496 g
$MnCl2.4H_2O$	0.278 g
$(NH_4)\ 6Mo_7O_24.4H_2O$	0.2 g
$NaNO_3$	16 g
$Na_2HPO_4.12H_2O$	7.2 g

With respect to light, the more the better is not true for microalgae. Most of them are adapted to low light intensities representing only a fraction of the full day light. After a linear increase of growth rate with increasing light intensity saturation is at about 100 µE/(m^2s). This is only 10% of the midday sunlight intensity in a European summer. Light in excess is wasted as fluorescence and finally heat (Posten, 2009). Applying dark/light cycles have a strong effect on algae growth. 8/16 dark/light cycle was applied to the culture since this period was the same as the time period of summer season at which microalgae can grow at highest rate.

Algae reproduce by cellular division, they divide until they fill whatever space they are in. They will initially enter en exponential growth phase, where cells grow and divide as a function of time as long as mineral substrates and light energy are supplied. When the concentration of algae is high enough that light does not penetrate through the entire culture, the algae move into the light limited linear growth phase. If they are not harvested algae will eventually reach terminal density and stop growing. Therefore, to choose proper time to harvesting of algae is very important. In order to identify proper time, pH, Optical Density (OD) and dry weight values were fallowed daily after each inoculation. Optical Density (OD) values of culture were read at 540 nm (Figure 3.4).

To identify dry weight, after properly homogenizing the microalgae culture, 20 ml of sample was taken from the photobioreactor. This sample was filtered to previously dried and weighted Whatman GF/C filter papers which has 0.45 µ pore sizes and by this way *Chlorella vulgaris* cells were separated from media. After keeping filter paper in drying oven during 2 hours at 105 ^{o}C, this paper was weighted by the help of weighter which has a sensivity of 0.0001 g.



Figure 3.3. Tintometer device that is used to measure OD values

To determine the types of species an Olympus Trinocular BX51 model microscope was used from the Environmental Engineering Department of Çukurova University (Figure 3.5).



3.4. Olympus Trinocular BX51 model microscope

3.3. Harvesting of Algae

Algae harvesting is one of the major factors that must be overcome in order for algae to be used as a fuel source. The problem is that microalgae mass cultures are dilute, typically less than 500 mg/l on a dry weight organic basis and the cells are very small. In order to be processed into biodiesel, the algae must be in the form of a paste that is 10% by solids.

Harvesting of microalgae from open pond or PBRs include several techniques. Normally harvesting of microalgae can be a single step process or two step process which involves harvesting and dewatering. Harvesting microalgae is difficult because of the small size of the algae. Choosing the effecting harvesting process for a particular strain is strongly related with size and features of that strain.

There are several ways of harvesting microalgae include settling or flocculation, centrifugation and filtration. These processes are provided by cell flocculation, either through the addition of chemical flocculants or through culture auto flocculation. Flocculation causes the cells to become aggregated into larger groups which are more easily filtered. Since high amounts of chemicals are required the cost of this method is very high.

During centrifugation process, the algae solution is pumped into a large centrifuge, which rotates around thousand rpm causing the algae to be pressed

against the outer wall which is a filter only a few microns in spacing. The water is forced out while the algae remain of the screen in the form of a paste around about 20% algae. This is a proven method that has been extensively used when working with microalgae. A Subitaş DE 225 model separator was used in order to seperate *Chlorella vulgaris* culture from water (Figure 3.5).



Figure 3.5. Seperator that is used to harvest *Chlorella vulgaris* culture

3.4. Oil Extraction

Results on microalgal oil extraction are rare and difficult to extrapolate to industrial scale. Algae oil extraction is very similar to soybean extraction. However soybean has a solid content around 90%. Hence to preserve consistency of the study, algal paste has to be dried up to a solid content of 90% before being processed in the oil mill or oil solvent. Comparison of different processes commonly used for wastewater treatment shows that belt dryer is one of the less demanding drying

processes able to reach a 90% solid content. Oil is separated from the biomass by counter-current circulation of a solvent, usually hexane: 2 g of hexane are lost for each kg of dry algae (Lardon, 2009).

In order to extract oil from microalgae a soxhlet extractor was used (Figure 3.6). In a soxhlet extractor, solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the soxhlet extractor. The soxhlet extractor is placed onto a flask containing the extraction solvent. The soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded (Anonymous, 2010).

At the end of oil extraction process, about 4g of oil was obtained before each inoculation (12-13 days period of time). In order to be able to determine fuel properties and conduct engine experiments at least 3000 g of oil is needed, this requires nearly 9000 days (about 25 years) of operation, or designing a larger PBR. Since required days exceed the thesis duration, microalgae oil was provided by Soley Biotechnology Institute (Figure 3.7).



Figure 3.6. Oil extraction process



Figure 3.7. Microalgae oil provided by Soley Biotechnology Institute

3.5. Process of Algal Oil into Biodiesel

After obtaining algae oil, in order to convert it into biodiesel, a process called transesterification should occur. Transesterification process is the reaction of a triglyceride (oil) with an alcohol to form esters and glycerol (Figure 3.8). During the esterification process, the triglyceride is reacted with alcohol in the presence of a

catalyst, usually a strong alkaline like sodium hydroxide. The alcohol reacts with the fatty acids to form the biodiesel. In most production methanol or ethanol is used as alcohol and potassium or sodium hydroxide are used as catalyst. Transesterification process is shown in the equation below;

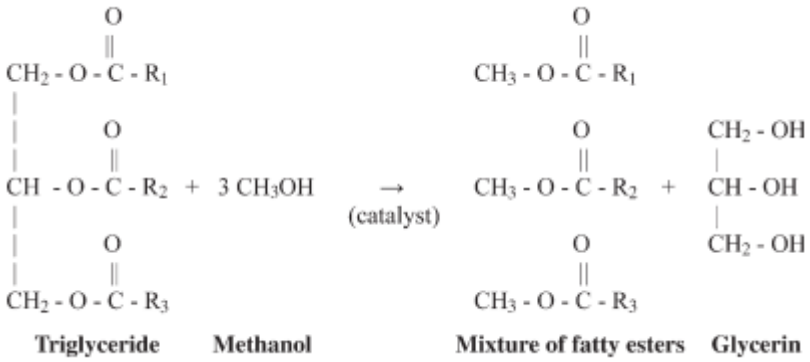


Figure 3.8. Transesterification reaction

where R1, R2, and R3 are long hydrocarbon chains, sometimes called fatty acid chains.

Methods commonly used for producing biodiesel are batch and continuous processes. In general, smaller capacity plants use batch systems. Continuous systems generally lead the operation on a 24x7 basis, requiring larger capacities to justify larger staffing needs and also requiring uniform feedstock quality. In the experiments a small scale laboratory reactor was used within small amounts of biodiesel production. Detailed view of small scale reactor can be seen in Figure 3.9.

The transesterification works well when the input oil is of high quality. However, quite often low quality oils are used as raw materials for bio-diesel preparation. In cases where FFA content of the oil is above 1%, difficulties arise due to the formation of soap, which promotes emulsification during the water washing stage. If the FFA content is above 2%, the process becomes unworkable.

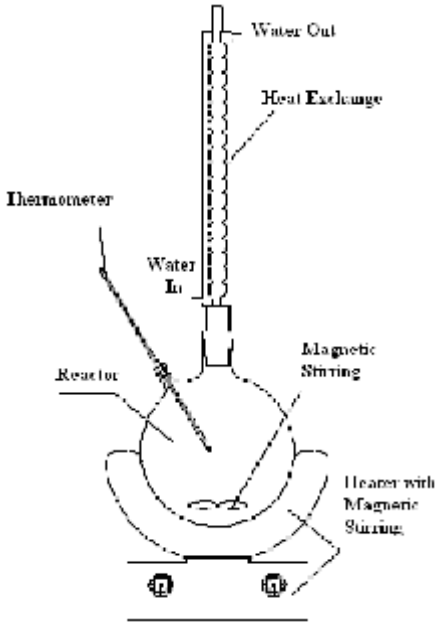


Figure 3.9. Small scale reactor (Özcanlı, 2009)

The factors affecting the transesterification process are:

1.Oil temperature

2.Reaction temperature

3.Ratio of alcohol to oil

4.Catalyst type and concentration

5.Intensity of mixing

6.Purity of reactants

The change in the esterification product composition vs time is shown in Figure 3.10. When these curves are examined, it can be seen that the methyl ester (ME) conversion started at 2 min; ME conversions of 30.28% at 6 min and 59.88% at 10 min were reached. After 10 min, no triglyceride (TG) was left in the mixture and diglycerides (DG) and monoglycerides (MG) formed in the following minutes were converted in ME. At the end of 38 min there were 97.32% ME, 2.27% DG, and 0.41% MG in the mixture. ME conversions of 98.18% in 42 min, 99.3% in 46 min, and 99.69% in 50 min were observed. Considering the energy requirement of the system and the conversion ratios, 38 min is a suitable reaction time (Karaosmanoğlu et. al. 1996).

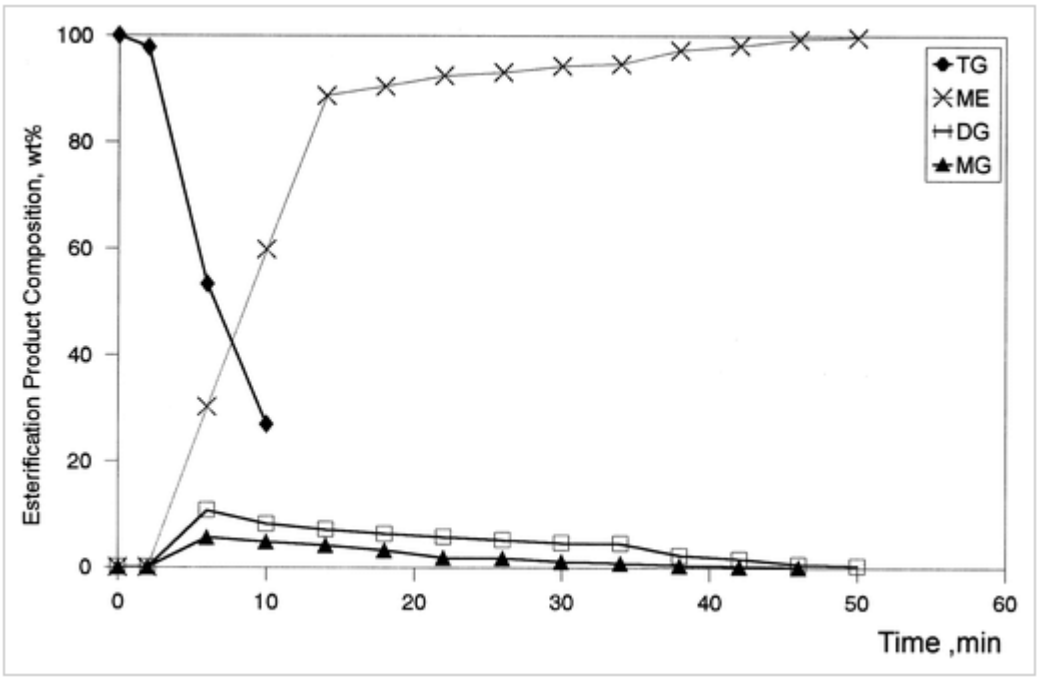


Figure 3.10. Change in esterification product composition with time (Karaosmanoğlu et. al. 1996).

