Biodiesel production with high quality from *Dunaleilla salina* under optimization factors according to ASTM.

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Abstract

MH medium was reprepared with the depriviation of nitrogen to 0.5g^{-1} KNO_3 ,0.5g^{-1} $MgCL_2$ and elevated the concentration of NaCL to 2.25M then, inoculated with *Dunaliella salina* in outdoor glass aquarium for three months. The culture was injected with 0.6 l^{-1}co_2 /day. The alga was semicontinuosly collected every four days. The collected samples were dried and used for biodiesel preparation. Results indicated that alga grown in modified medium shifted the biosynthesis of fatty acids towards the saturation level rather than unsaturated one. Since the relative percentages of saturated fatty acid (SFA) was 92.2% more than its corresponding control (basal medium) 65.6%. Analysis of fatty acid profile obtained through gas liquid chromatography (GLC) revealed that the fatty acid methyl esters obtained from treated alga (FAME) contains palmitic acid (C16:0, 54%),myristic(C14:0, 23%) and stearic (C18:0, 3.2%) with the high ratios rather than their corresponding ratios in control sample. Physical and chemical properties of biodiesel extracted from modified medium including cetane number, kinematics viscosity, flash point, pour point and cloud point are coping perfectly with ASTM. ASTM is an international agency, known until 2001 as the American Society for Testing and Materials. Meaning that biodiesel of *Dunaliella salina* treated with modified medium is a high quality biodiesel according to ASTM.

Keywords: Biodiesel - *Dunaleilla salina* - ASTM – Saturated Fatty acids- FAME.

1.Introduction.

Nowadays, the need of energy consumption is increasing continuously due to increases in industrialization and population. Accompanied by fossil fuel depletion, has led to a search for alternate biofuel sources. The basic sources of fossil fuels are petroleum, natural gas and coal **(Kulkarn and Dalai, 2006).**

Biodiesel is an attractive fuel for diesel engines that it can be made from any vegetable oil (edible or non-edible oils) **Paynich, (2007)**, used cooking oils (**DePoola *et al*., 2009)**, animal fats (**Hilbert *et al.*, 2005*)*** as well as microalgae oils. Biodiesel is defined by ASTM International as a fuel composed of mono alkyl esters of long-chain fatty acids derived from renewable vegetable oils or animal fats meeting the requirements of ASTM D6751 **(ASTM2008a)**. Biodiesel is acceptable fuel due to it has non-toxic emissions, it is a clean energy, renewable, non-toxic and sustainable alternative to petroleum based is burned in a diesel engine **(Fukuda *et al.*, 2001)**.

In order to decrease greenhouse gas emissions from industrial combustions and

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transports, biodiesel demand is constantly increasing, but oil crops are not able to satisfy it because of their high cost of performance. This high cost is due to the competition of biofuels with the food industry. Precisely, microalgae as the third generation biofuels, have received immense attention due to microalgae are photosynthetic microorganisms that convert sunlight, water and carbon dioxide to sugars, from which biological macromolecules, such lipids, can be obtained **Wilhelm and Jakob, (2011)**. They have been suggested as very good candidates for fuel production. The production of biodiesel from algae has received a lot of publicity (**Christi, 2007, Christi, 2008, Greenwell *et al,* 2010, Gordon, and Polle, 2007)**. The lipid content is effected by many environmental factors, there are a variety of ways of doing this in different algae species, including nitrogen deprivation, light, pH stress, CO_2 aeration **(Chiu and Kao*,* 2009)** and osmotic stress **(Takagi *et al,*. 2006)**. In this regard**,** some algal species are halo tolerant and produce lipids as compatible solutes to cope with high or fluctuating salinities **(Edwards, 1990)**. *D. salina* is a halophile green micro alga, which can be found in sea salt fields, salty ponds and marine waters. Because of its high content of carotenoids, it is a good source of foods with antioxidant activity **(Georgianna *et al*., 2013 and Oren, 2005**). Furthermore, it can be used for biodiesel production. **Tang *et al*., (2011)** showed the potency of producing biofuels by methylation of different fatty acids such as linolenic and palmitic acids and introduced *Dunaliella* as a good feedstock for biofuel production **(Beetul *et al*., 2014)** . *D.salina* is a naturally isolated strain and has unknown features and its fatty acids content and fast growth rate so that it is suitable to be used as biodiesel feedstock. **Abd El-Baky *et al.,* (2004), Weldy and Huesemann (2007)** reported that *D. salina* could produce high amounts of lipids in the range from 38 to 44% in terms of dry weight.

