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Biodiesel production from microalgae and determine properties of produced fuel using standard test fuel

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Abstract

Microalgae with their higher growth rate and oil volume can be counted on as a new source of producing biodiesel. *Chlorella vulgaris* microalgae have been used in this study for producing biodiesel. From one germinator device was used as the culture room. The optimum temperature for cultivation was adjusted, 25°C; pH, 7; Light intensity, 3500 Lux. Due to stiffness of cell envelope of this type of microalgae, the cell envelope was broken using Ultrasonic device in 40°C. The biodiesel produced from transesterification methods was analyzed by gas chromatograph (GC). The biodiesel samples were characterized in accordance with American standard specification for biodiesel (ASTM D6751) and European standard specifications for biodiesel (EN14214) protocols. 18-carbon fatty acids with 51.3% constitute the most fatty acids presence. Olecic acid with 28.10%, Palmitic acid with 24%, Linolelaidic acid with 16.7%, Linolenic acid with 10.6% and Linoleic acid 10% assign the biggest share to themselves respectively. The resultant biodiesel was evaluated for physic-chemical properties namely kinematic viscosity (4.8 mm^2/s), density (870 kg/m^3), flash point (140 °C), cetane number (60), cloud point (0 °C), pour point (-11 °C), heating value (41MJ/kg). Although the presence of some unsaturated fatty acids increased the pour and cloud point of the biodiesel, the microalgae with its high growth rate however can be considered as a proper source of biodiesel production.

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Introduction

Continued use of petroleum sourced fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment. Renewable, carbon neutral transport fuels are necessary for environmental and economic sustainability (Chisti 2007). Biodiesel can be carbon neutral and produced intensively on relatively small areas of marginal land (Demirbas *et al.*, 2010).

Biodiesel is a non-petroleum-based diesel fuel, principally saturated, monoenoic and dienoic C16, C18 and/or C22 alkyl (usually methyl) esters, made by transesterification of plant oils. It should not be confused with hydrotreated vegetable and animal oils (Huber *et al.*, 2007), sometimes known as green diesel, which are principally hydrocarbons, but are only beginning to be commercialized (Kalnes *et al.*, 2007; Neste oil, 2008). Biodiesel can be used alone (when it is known as B100), or blended with conventional petroleum diesel (e.g., 5% biodiesel in petroleum diesel is known as B5), in relatively unmodified diesel-engine vehicles, although few manufacturers warrant their engines for fuels containing more biodiesel than B5 (National Biodiesel Board, 2008). Fatty acid methyl esters were probably first used on a substantial scale in South Africa in the 1970, when the ethyl ester of sunflower oil was investigated as a transportation fuel, but research stopped with the fall of apartheid (Goering, 1981).

Biodiesel is a mixture of mono-alkyl esters, and is distinct from the initially harvested triglycerides. It is typically made by transesterification of triglycerides with methanol and an alkaline catalyst to liberate the methyl esters (Ma and Hanna, 1999), although enzymatic options are under investigation (Ranganathan *et al.*, 2008), and may offer environmental benefits (Harding *et al.*, 2008). The product is thus sometimes known as FAME (Fatty acid methyl esters) or VOME (Vegetable oil methyl esters). Although the transesterification works on triglycerides of animal or plant origin, virgin or used (e.g., discarded frying oils (Encinar *et al.*, 2005)), production from fresh plant oils dominates the industry.

Algae have demonstrated to be an efficient bio energy source (Chisti, 2007) because in contrast to sugarcane, soybean, canola and oil palm, algae are not edible, they are less expensive to producte, grow faster, allow higher yield and production rate per hectare (Sivakumar *et al.*, 2010), do not require clean water to grow, and have the potential of reducing carbon emission (Danquah *et al.,* 2009). This is the reason why developed countries have turned to algae as an efficient and adequate choice for the global energy crisis.

Several of microalgae, e.g., *Chlorella, Dunaliella and Spirullina* could accumulate considerable amount of oil (up to 60% of dry weight) when cultured under certain environmental conditions, such as high in temperature, light intensity and hyper salinity (El-Baky *et al.*, 2004).

Chlorella is a microalga that can grow photoautotrophically or heterotrophically under different culture conditions. Heterotrophic growth of *Chlorella* supplied with acetate, glucose, or other organic compounds as carbon source, results in high biomass and high content of lipid in cells (Endo *et al.*, 1977; Wu *et al.*, 1994).