During the microalgae biodiesel production, the necessary amount of catalyst (NaOH) for the transesterification reaction (0.4 % by weight of the oil) was dissolved in methanol and added to the reactor after heating the microalgae oil to 65 °C; the reaction was performed at 60-61 °C and the mixture was stirred by the help of a magnetic stirrer at about 600 rpm during 1 h. After completion of the transesterification reaction, the mixture was cooled to room temperature and then transferred to a separatory funnel and separation of the ester and glycerin phases was performed by letting them stand for 8 h in the separatory funnel. The crude ester phase was washed 3 times with hot water at 1/5 water to ester phase ratio. After washing process the mixture was waited in seperatory funnel during 30 min and by this way water is separated from methyl ester. Since purity level has strong affects on fuel properties, in order to provide water content to be less than 0.1, drying process was conducted by heating the biodiesel to 105^{o}C during 1 h until bright color occurred. Finally, filtering process was done in order ensure that the end product is of excellent quality (Figure 3.11).

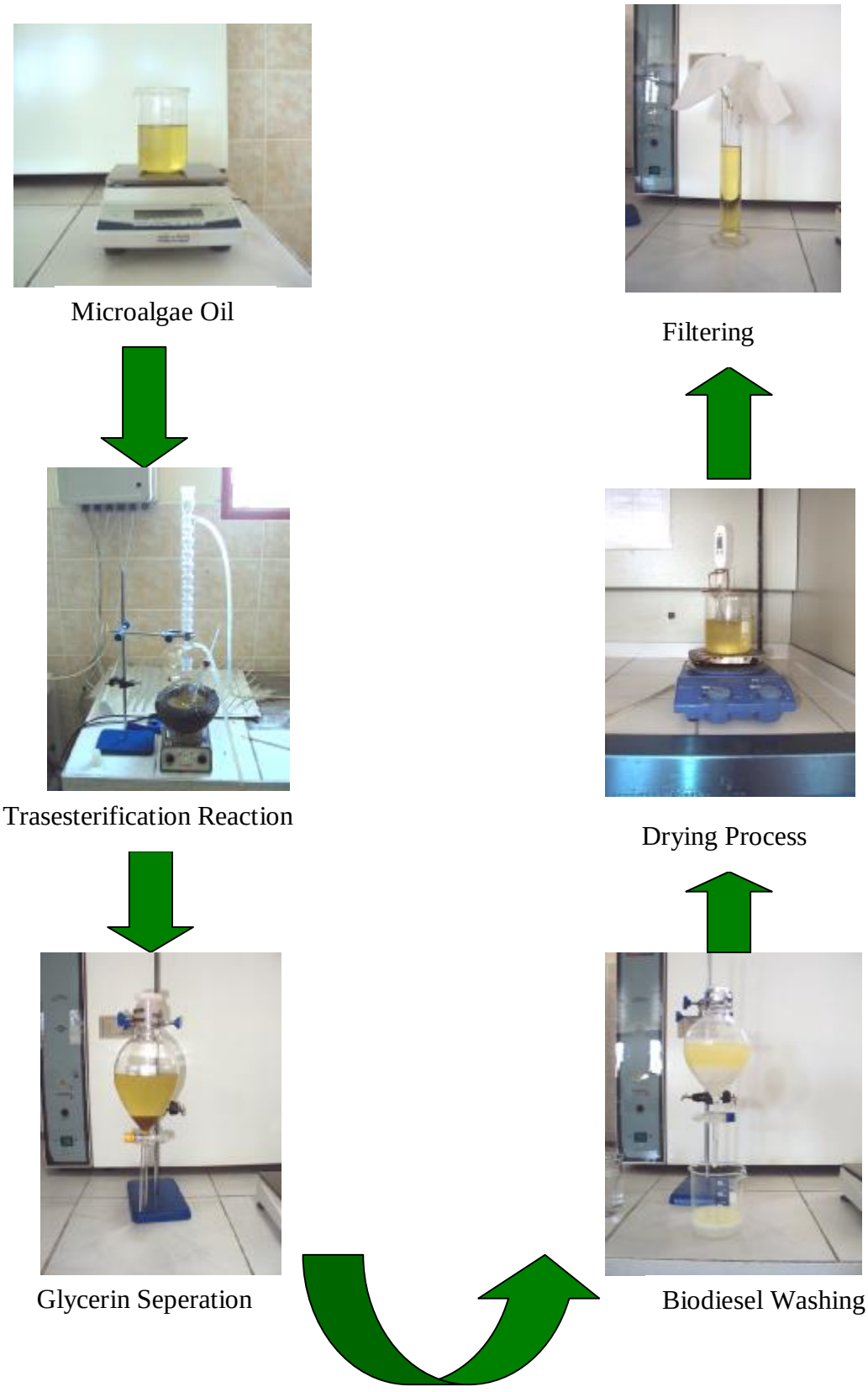


Figure 3.11. The entire biodiesel production process

3.6. Preparation of Fuel Blends

In this study, 5 different fuel blends were prepared by mixing microalgae biodiesel, ricinus communis biodiesel and diesel fuel. The fuel contents of different blends are presented in Table 3.2.

Table 3.2. Prepared fuel blends

Samples	Microalgae Biodiesel (ml)	Diesel fuel (ml)	Ricinus Communis Biodiesel (ml)	Total (ml)
B5	30	570	-	600
B10	60	540	-	600
B20	120	480	-	600
B50	300	300	-	600
MB95R5	570	-	30	600

3.7. Determination of Fuel Properties

The fuel quality measurements of the blends were done according to TS EN 14214. The testing equipments and their measuring methods were briefly explained below.

3.7.1. Density Measurement

The density measurement was done using Kyoto Electronics DA-130 type densimeter (Figure 3.12). This densimeter uses the resonant frequency method to measure the densities. With the densimeter we can also measure the specific weight, API gravity, % Brix, volume and mass alcohol rates. The measurement interval of the device is 0 to 2 g/cm^3 and 0 to 40 ºC. The device has a sensitivity of ±0.001 g/cm^3, and a stability of 0.0001 g/cm^3. The device is measuring according to the standards of TS 6311, ASTM D 4052-96 (Abdulvatihoğlu, 2009).



Figure 3.12. Kyoto Electronics DA-130 portable densimeter

3.7.2. Cetane Number Measurement

The Cetane number and indexes were measured by Zeltex ZX440 (Figure 3.13) type device, which works under the close infrared spectrometer (NIR) principal. With the help of this principal the Cetane number measurement experiment became very fast and cheap with only 3% error compared to the time consuming expensive motor tests (Abdulvahitoğlu, 2009).



Figure 3.13. Zeltex ZX440

3.7.3. Viscosity Measurement

The viscosities of the fuels were measured with Saybolt Universal Viscosimeter (Figure 3.14) produced from Ubbelohde tube with ASTM D88 standards. The meaurement results were recorded in seconds. Then using a conversion table the results were converted from SSU (saybolt universal second) to centistokes (cSt) unit. The measurements were done at 40 ºC according to the TS EN 14214 standard (Abdulvahitoğlu, 2009).



Figure 3.14. Saybolt Universal Viscosimeter

3.7.4. Cold Flow Measurement

A fuel property that is particularly important for the low temperature operability of diesel fuel is the cloud point. The cloud point is the temperature at which a cloud of wax crystals first appears in a liquid upon cooling. Therefore, it is an index of the lowest temperature of the fuel's utility under certain applications. Operating at temperatures below the cloud point for a diesel fuel can result in fuel filter clogging due to the wax crystals. As described in ASTM D 2500, the cloud point is determined by visually inspecting for a haze in the normally clear fuel, while the fuel is cooled under carefully controlled conditions.

A second measure of the low temperature performance of diesel/biodiesel fuels is the pour point. The pour point is the lowest temperature at which a fuel sample will flow. Therefore, the pour point provides an index of the lowest temperature of

the fuel's utility for certain applications. The pour point also has implications for the handling of fuels during cold temperatures. The standard procedure for measuring the pour point of fuels is ASTM D 97.

Tanaka MPC-102 (Figure 3.15) was used to determine pour point of fuels. Pour Point measurement is made utilizing a new ASTM test method, namely "Air Pressure Method" (ASTM D6749 on "Standard Test Method for Pour Point of Petroleum Products (Automatic Air Pressure Method 1)), which yields eventually no bias against the conventional test method, repeatability/reproducibility of $1^{o}C$ /$2^{o}C$ and 2-3 times faster determinations. The device can measure according to the ASTM D6749/D97, ISO 3016 (PP), ASTM D2500, ISO 3015 (CP). Measuring range is +51 ^{o}C to – 40 ^{o}C with tap water of 20 ^{o}C and +51 ^{o}C to – 65 ^{o}C with cooling liquid of -35 ^{o}C. Amount of applied air pressure for pour point detection, to accommodate different sample types: L (low) for diesel fuels, H (high) for lube oils, VH (very high) and UH (ultra high) for residual fuels and similar samples. Testing intervals: 1.0 ^{o}C, 2.5 ^{o}C, 3.0 ^{o}C (Abdulvahitoğlu, 2009).



Figure 3.15. Tanaka MPC-102L

3.7.5. Flash Point Measurement

Tanaka Automated Pensky-Martens Closed Cup Flash Point Tester, model APM-7 was used to determine flash point of test fuels. It is conforming the standarts,ISO 2719, ASTM D93, IP 34, ETC. Measuring range is from ambient temperature to 370 ^{o}C (Abdulvahitoğlu, 2009).



Figure 3.16. Tanaka APM-7

3.8. Diesel Engine Test Rig

In this study, experiments were performed on a Mitsubishi Canter 4D34-2A (Figure 3.17), four stroke diesel engine with four cylinders. Specifications of the engine are presented in Table 3.3.