2.Materials and Methods

The organism and culture medium: *D.salina*: is a unicellular species of green algae without cell wall **(Hounslow, 2010)**. It lives in areas of fluctuating salinity and can tolerate extreme salinities. This microalga is quite easy to cultivate and has a relatively high growth rate and lipid content **(Tang *et al*., 2011)**. The strain was obtained from the Culture Collection of Algae at the (**Alexandria University)**. The alga *D.salina* was aseptically grown on (MH) basal medium and (MH) modified basal medium prepared according to **(Loeblich, 1982). Modified** medium was prepared including the optimization factors (2.25M salinity, pH=8.5, $0.5g^{-1}$ $MgCl_2$, $0.5g^{-1}KNO_3$) after some preliminary experiments.

Mass culture experiments.

Mass culture of both (MH) modified and basal medium were prepared using glass aquarium with the dimensions of (170 cm long, 80 cm wide and 30 cm height). The glass aquarium is entirely made up of transparent glass of 6mm thick. Each aquarium contains 20 liters of algal culture that exposed to illumination of 5000 lux. A water pump is important for mixing the culture and spread the nutrients regularly. The aquarium also provided with oxygen pump with a perforated tube.

Sampling of algal biomass.

Before taking the samples the culture of each aquarium firstly stirred thoroughly using a glass rod to insure complete mixing of the algal culture and homogenous cell density distribution. The least generation time represent the time for taking the samples from each aquarium, where 2 liters were taken at the elapse of each generation time. The algal residue was collected by centrifugation at 5000 rpm. Then the fresh samples were dried at 70 °c. The different dry weight of each medium was mixed and weighted for extraction of lipid and any further analysis.

Renewal of the nutrients of the culture media of the ponds.

For continuation the algal growth for a long period, 2 liters of fresh prepared medium were added to each aquarium for regaining the loss of the medium as well as for renewable the nutrients within the aquaria. Then, the culture of each, restore the original volume of the aquarium (20L) using 2L**(MH)**. Then, the pond thoroughly stirred using glass rod, this to insure complete mixing of the algal culture and homogenous nutrients distribution**.**

Determination of lipid content by gravimetric method.

Oil content of ***D. salina*** was obtained by soxhlet apparatus using n-hexane as extraction solvent for 6 hours under reflux **(Frenz *et al.,* 1989).** Hexane free residue was weighted and expressed as mg lipid/ 100 g dry wt. determined according to **(Sadasivam and Manickam, 1996)**

Extraction and determination of fatty acids.

Separation of fatty acids:

The lipid samples were saponified over-night with ethanoic KOH (20%) at room temperature. The fatty acids were freed from their potassium salts by acidification with hydrochloric acid (5N), followed by extraction with ether or petroleum ether 40-60^{O}C. The ether extract was washed three times with distilled water then dried over anhydrous sodium sulfate, and filtered off **(Vogel, 1975).**

Methylation of fatty acids with diazomethane:

Fatty acids produced from lipid samples as well as standard fatty acids were dissolved in a little anhydrous methanol and the ethereal solution of diazomethane was added in a small portion until gas evolution ceased. The mixture acquired a pale yellow color indicated the addition of excess of diazomethane, the reaction mixture was left for 10 minutes and ether was evaporated under nitrogen stream at room temperature. Two drops of redistilled chloroform solution was added to dissolve the fatty acids methyl esters.