In the same context, the current study attempts to examine the capability of biodiesel production from microalgae. The main goal of this paper is to evaluate and measure the amount of biodiesel and fatty acid composition produced by strains of *Chlorella vulgaris,* and determine properties of produced fuel using standard test fuel.

Materials and methods

Cultivation of Microalgae

The microalgae used in this study was *Chlorella vulgaris.* Microalgae were obtained from a collection of microalgae in Biotechnology Research Center, Tabriz. For the cultivation of *Chlorella vulgaris* was prepared five one-liter culture mediums in total sterile conditions. The first culture medium was included: Nacl, 500 mg; $CaCl_2$, 40 mg; $MgSo_4$, 50 mg; and the second medium culture was included 1ml for per liter of KH_2PO_4 (100mM); and the third medium culture was included 10ml for per liter of KNO_3 (1M); and the fourth medium culture was included 10 ml for per liter of trace elements, Including: H_3Bo_3, 286 mg; $MnSo_4.4H_2o$, 130 mg; $ZnSo_4.7H_2o$, 320 mg; $CuSo_4.5H_2O$, 183 mg; $Na_2MoO_4.4H_2O$, 2.1 mg; $5CoCl_2.6H_2O$, 2.1 mg and the fifth medium culture was included 1 ml for per liter of EDTA, 150 mg/100ml; $FeSo_4.7H_2O$, 2500 mg/100ml. All chemicals used in this study were purchased from Merck Company. Primary cultivation was performed in 250 ml Erlenmeyer flask with 10% of Stock microalgae insemination. From one germinator device (Grouc Company Model, 400 GC, Iran) which is equipped with light, temperature, humidity and ventilation control systems was used as the culture room. The optimum temperature for cultivation was adjusted, 25°C; pH, 7; Light intensity, 3500 Lux (light intensity was measured by a light meter pro, model of TES 1339). For measuring electrical conductivity was used the EC meter (Metrohm, 712). During the cultivation, aeration was designed as continuously by an air pump.

Growth Evaluation

Algal growth was determined by measuring both optical density at 680 nm with a spectrophotometer (JENWAY, 6305) and cell numbers counted with a hemacytometer (Lam Neubauer) under an OLYMPUS microscope (Optical Analysis Corp., Nashua, NH). With the growth of microalgae, the cultivation concentrations are increased and after 11days the logarithmic growths of microalgae have reached a maximum and after this microalgae growth stage were stopped and their density is practically remained constant and harvesting time was started. The cultures were harvested by centrifugation at 5000 rpm for 15 min. Pellets were then washed twice with distilled water and dehydrated at 30˚C until a constant weight was achieved. The dry weight of the algal biomass was estimated from the average of at least three representative samples.

Oil Extraction

Oil extraction from microalgae biomass was performed on the methods of Bligh and Dyer (1959). Concentrated algae was frozen at -20 ºC for 24 h, and then 5 g of algae was disrupted with glass beads in a vortex mixer for 10 min. After that, the lipid was extracted with 5 mL of chloroform: methanol (2:1), and then the extracted lipid was centrifuged at 5000 rpm for 10 min to separate into three layers. The upper layer (methanol layer including water) was removed and the chloroform layer including lipid was collected. The residues were subjected to repeated extraction twice. All extracts obtained were then mixed together to form the crude oil extract from which the chloroform was evaporated to yield the resultant algae oil.

Biodiesel Production

The biodiesel was synthesized the conventional base catalyzed transesterification using unrefined oil from microalgae biomass (Han *et al*, 2006). Prior to the reaction, methanolic NaOH was prepared by dissolving 1.0 g NaOH in 40 g methanol in a closed container. The transesterification reaction was then carried out by reacting 2 g of microalgae oil with the methanolic NaOH in a round-bottom flask with a magnetic stirrer for 2 h at 60–65 ºC under reflux. After the reaction, the crude biodiesel layer was separated from the glycerin layer after allowing the reaction mixture to settle overnight. The biodiesel was then washed at least four times with 100 ml distilled water. The purified biodiesel was obtained after centrifugation, drying with anhydrous and filtration. The biodiesel produced from transesterification methods was analyzed by gas chromatograph (GC).