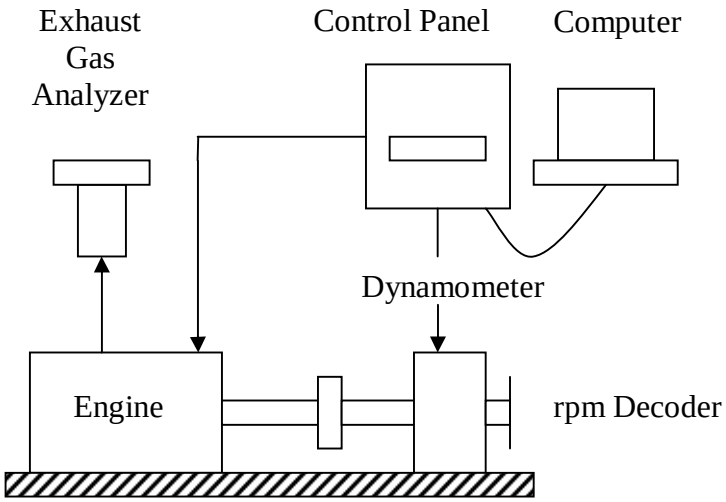


Figure 3.17. Layout of experimental setup

Table 3.3. Technical specifications of the test engine

Brand	Mitsubishi Canter
Model	4D34-2A
Configuration	In line 4
Type	Direct injection diesel with glow plug
Displacement	3907cc
Bore	104mm
Stroke	115mm
Power	89kW @ 3200rpm
Torque	295Nm @ 1800rpm
Oil Cooler	Water cooled
Weight	325kg

The engine torque was measured by the help of a hydraulic dynamometer. Table 3.4 shows technical specifications of the dynamometer.

Table 3.4. Technical specifications of the dynamometer

Torque range	0-1700 Nm
Speed range	0-7500 rpm
Body weight	45 kgf
Total weight	110 kgf
Body diameter	350 mm
Torque arm length	350mm

The speed sensor used to detect prime mover speed is the magnetic pickup (MPU). When a magnetic material (usually a gear tooth driven by the prime mover) passes through the magnetic field at the end of the magnetic pickup, a voltage is developed. The frequency of this voltage is translated by the speed into a signal which accurately depicts the speed of the prime mover. S type load cell is used to measure the torque of dynamometer (Stainless steel which has a sensivity of 1/3000. Dynamometer control unit was used for receiving and collecting all the data that was used by the system.

TESTO 350 XL gas analyzer is used to measure exhaust emissions. Emission data was collected by the help of a computer program. Measurement accuracy of the gas analyzer is ±10 ppm for CO, 1% for CO_2 and ±1 ppm for NO_x. Measurement capacity of the device is 0-10000 ppm for CO, 0-50% for CO_2 emission and 0-3000 ppm for NO_x.

4. RESULTS AND DISCUSSION

4.1. Cultural Characteristics Determination

The microalgal strain used in this study was *Chlorella vulgaris* obtained from the Çukurova University Faculty of Aquaculture. To improve the yield of algal oil and minimize the cost, optimizing the medium to achieve a maximum performance of microalgae growing seems to be of critical importance, because medium composition can significantly affect the yield and cost of products. The key factors associated with oil yield are nitrogen content, C/N (ratio of carbon source/ nitrogen source), and carbon source.

Although the *Chlorella vulgaris* species was cultured during this study, the culture was contaminated by wild species after 4 months, since the manufactured PBR was not fully isolated from the ambient. The microscopic observations revealed that, *Scenedesmus dimorphus* became as dominant species after a while. The picture of mixed culture is shown in Figure 4.2.

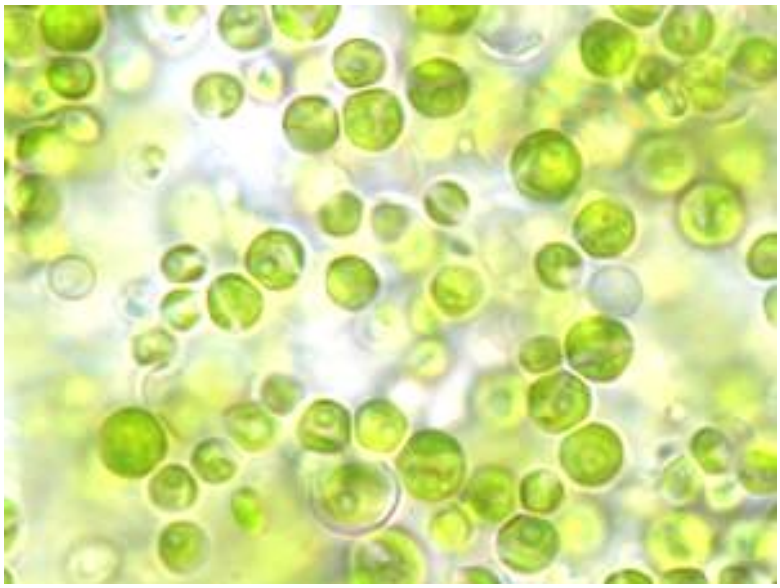


Figure 4.1. *Chlorella vulgaris*

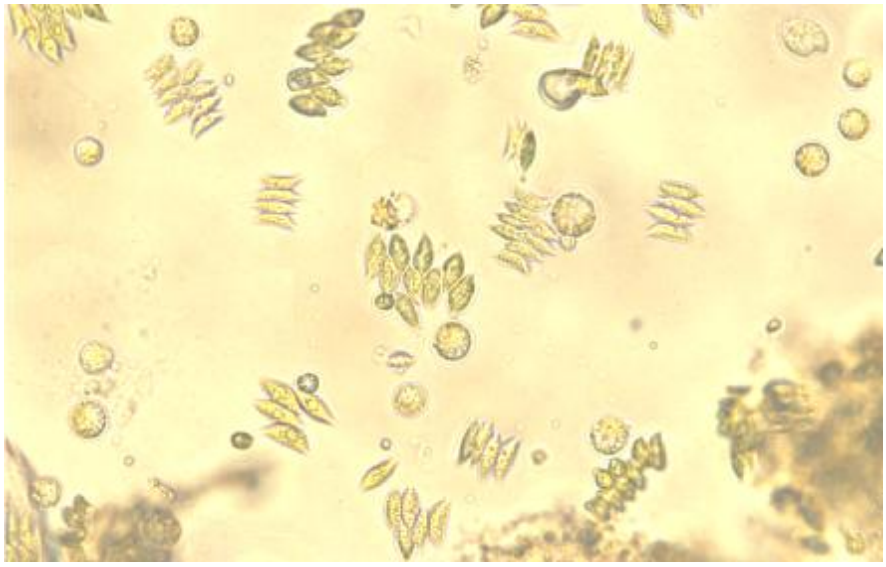


Figure 4.2. The picture of mixed culture (taken at 400x microscopic magnification)

Four different microalgae culture experiments were conducted to examine the cultural characteristics. The temperature of the culture media was kept at 25^{o}C. In the first experiment microalgae culture was grown at normal conditions (control group), in the second experiment microalgae culture was grown in Nitrogen (N) deficient media, in the third experiment the culture was grown in CO_2 deficient media and in the fourth experiment nitrogen gas which has concentration 20% to air was given directly to the culture by the help of air stone. During all four experiments optical density, dry weight and pH values were measured daily.

4.1.1. Optical Density Values

The highest and the lowest optical density values, which were obtained from the control group, were found 0.2977 and 2.2749, respectively. As expected, OD value of the culture increased day by day and the maximum OD value was obtained at the end of 12 th day, the time when the culture was harvested. The variation of optical density values of control group is shown in Figure 4.3.

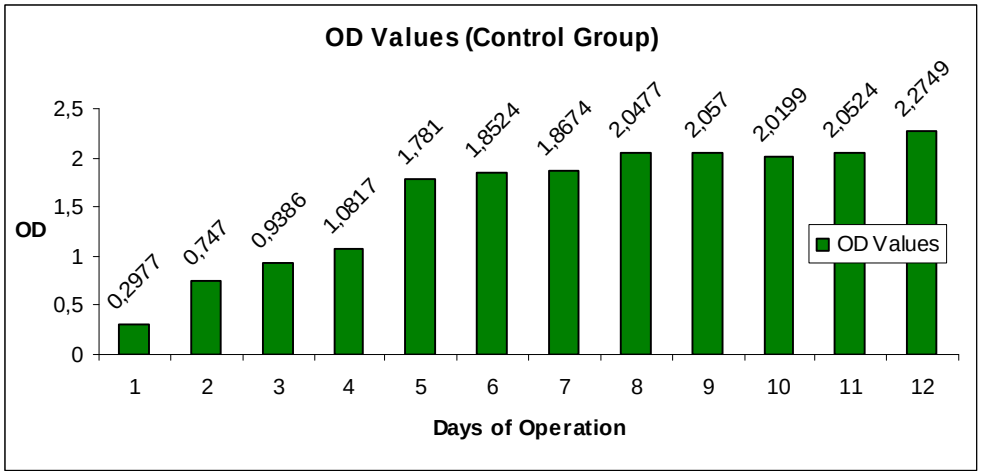


Figure 4.3. Optical density values of *Chlorella vulgaris* (control group)

For the *Chlorella vulgaris* culture which was grown in N deficient media, optical density values were read 0.3554 as minimum and 2.5337 as maximum. The variation of optical density values of *Chlorella vulgaris* culture which was grown in N deficient media is shown in Figure 4.4.

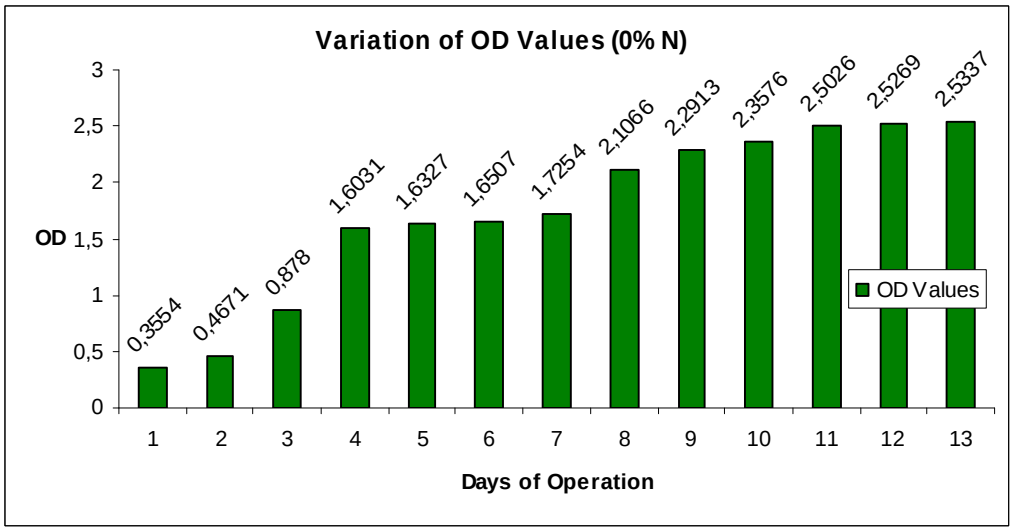


Figure 4.4. Optical density values of *Chlorella vulgaris* (0% N)

The optical density values vary between 0.39587 and 2.3903 for *Chlorella vulgaris* culture which was grown without CO_2 gas sparking. The variation of optical density values of *Chlorella vulgaris* culture which was grown without CO_2 gas sparking is shown in Figure 4.5.