Fuel Property Measurements

The internal combustion engine was invented more than 100 years ago, and numerous improvements have been made since its invention. The development of fuels paralleled the development of the engine. Many standards concerning the required properties of engine fuels and tests for measuring those properties have been set. Most of the standards were developed through the cooperative efforts of the American Society for Testing Materials (ASTM), the Society of Automotive Engineers (SAE), and the American Petroleum Institute (API), (Goering, 2006). The biodiesel samples were characterized in accordance with American standard specification for biodiesel (ASTM D6751, 2008; Moser, 2009) and European standard specifications for biodiesel (EN 14214, 2003; Moser, 2009) protocols and the results were compared with diesel fuel. The tests included Flash point (D93), Kinematic viscosity at 40 °C (D445), Cetan Index (D979), Cloud point (D2500), Pour point (D97), Heat of Combustion (D240), Density (D4052).

Results and discussion

Growth rate and microalgae biomass

The strain under study grew in optimal condition. Growth curve is shown in Fig. 1. The phase of adaptation to the culture medium was short, this stage of growth has occurred in the first three days. From the fourth to the ninth day, the cells entered the exponential phase and after the eleventh day, the slope of the line is almost horizontal. After the thirteenth day, growth has been decrease and it seems to have entered a phase of death. The growth curve are consistent with the research report of Richmond (2004). biomass production within two weeks estimated about 0.6 (g/L) dry weight and a relatively low oil content of 20%.

Table 1. Fatty acid methyl esters (FAMEs) in biodiesel from *C. vulgaris* microalgae.

No	C:D	Molecular formula	Fatty acid methyl ester	content(%)
1	C12:0	$C_{12}H_{24}O_2$	Lauric acid metly ester	0.25
2	C14:0	$C_{14}H_{28}O_2$	Myristic acid metly ester	0.6
3	C16:0	$C_{16}H_{32}O_2$	Palmitic acid metly ester	24
4	C16:1	$C_{16}H_{30}O_2$	Palmitoleic acid metly ester	1.08
5	C17:0	$C_{17}H_{34}O_2$	Margaric acid metly ester	1.02
6	C18:0	$C_{18}H_{36}O_2$	Stearic acid metly ester	2.6
7	C18:1	$C_{18}H_{34}O_2$	Oleic acid metly ester	28.10
8	C18:2c	$C_{18}H_{32}O_2$	Linoleic acid metly ester	10
9	C18:2t	$C_{19}H_{34}O_2$	Linolelaidic acid metly ester	16.7
10	C18:3	$C_{18}H_{30}O_2$	Linolenic acid metly ester	10.6
11	C20:4	$C_{20}H_{32}O_2$	Arachidonic acid metly ester	0.2
12	other	-	-	4.85
∑ SFA[a]				28.47
∑ MUFA[b]				29.18
∑ PUFA[c]				37.5

[a] Saturated Fatty acids: C12:0 + C14:0 + C16:0 + C17:0 + C18:0

[b] Monounsaturated Fatty Acids: C16:1 + C18:1

[c] MPolyunsaturated Fatty Acids: C18:2c + C18:2t+ C18:3 + C20:4.

Fatty Acid Composition

The fuel properties of biodiesel are highly dependent on the fatty acid profile of the vegetable oil or animal fat starting material. Primarily, the chain lengths and degree of unsaturation in fatty acid methyl esters (FAME) dictate the physico–chemical properties of the biodiesel (Moser, 2009).

The major fatty acid metly esters composition of the microalgae was determined using a GC analysis; results are listed in Table 1. Palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and α-linolenic (C18:3) acid are present the highest amounts in good agreement with works found in literature for the fatty acids composition of *Chlorella vulgaris* (Petkov and Garcia, 2007). There were fifteen fatty acid derivative, and the most abundant composition was 18-carbon fatty acids with the content of 51.3%. Olecic acid with 28.10%, Palmitic acid with 24%, Linolelaidic acid with 16.7%, Linolenic acid with 10.6% and Linoleic acid 10% assign the biggest share to themselves respectively (table 1). This resulted in the appropriate quality of the biodiesel.

Fuel property

To assess the potential of biodiesel as a substitute of diesel fuel, the properties of biodiesel such as Kinematic viscosity, Density, Flash point, Cetane number, Cloud point, Pour point, Heat of combustion were determined. A comparison of these properties of diesel fuel, biodiesel from microalgae oil and ASTM and EN biodiesel standard are shown in Table 2.

Table 2. Comparison of properties of biodiesel from *C. vulgaris* microalgae, ASTM and EN biodiesel standard and diesel fuel.

Properties	*C. vulgaris* Microalgae Biodiesel	ASTM[a] biodiesel standard	EN[b] biodiesel standard	Diesel[c] fuel
Kinematic viscosity at 40 °C (mm^2/s)	4.8	1.9-6	3.5-5	2-4.5
Density kg/m$^{3)}$	870	-	860-900	820-860
Flash point (°C)	140	130 min	120 min	55
Cetane number	60	47 min	51 min	51
Cloud point (°C)	0	-3 to12	-	-18
Pour point (°C)	-11	-15 to10	-	-25
Heating Value (MJ/kg)	41	-	-	40-45

[a] ASTM D 6751-01 for biodiesel (B100)

[b] EN 14214

[c] The data about diesel fuel was taken from published literature as indicated in the text (Canaki and Sanli, 2008).

Kinematic viscosity

Viscosity is a measure of the flow resistance of a liquid. Fuel viscosity is an important consideration when fuels are injected into combustion chambers by means of high-pressure injection systems, as in diesel engines. The viscosity must be high enough to ensure the proper lubrication of the injector pump. If viscosity is too low, the fuel will flow too easily and will not maintain a lubricating film between moving and stationary parts in the pump. If viscosity is too high, the injectors may not be able to atomize the fuel into small enough droplets to achieve good vaporization and combustion. Injection line pressure and fuel delivery rates also are affected by fuel viscosity (Goering and Hansen, 2004).

The kinematic viscosity of biodiesel from microalgae oil at 40°C were comparable to theses of ASTM biodiesel standard and diesel fuel (table, 2). the kinematic viscosity of the biodiesel from microalgae oil was determined as equal to 4.8 mm^2/s, whereas the kinematic viscosities of ASTM biodiesel standard and diesel fuel are within the range of 3.5 to 5.0 mm^2/s and 1.9 to 4.1 mm^2/s respectively. Therefore, the kinematic viscosity of biodiesel from microalgae oil comply with the limits established by ASTM related to biodiesel quality, although this value is higher than diesel. The results show that the kinematic viscosity of biodiesel from microalgae oil is suitable.

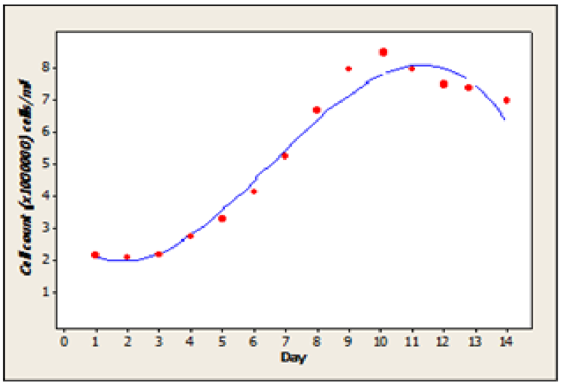

Fig. 1. Daily growth Curve of *Chlorella vulgaris* microalgae within two weeks.

Density

Specific gravity is a measure of the density of liquid fuels. It is the ratio of the density of the fuel at 15.6°C (60°F) to the density of water at the same temperature. The density of water is 1 kg/L (8.34 lb/gal) and thus, in SI units, the specific gravity of a fuel is equal to its density in kg/L. Density of liquids decreases slightly with increasing temperatures. Therefore, densities must be measured at the standard temperature of 15.6°C (60°F) or must be corrected to that temperature (Goering and Hansen, 2004).The density of fuel has some effect on the breakup of the fuel injected into the cylinder. In addition, more fuel is injected by mass as the fuel density increases. All biodiesel fuels regardless of produced from vegetable oils or from fats are denser and less compressible than the diesel fuel (Canakci and Gerpen, 2003; Graboski and McCormick, 1998; Knothe, 2005). The density and compressibility have very important influences on the engine fuel injection system. The injected fuel amount, injection timing and injection spray pattern are directly affected by these parameters (Lee *et al.*, 2002). The density can be correlated with particulate matter (PM) and NO_x emission. The fuel which has high density generally causes an increase in PM and NO_x emission in diesel engines (Ali *et al.*, 1995; Szybist, 2007). The European standard specifications (EN) limits for density in biodiesel fuel are 860-900 Kg/m^3, but density limits for biodiesel fuel have not been established by the ASTM. The results of table 2, indicated that the density value of *Chlorella Vulgaris* microalgae oil based biodiesel (870 kg/m^3) was found to be in the range of EN specifications (860-900 kg/m^3).