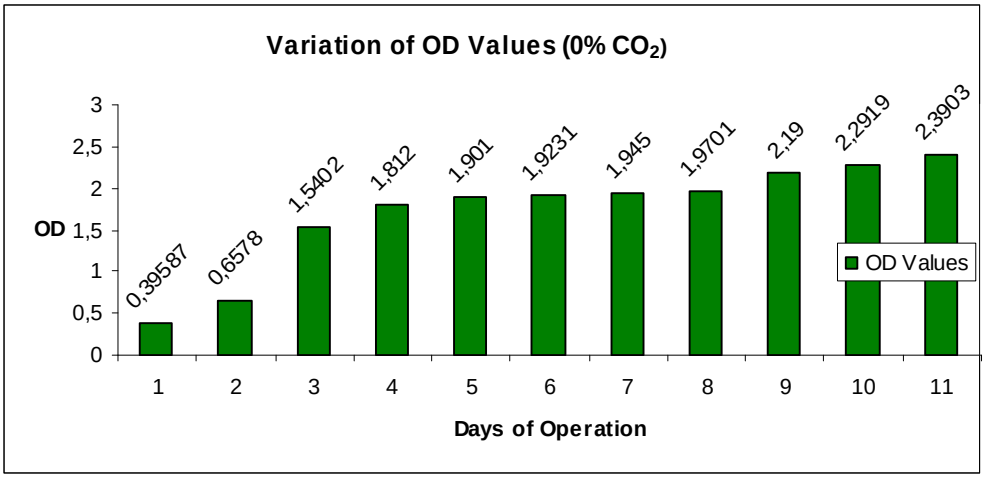


Figure 4.5. Optical density values of *Chlorella vulgaris* (0% CO_2)

The highest and the lowest optical density values, which were obtained from the *Chlorella vulgaris* culture grown with 20% v/v nitrogen gas sparking, were found 0.456 and 1.9582 respectively. The variation of optical density values of fourth experiment is shown in Figure 4.6.

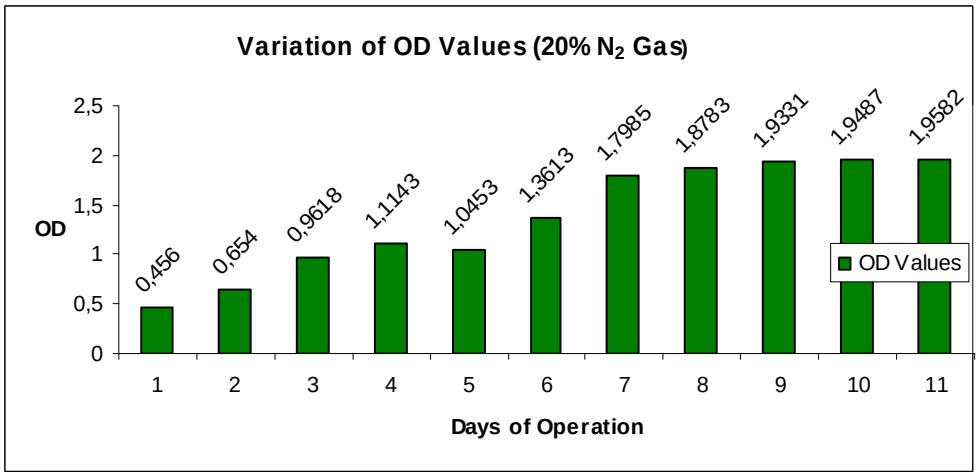


Figure 4.6. Optical density values of *Chlorella vulgaris* (20% N_2 gas)

Among four experiments the highest optical density value was obtained for culture which was grown in N deficient media (1.74 mean value) and the lowest value was obtained for the culture which was grown with 20% v/v nitrogen gas sparking (1.37 mean value). Therefore, it can be concluded that, microalgae shows the best growing characteristics in N deficient media and growing microalgae with direct nitrogen gas has negative affects on algae growing.

4.1.2. Dry Weight Values

For the control group, dry weight values were read 0.63 gL^{-1} as minimum, 4.3 gL^{-1} as maximum. The variation of dry weight values of control group is shown in Figure 4.7.

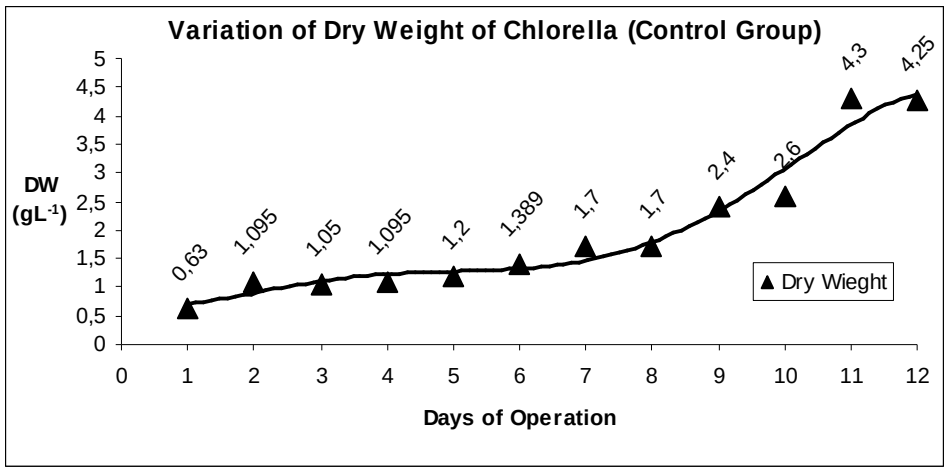


Figure 4.7. Dry weight values of *Chlorella vulgaris* (control group)

For the *Chlorella vulgaris* culture which was grown in N deficient media, dry weight values were read 0.5235 gL^{-1} as minimum and 4.6555 gL^{-1} as maximum. The variation of dry weight values of *Chlorella vulgaris* culture which was grown in N deficient media is shown in Figure 4.8.

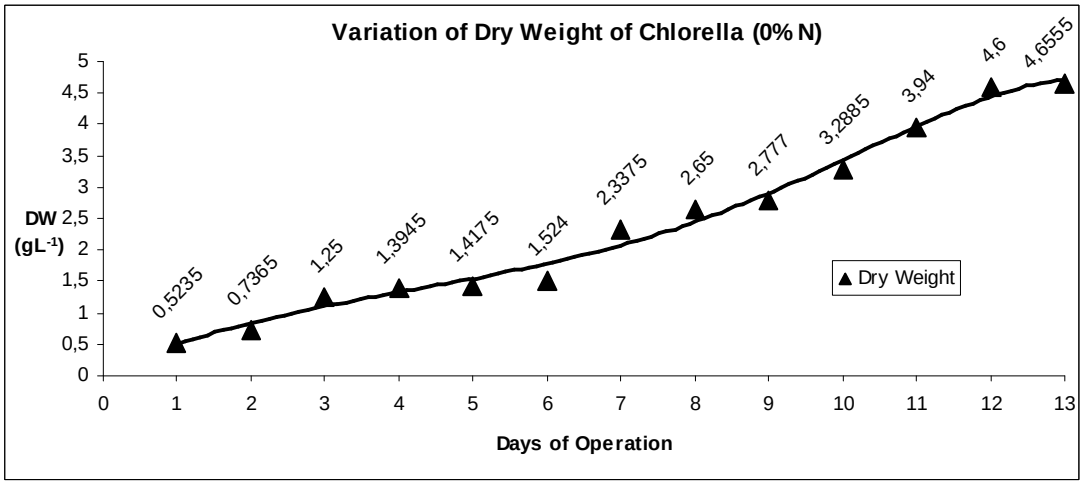


Figure 4.8. Dry weight values of *Chlorella vulgaris* (0% N)

The dry weight values vary between 0.676 gL^{-1} and 3.05 gL^{-1} for *Chlorella vulgaris* culture which was grown without CO_2 gas sparking. The variation of dry weight values of *Chlorella vulgaris* culture which was grown without CO_2 gas sparking is shown in Figure 4.9.

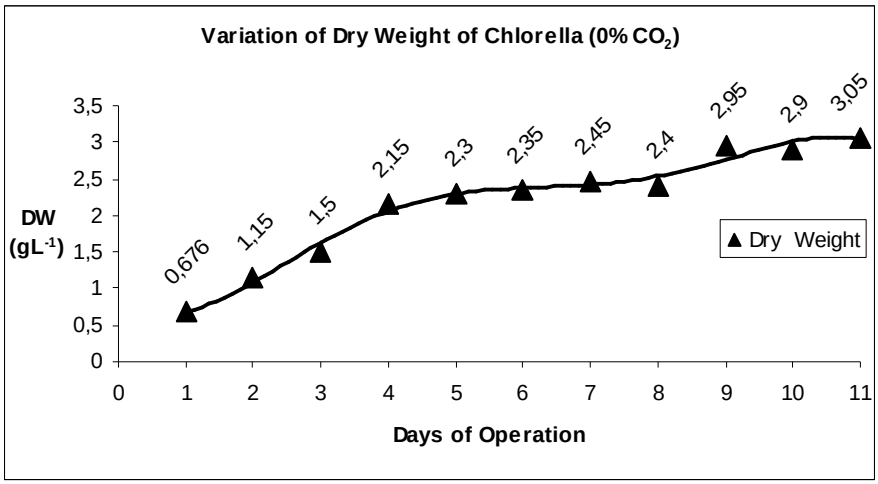


Figure 4.9.Dry weight values of *Chlorella vulgaris* (0% CO_2)

The highest and the lowest dry weight values, which were obtained from the *Chlorella vulgaris* culture grown with 20% v/v nitrogen gas sparking, were found 0.6 gL^{-1}, 2.05 gL^{-1} respectively. The variation of dry weight values of fourth experiment is shown in Figure 4.10.

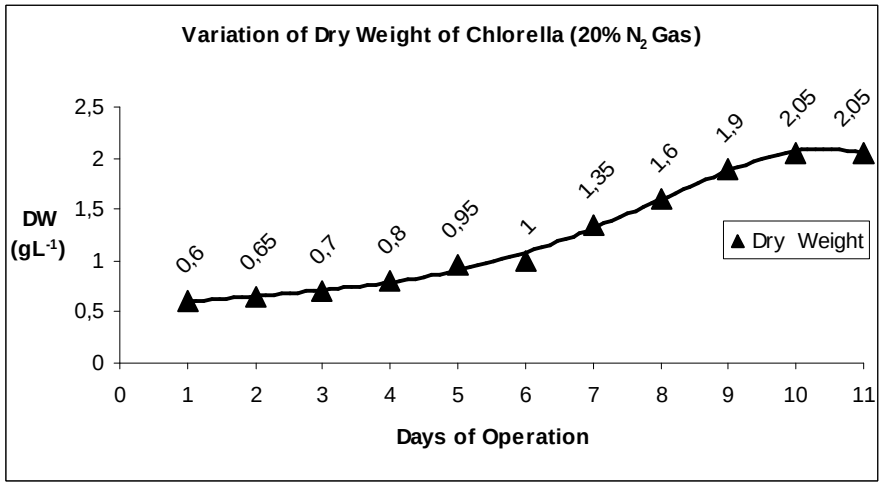


Figure 4.10. Dry weight values of *Chlorella vulgaris* (20% N_2 gas)

Variation of dry weight values shows similar characteristics to optical density values as expected. Namely, the highest dry weight value was obtained for culture which was grown in N deficient media (2.39 gL^{-1} mean value) and the lowest value was obtained for the culture which was grown with 20% v/v nitrogen gas sparking (1.24 gL^{-1} mean value).

4.1.3. pH Values

Because microalgae are primitive plants, they both exhale and inhale oxygen in the photosynthesis process. Algae produce carbon dioxide during darkness and produce oxygen during daylight. The production of carbon dioxide can effect pH of the water. Carbon dioxide dissolves in water to form carbonic acid (H_2CO_3) that has a pH of less than 7, therefore; pH will tend to fall when carbon dioxide is high ($CO_2 + H_2O \rightleftharpoons H_2CO_3$). Therefore concentration of CO_2 in the PBR is directly related to pH value.

The highest and the lowest pH values, which were obtained from the control group, were found 8.7 and 10.3 respectively. The variation of pH values of control group is shown in Figure 4.11.

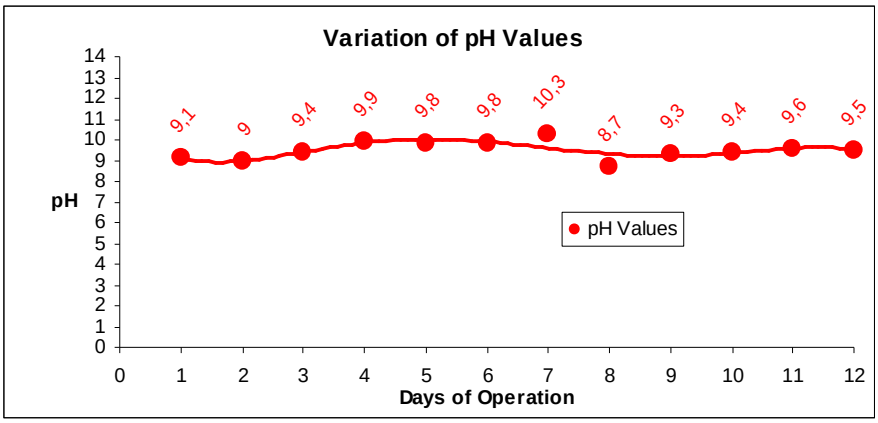


Figure 4.11. pH values of *Chlorella vulgaris* (control group)

For the *Chlorella vulgaris* culture which was grown in N deficient media, pH values were read 7.8 as minimum and 9.1 as maximum. The variation of dry weight

values of *Chlorella vulgaris* culture which was grown in N deficient media is shown in Figure 4.12.

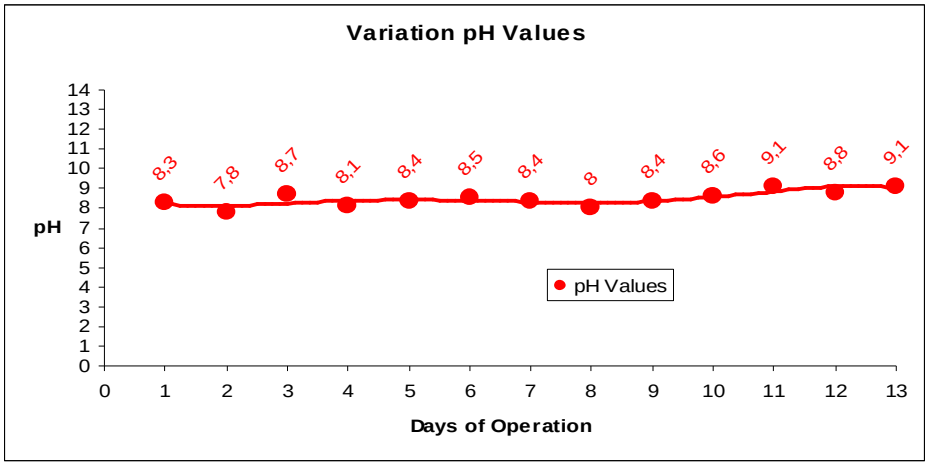


Figure 4.12. pH values of *Chlorella vulgaris* (0% N)

The pH values vary between 8.9 and 10 for *Chlorella vulgaris* culture which was grown without CO_2 gas sparking. The variation of pH values of *Chlorella vulgaris* culture which was grown without CO_2 gas sparking is shown in Figure 4.13.

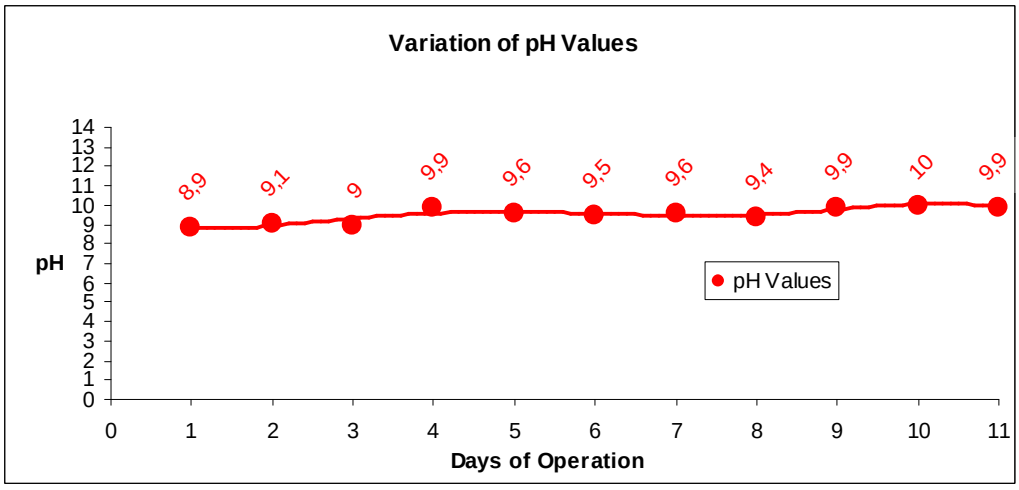


Figure 4.13. pH values of *Chlorella vulgaris* (0% CO_2)

The highest and the lowest pH values, which were obtained from the *Chlorella vulgaris* culture grown with 20% v/v nitrogen gas sparking, were found

7.6, 9.6 respectively. The variation of pH values of fourth experiment is shown in Figure 4.14.

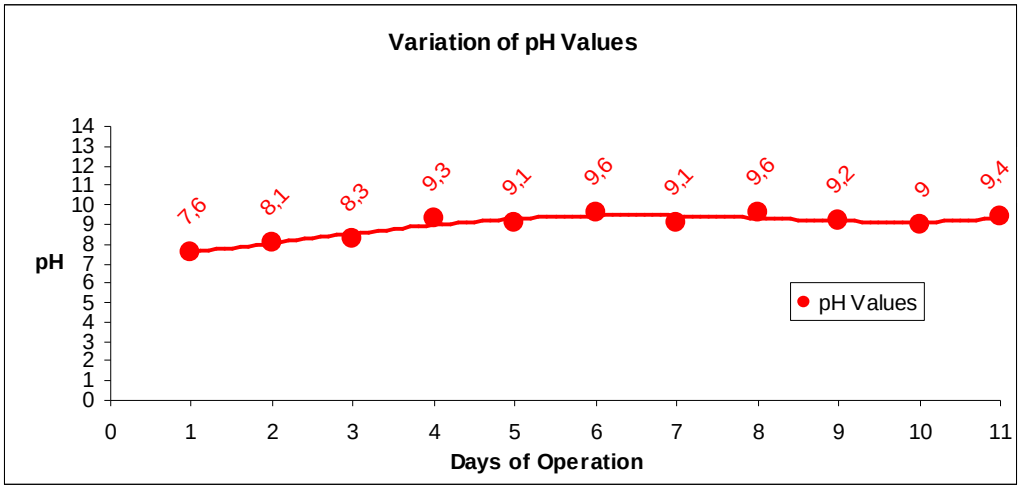


Figure 4.14. pH density values of *Chlorella vulgaris* (20% N_2 gas)

As stated above there is an inverse proportion between pH value and CO_2 concentration, since CO_2 dissolves in water and form carbonic acid, as a result the water becomes acidic. The same result was obtained during the experiments, and among all experiments, the highest pH value (9.53) was obtained for *Chlorella vulgaris* culture which was grown without CO_2 gas sparking.

4.2. Fuel Properties

In this study, mixtures consisting of methyl ester produced from microalgae oil and diesel fuel were used as alternative fuel. 100% diesel fuel was also used as reference. MB (microalgae biodiesel) fuel and diesel fuel were mixed in the volumetric ratios of 5%, 10%, 20% and 50%. Mixtures were prepared just before the tests. Fuel properties of diesel fuel, microalgae biodiesel and the mixtures were determined in Çukurova University Automotive Engineering Department Laboratories. The tests were performed three times, and averages of the three results were taken. Fuel property measurement results are shown in Table 4.1.