Flash point

The flash point is the lowest temperature to which a fuel must be heated to produce an ignitable vapor-air mixture above the liquid fuel when exposed to an open flame. At temperatures below the flash point, not enough fuel evaporates to form a combustible mixture. The flash point is the temperature at which the fuel will start to burn when it comes to contact with fire (Ali *et al.*, 1995). It is an important temperature from the safety point of view during storage and transportation. This temperature is correlated with its volatility, which is an important fuel feature for an engine's starting and warming. The combination of high viscosity and low volatility of a fuel causes bad cold engine start up, misfire and ignition delay (Szybist, 2007; Canakci and Sanli, 2008). A fuel with high flash point may cause carbon deposits in the combustion chamber. The Flash point of the biodiesel from microalgae oil was determined as equal to 140 °C (table 2). It is evident that flash point of Chlorella Vulgaris microalgae oil based biodiesel is well within the specifications of ASTM (>130) and EN (>101) standards.

Cetane number

This is an index correlated to the pressure at which the fuel–air mixture burns in the absence of a spark or flame. The cetane number is the prime indicator of fuel ignition quality and the opposite of octane number of a gasoline fuel. It can be defined as the measure of knock tendency of a diesel fuel. The cetane number is related to the ignition delay time that is the time between the start of injection and start of combustion. As the cetane number increases, the ignition delay decreases and the main combustion phase (diffusion controlled combustion) increases. Long ignition delay is not acceptable since it causes diesel knock (Canakci and Sanli, 2008).

The ASTM and EN minimum for Cetan number of biodiesel is 47 and 51, as listed in table 2. The results of table 2, indicated that the cetan number of *Chlorella vulgaris* microalgae oil based biodiesel (60) was found to be in the range of ASTM and EN specifications.

Cloud and pour point

As a liquid is cooled, a temperature at which the larger fuel molecules begin to form crystals is reached. With continued cooling, more crystals form and agglomerate until the entire fuel mass begins to solidify. The temperature at which crystals begin to appear is called the cloud point, and the pour point is the highest temperature at which the fuel ceases to flow. The cloud point typically occurs between 5°C and 8°C (10°F and 15°F) above the pour point (Goering and Hansen, 2004). ASTM D6751 EN14214 does not include minimum values for cloud point and pour point, although it requires that cloud point should be reported. cloud point is standard tests used to assess the flow properties of biodiesel or any other fuel during cold weather operations (Knothe, 2005). The Cloud and pour point values of of Chlorella Vulgaris microalgae biodiesel were obtained at 0 and -11 °C respectively (table 2).

Heating Value

The purpose of fuels is to release energy for doing work. Thus, the heating value of fuels is an important measure of their worth. The heating value affects the performance curve of the engine: specific consumption increases and power decreases (Freedman *et al.*, 1989). The Heating value of biodiesel was determined as equal to 41 MJ/kg (table 2).

Conclusion

The microalgae used in this study was *Chlorella vulgaris*. The strain under study grew in optimal condition. Biomass production within two weeks estimated about 0.6 (g/L) dry weight and a relatively low oil content of 20%. Biodiesel which was obtained from *Chlorella vulgaris* microalgae oil by transesterification. The gas chromatograph of biodiesel indicated that there were fifteen fatty acid methyl esters derivative in the biodiesel, and the most abundant composition was 18-carbon fatty acids with the content of 51.3%. Olecic acid with 28.10%, Palmitic acid with 24%, Linolelaidic acid with 16.7%, Linolenic acid with 10.6% and Linoleic acid 10% assign the biggest share to themselves respectively. To assess the potential of biodiesel as a substitute of diesel fuel, the properties of biodiesel were determined. The kinematic viscosity of biodiesel from microalgae oil comply with the limits established by ASTM related to biodiesel quality, although this value (4.8 mm^2/s) is higher than diesel. The results show that the kinematic viscosity of biodiesel from microalgae oil is suitable. The density value (870 kg/m^3) was found to be in the range of EN specifications (860-900 kg/m^3). It is evident that flash point (140 °C) is well within the specifications of ASTM (>130) and EN (>101) standards. The cetane number (60) was found to be in the range of ASTM and EN specifications. The Cloud and pour point values were obtained at 0 and -11 °C respectively. The Heating value of biodiesel was determined as equal to 41 MJ/kg. Although the presence of some unsaturated fatty acids increased the pour point and cloud point of the biodiesel, the micro algae with its high growth rate however can be considered as a proper source of biodiesel production.

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