Table 4.1. Properties of test fuels

Properties	Diesel Fuel	B5	B10	B25	B50	MB	European Biodiesel Standard (EN 14214)
Density (kg/L)	0.833	0.835	0.838	0.843	0.859	0.886	0.860–0.900
Cetane Number	56.46	56.04	55.08	54.19	51.55	48.31	>51
Viscosity (at 40°C) (mm^2/s)	2.37	2.62	2.84	2.88	3.51	4.47	3.5 - 5.0
Pour Point (°C)	-10	-12	-12	-12	-12	-12	Summer < 4.0 Winter < -1.0
Flash Point (°C)	58.5	68.5	78.5	78.5	78.5	165.5	>120

The density of microalgae biodiesel (MB) is higher than that of diesel fuel, which are 0.886 kg/l and 0.833 kg/l respectively. Due to the higher density of MB in accordance with the diesel fuel, blending with MB was caused an increase in the density values. Any increase in the percentage of the MB caused a rise in the blend density. Density values of all blends meet European Biodiesel Standard.

Generally cetane number is an indicator of the ignition quality of a diesel fuel. If a cetane number is too high, combustion can occur before the fuel and air are properly mixed, resulting in incomplete combustion and smoke. If a cetane number is too low, incomplete combustion occur (Gerpen 2005). Although all other properties of MB stand in European Biodiesel Standard, cetane number of the MB stands lower than desired. As it can be seen from Table 4.1, cetane number can be improved by mixing MB with diesel fuel. (All of the blends satisfy European Biodiesel Standard.)

Viscosity, which is a measure of resistance to flow of a liquid due to internal friction of one part of a fluid moving over another, affects the atomization of a fuel upon injection into the combustion chamber and thereby, ultimately, the formation of

engine deposits. The general rule is; the higher the viscosity, the greater the tendency of the fuel to cause such problem (Gerpen 2005). Fuel property experiments revealed that, MB has higher viscosity value than diesel fuel (4.47 and 2.37 mm^2/s respectively), however; the viscosity value of MB stand between European Biodiesel Standards. It is observed that, MB addition to diesel fuel caused increments in the viscosity values.

The temperature at which crystal formation is extensive enough to prevent free pouring of fluid is determined by measurement of its pour point (PP). If pour point of a fuel is not low enough, some concerns with storing, transferring and using biodiesel in cold weather can occur. Methyl ester derived from micro algae has PP of around -12 ^oC. In addition, PP's of all MB blends were measured as -12 ^oC and this value satisfy European Biodiesel Standard both for summer and winter.

The flash point is the temperature at which the fuel will give off enough vapor to produce a flammable mixture. This is important information for the safe transportation, storage of the fuel. The experiments showed that MB has flash point of 165,5 ^oC which is acceptable according to European Biodiesel Standards.

4.3. Engine Performance

In this study, experiments were performed on a Mitsubishi Canter 4D34-2A, four stroke diesel engine with four cylinders. The engine speed was changed between 1200 rpm and 2800 rpm and the tests were done at full load conditions. Before starting to the experiment, the engine was operated with the new fuel for sufficient time to clean out the remaining fuel from the previous experiment. Engine performance values such as torque and brake power were read by the help of a computer program of dynamometer control unit which can take values in two second time intervals and exhaust emissions such as CO and CO_2 were obtained by the help of another computer program.

4.3.1. Brake Power Output

Figure 4.15 shows the variation of brake power according to different engine speed values. As it can easily be seen in Figure 4.15, power of the engine was reduced with increasing percentage of MB in the blends (There is an average of about 6% reduction for B100 compared to diesel fuel). The reduction can be resulted from incomplete combustion of the fuel due to low cetane number of MB. For all fuels the maximum power output was obtained at about 2400 rpm.

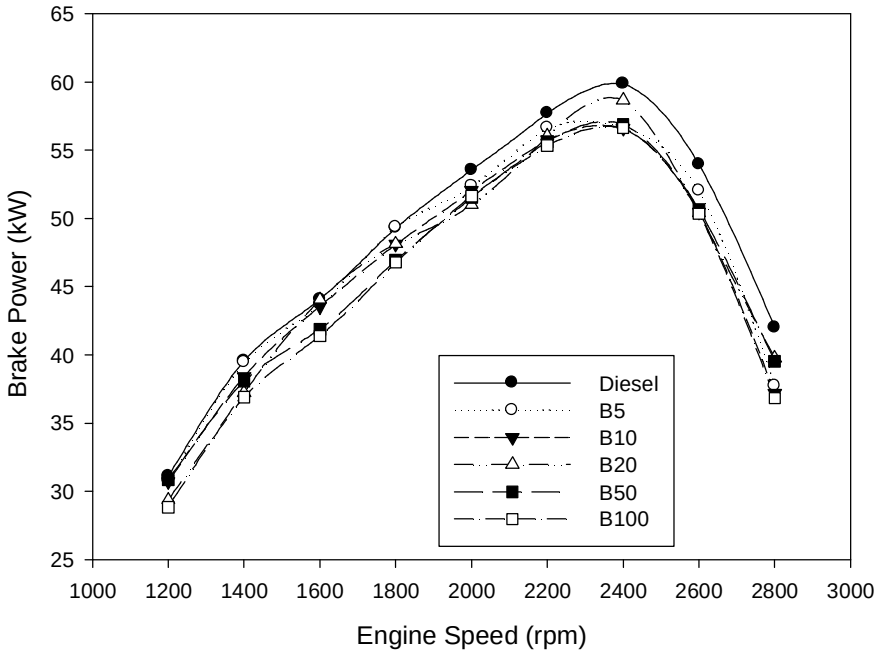


Figure 4.15. Brake power output versus engine speed for the test fuels

4.3.2. Torque Output

The torque output of test fuels is shown in Figure 4.16. Torque output values reduced with the increased concentration of MB in the blends (There is an average of about 5.3% reduction for B100 compared to diesel fuel). The value of the power reduction amount is higher at high engine speeds (about 13 % at 2800 rpm) than that of at lower engine speeds (about 3.8% at 1200 rpm). The maximum torque values for all fuels were obtained at an engine speed of 1400-1600 rpm. The torque output is reduced through higher engine speeds.

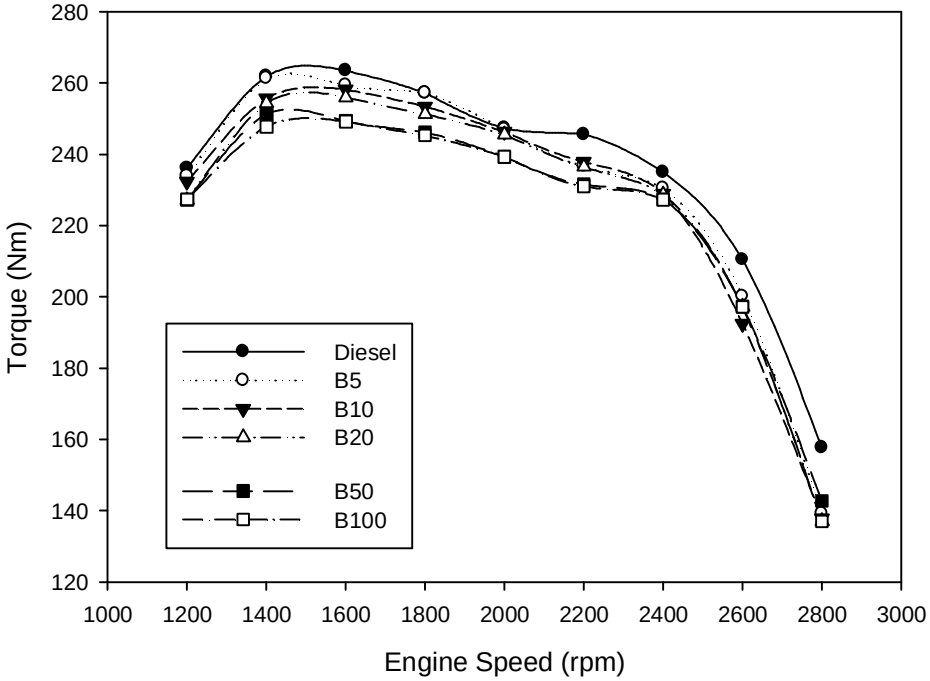


Figure 4.16. Torque output versus engine speed for the test fuels

4.4. Exhaust Emission Values

4.4.1. CO and CO_2 Emissions

CO is an intermediate combustion product and is formed mainly due to incomplete combustion of fuel. If the fuel is combusted completely, CO is converted to CO_2. If the combustion is incomplete due to shortage of air or due to low gas temperature, CO will be formed. Usually high diesel CO emissions formed with fuel-rich mixtures, but as diesel combustion is occurred with lean mixture and has an abundant amount of air, CO from diesel combustion is low. Generally a reduction in CO emission values occur when biodiesel is used instead of diesel fuel since biodiesel contains additional oxygen and this additional oxygen enhances complete combustion.

The variation of CO emissions for different fuels is compared in Figure 4.17. It can easily be seen from the figure that there is an average of 9.4% reduction in CO emission values when B100 was used instead of diesel fuel. These lower CO

emissions of biodiesel may be due to its high oxygen content as compared to diesel. The extra oxygen molecule present in the biodiesel chain might have been used to convert some of the CO into CO_2 during combustion, thus CO formation is reduced. For all of the fuels, CO emissions increased with increasing engine speed. For high engine speeds, CO emission values were higher for some blends (B50, B20, B10); this increase might have resulted from incomplete combustion of the fuel.

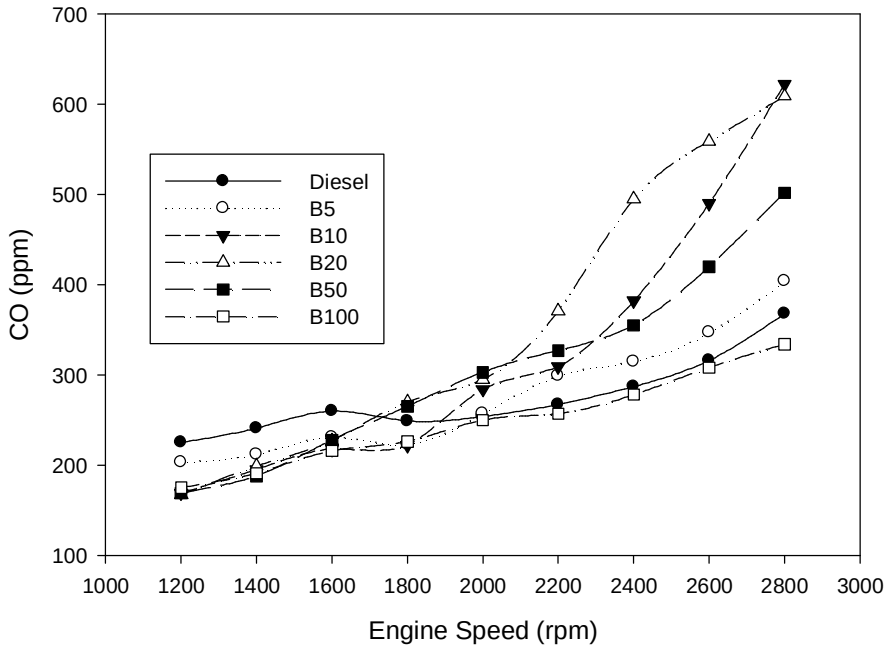


Figure 4.17. Comparison of CO values for diesel, MB and its blends

Figure 4.18 demonstrates the variation of measured CO_2 emission values of test fuels for different engine speeds. There is an average of 4.8% reduction in CO_2 emission values when B100 is used instead of diesel fuel. This reduction might be due to lower elemental carbon to hydrogen ratio of biodiesel against to diesel fuel. For all test fuels, maximum CO_2 emission values were measured at about 1600 rpm where maximum torque values were obtained.

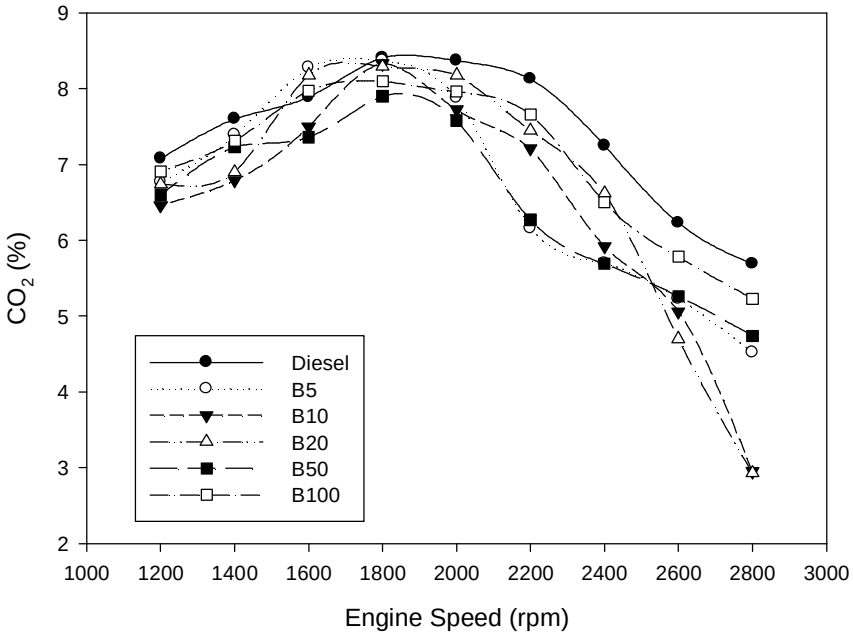


Figure 4.18. Comparison of CO_2 values for diesel, MB and its blends

4.4.2. NO_X Emissions

Naturally NO_X emission increases with the increase in engine torque. It is well known that nitrogen is an inert gas, but it remains inert up to a certain temperature (1100 ^oC) and above this level it does not remain inert and participate in chemical reaction. At the end of combustion, gas temperature inside cylinder arises around 1500 ^oC. At this temperature oxidation of nitrogen takes places in presence of oxygen inside the cylinder. On the other hand, since the formation of nitrogen oxides do not attain chemical equilibrium reaction; then after the end of expansion stroke when the burned gases cool and the formation of NO_x freeze, the concentration of the formed NO_X in the exhaust gas remain unchanged (Nabi et. al.)

The variation of NOx emission values for different test fuels is presented in Figure 4.19. On an average a 11.7% reduction in NOx was obtained for biodiesel and as compared to diesel. The reductions in NO_X emission could be due to complete combustion of MB as compared to diesel.

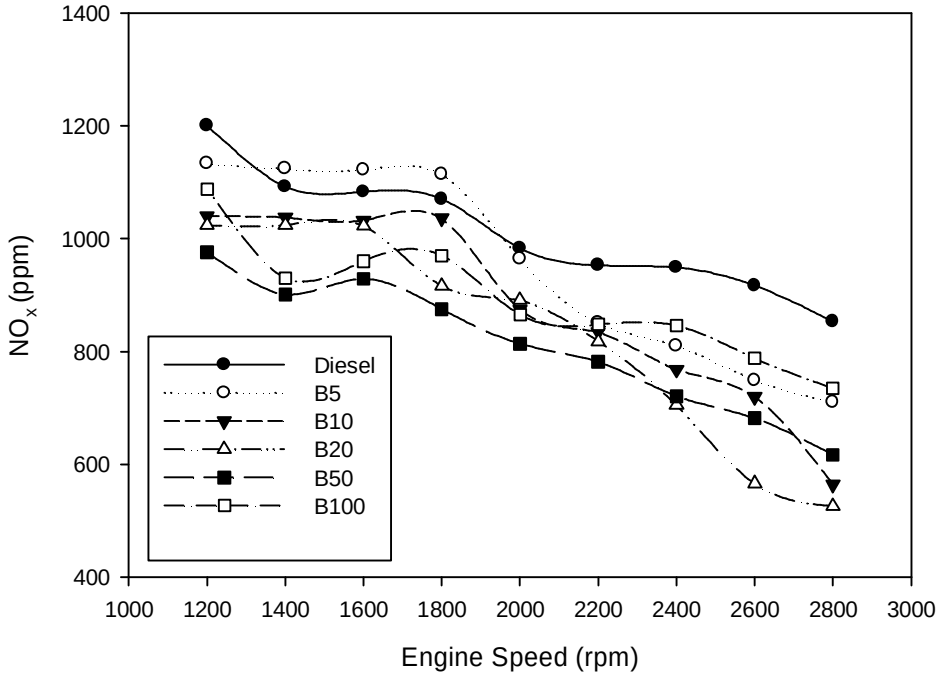


Figure 4.19. Comparison of NO_x values for diesel, MB and its blends

4.5. Using Ricinus Communis Biodiesel as Additive

In order to increase Cetane number of microalgae biodiesel, ricinus communis biodiesel was added at volumetric ratio of 5%. The fuel properties of microalgae biodiesel. ricinus communis biodiesel and the blend are presented in Table 4.2. As it can be seen in Table 4.2, Cetane number of the blend was found as 52.43, therefore all of the fuel properties of the blend satisfy European Biodiesel Standard.

Table 4.2. Properties of blends

	MB	MB95R5	Ricinus Communis Biodiesel	European Biodiesel Standard (EN 14214)
Density (kg/L)	0.886	0.89	0.92	0.860–0.900
Cetane Number	48.31	52.43	80	>51
Viscosity (at 40°C) (mm^2/s)	4.47	5.0	11.5	3.5 - 5.0
Pour Point (°C)	-12	-12	-30	Summer < 4.0 Winter < -1.0
Flash Point (°C)	165.5	>130	>130	>120

Figure 4.20 shows the variation of brake power according to different engine speed values. As it can easily be seen in Figure 4.20, the power output of the engine did not change after ricinus communis biodiesel addition.

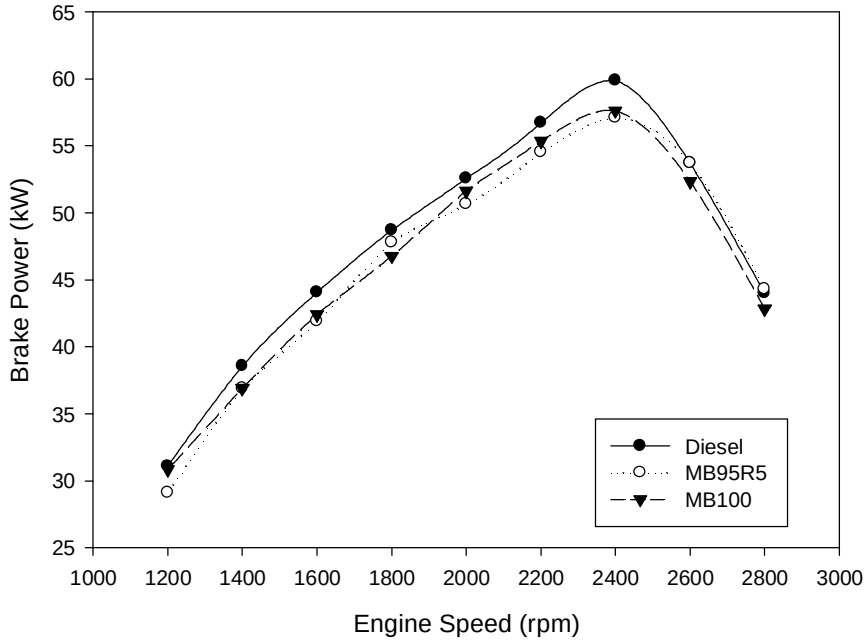


Figure 4.20. Brake power output versus engine speed for diesel, MB and MB95R5

The torque output of the engine is compared in Figure 4.21. The results showed that, torque output has increased about 1% with ricinus communis biodiesel addition.

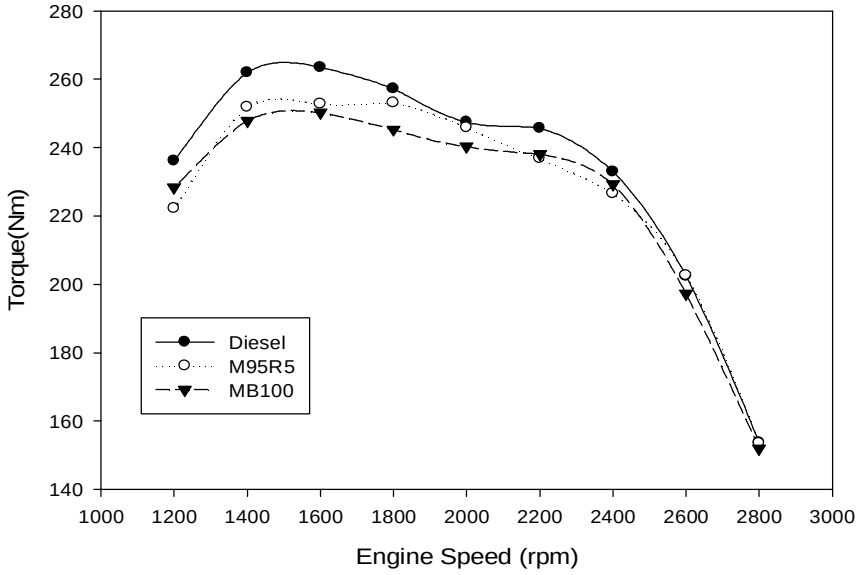


Figure 4.21. Torque output versus engine speed for diesel, MB and MB95R5

Figure 4.22 demonstrates the variation of measured CO emission values of test fuels for different engine speeds. There is an average of 8,5% increase in CO emission values after ricinus communis biodiesel addition.

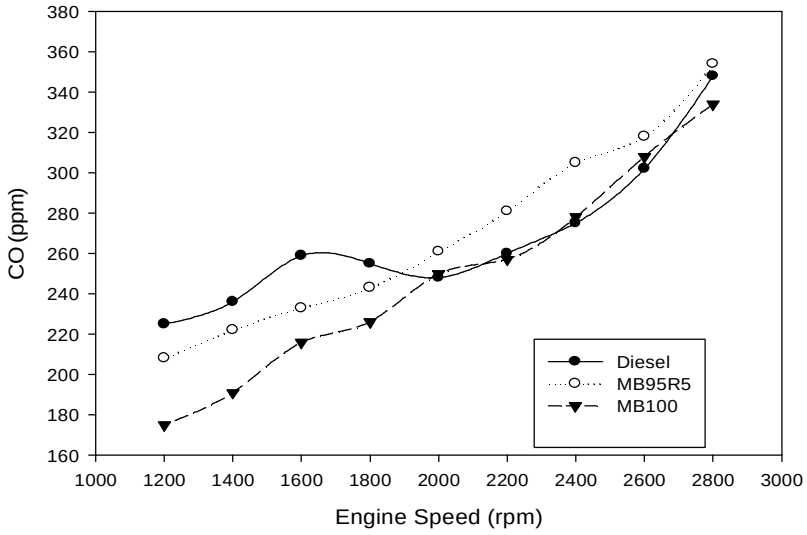


Figure 4.22. Comparison of CO values for diesel, MB and MB95R5

The variation of CO_2 emissions for different fuels is compared in Figure 4.23. It can be seen from the figure that there is an average of 4% reduction in CO_2 emission values after ricinus communis biodiesel addition.

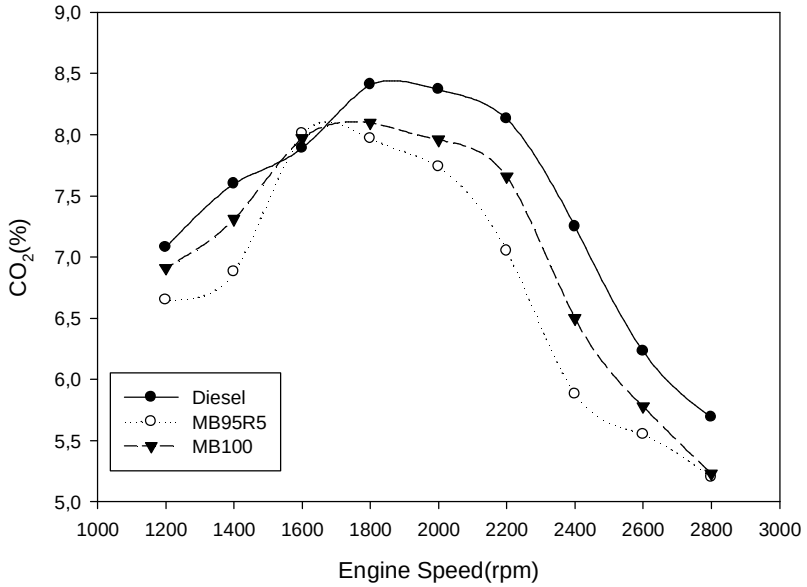


Figure 4.23. Comparison of CO_2 values for diesel, MB and MB95R5

The variation of NOx emission values for different test fuels is presented in Fig. 4.24. On an average, a 5.2% increase in NOx was obtained for MB95R5.

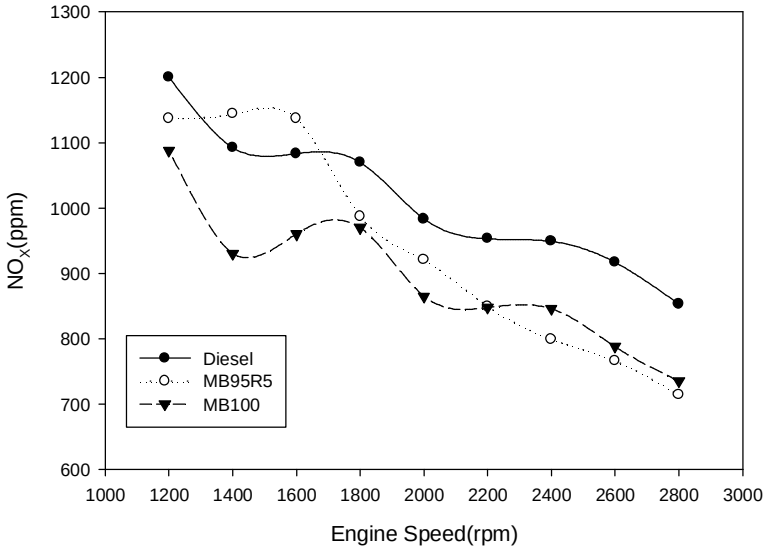


Figure 4.24. Comparison of NO_x values for diesel, MB and MB95R5

4.6. Cost analysis of Microalgae Biodiesel

Biodiesel production in Turkey started in 2000, and in 2–3 years time, it had reached a sufficient level to put Turkey in second place in Europe for its production, after Germany (Arslan et al. 2010). However, since 2007 total biodiesel production capacity of the country has been constant at about 1,000,000 tons per year (1,071,361 tons in 2007; 1,065,924 tons in 2008; 918,199 tons in 2009) and despite its high biodiesel capacity, unfortunately today's biodiesel production of Turkey is approximately 60,000 tons/year (Balat 2010, EMRA 2009). The main reason of the reduction of biodiesel production is high cost of oil. With addition of extra taxes to this price total production cost reaches to 3.79 TL/L . If it is considered that today petro diesel price is about 3.00 TL/L in Turkey, for biodiesel it is impossible to compete with petro diesel. In order to reutilize the biodiesel production facilities, microalgae seems to be a proper source of oil since with today's technology it is possible to obtain microalgae oil down to around 0.2 TL/L and microalgae biodiesel total production cost of 1.48 TL/L (Table 4.3).

Table 4.3. Biodiesel Production Cost in Turkey

Material	Canola Biodiesel				Microalgae Biodiesel			
	Amount (L)	Price (TL/L)	Cost (TL)	Rate (%)	Amount (L)	Price (TL/L)	Cost (TL)	Rate (%)
Oil	1	2.16	2.16	57	1	0.2*	0.2*	13.5
Methanol	0.18	1	0.18	4.7	0.18	1	0.18	12.2
Other Expenditures	-	-	0.15	4	-	-	0.15	10.1
Cost of Biodiesel (TL/L)	-	-	2.49	65.7	-	-	0.53	35.8
PCT (Private Consumption Tax) (TL/L)	-	-	0.72	19	-	-	0.72	48.6
VAT (Value Added Tax) (18%)	-	-	0.58	15.3	-	-	0.23	15.6
Total Cost (TL/L)	-	-	3.79	100	-	-	1.48	100

* The cost of 1 L oil was taken from Soley Biotechnology Institute

4.7. Concluding Remarks

Four different microalgae culture experiments were conducted to examine the cultural characteristics, improve the yield of algal oil, and therefore achieve a maximum performance of microalgae growing. Among four experiments the highest optical density and dry weight values were obtained for culture which was grown in N deficient media.

Fuel properties of diesel fuel, MB and their blends such as density, Cetane number, viscosity, pour point and flash point were determined. It is found that except its low Cetane number, microalgae biodiesel satisfies European Biodiesel Standars (EN 14214). For 100% biodiesel usage, ricinus communis biodiesel was added at volumetric ratio of 5% in order to increase Cetane number of microalgae biodiesel and the fact that the blend satisfies all EN14214 standards was provided.

The engine performance test results showed that, although the average power and torque output of engine fueled with MB decreased by 6% and 5.3% respectively compared with diesel fuel, the emission values were improved with MB usage: CO and CO_2 emissions decreased by 9.4% and by 4.8% respectively, in addition to NO_x emissions decreased by 11,7.

Finally, it can be concluded from the experiments that, MB can be used as alternative fuel in conventional diesel engines without any major modification, by this way exhaust emission values can be improved.

5. CONCLUSIONS

In recent years, the use of liquid biofuels in the transport sector has shown rapid global growth, driven mostly by policies focused on achievement of energy security and reduction of greenhouse gases emissions. Therefore, the usage of biodiesel as an alternative energy source instead of fossil-based fuels becomes very popular because biodiesel is totally renewable and has more favorable combustion emission profile. Although more than 350 oil-bearing crops are identified, they do not have the potential of totally replacing fossil fuels since biodiesel produced from agricultural products affect food production and have high water and fertilizer requirement. Microalgae, which are sunlight-driven, cell factories that convert carbon dioxide to biofuels; can be a potentially greener fuel feedstock, since they produce more fuel per farmed area of land, and can be grown on nonarable lands. Microalgae are estimated to produce biomass at a rate 50 times greater than the fastest growing terrestrial plant.

Due to the high economic development rate of Turkey, the energy demand of the country was doubled between the years 2000–2010 and expect to fivefold between 2000 and 2025. Turkey's annual crude oil production can meet only 10% of the national demand for oil and the remainder is imported from other countries. At 42%, oil is the most important component of Turkish energy consumption and accounts for over 61% of energy imports, as a result diesel fuel prices raised dramatically between the years 2005. The most feasible way to meet this growing demand is by utilizing alternative fuels. By this way, dependency level, unemployment rate and greenhouse gases emissions of the country will be decreased. From this aspect, microalgal biodiesel can be a good choice since it is sustainable and environmentally friendly. In addition, Turkey is a very suitable country for microalgae production because three sides of it are surrounded by the sea; furthermore the country is sunny, has a mild climate and for most of the regions, temperature does not decrease below 15^{o}C throughout the year.

Although using microalgae as biofuel sources has many advantages, due to high cost of production and harvesting methods, algal biofuel production has not

been commercialized currently. In addition to, there have been no reports on investigation the effect of MB on performance and exhaust emissions of a diesel engine, to best of our knowledge. Therefore the aim of this study was to examine cultural characteristics of microalgae in different medias and to interpret performance and exhaust emission results of an engine using MB.

The cultural characteristics determination experiments showed that, the highest dry weight and optical density values were obtained for culture which was grown in N deficient media, on the other hand the lowest values were obtained for the culture which was grown with 20% v/v nitrogen gas sparking.

According to fuel properties determination experiments, it was found that; whereas density,viscosity, pour point and flash point properties of MB satisfy EN 14214 standards the Cetane number of MB stays lower than desired. The engine performance and exhaust emission measurement experiments revealed that whereas the power and torque output of engine fueled with MB decreased by 6% and 5.3% respectively, the emission values were improved with MB usage, namely CO,CO_2 and NO_X emissions decreased by 9.4%, 4.8% and 11.7%, respectively.

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