Gas liquid chromatography (GLC):

One µl of fatty acid methyl ester was injected into a 6 feet × 1/8 inch internal diameter column packed with 20% diethylene glycol succinate (DEGS) on chromsorb 60-80 mesh by using Hewellet-Packard (model:HP-GC-MS) according to the standard conditions:

Identification of fatty acids:

A set of standard fatty acids of 10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1 and 22:0 with purity of 99% was purchased from Nu-check Prop. The purity of each fatty acid methyl ester was checked by GLC and gave one peak.

Quantification of fatty acids:

The response of each fatty acids separated on the chromatography was determined as peak area per unit weight of sample, as recommended by **Radwan (1978).**

Peak area = height x peak width (at the peak height). The area so obtained is 0.94 times the true area. This method widely used as it is highly reproducible.

True area = peak / 0.94.

3. Results and Discussion

3.1 Results:

Table (1): Composition of (MH) basal medium and (MH) modified one.

a. Macronutrients:

Stock	Basal Medium		Modified medium	
	Component	g/l	Component	g/l
1	NaCL	73.050 g	NaCL	131.5 g
2	$MgSO_4.7H_2O$	0.500 g	$MgSO_4.7H_2O$	0.500 g
3	KNO_3	1.000 g	KNO_3	0.5 g
4	$MgCl_2.6H_2O$	1.500 g	$MgCl_2.6H_2O$	0.5 g
5	KH_2PO_4	0.035 g	KH_2PO_4	0.035 g
6	$CaCl_2.2H_2O$	0.200 g	$CaCl_2.2H_2O$	0.200 g
7	KCl	0.200 g	KCl	0.200 g
8	$NaHCO_3$	0.043 g	$NaHCO_3$	0.043 g

b. Micronutrients: In both basal and modified medium

Stock	Component	g/l
9	$ZnCl_2$	0.041 mg
	$MnCl_2.4H_2O$	0.410 mg
	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.300 mg
	$CuCl_2.2H_2O$	0.041 mg
	$CoCl_2.2H_2O$	0.015 mg
	H_3BO_3	0.610 mg
	EDTA	1.890 mg
	$FeCl_3.6H_2O$	2.440 mg

pH was adjusted at 7.5 in basal medium and at 8.5 in modified medium.

- Di hydrogen Potassium phosphate solution was autoclaved separately and added aseptically to the sterilized medium to avoid phosphate precipitation.

Gas Liquid chromatography (GLC) analysis of biodiesel extracts from *D.salina*grown in basal medium and modified one.

Gas Liquid chromatography analysis of fatty acid methyl esters of *D.salina* grown in basal medium and the other was in modified medium which contain the optimum concentration of different nutrients for lipid production were shown in table (2). Ten fatty acids were identified from Lauric (C12:0) to Arachidic (C20:0) fatty acids. It was found that treating the alga with modified medium resulted in an obvious increase in the relative percentages of saturated fatty acid (SFA) more than its corresponding control (basal medium). The increase in SFA is due to increase in the content of Myristic acid (C14:0) and Palmitic acid (C16:0). On the other hand, there was a decrease in unsaturated fatty acid (USFA). Other interesting observation include that there was a decrease in saturated fatty acid in the basal medium. Results revealed that the most physicochemical properties of biodiesel produced by *D.salina* were coincident with the international properties in ASTM. Through this standard it could be obvious that cetane number of biodiesel obtained from alga collected from modified medium reach (92.2 %) whereas its value obtained from the alga collected from basal medium was (65.68) %. These results manifested that the ratio of saturated fatty acid to the unsaturated one of modified and basal medium was 11.8 and 1.9 respectively. This mean that the highest ratio the best quality of biodiesel. Another conformational data for the good quality of biodiesel is the highest values of both C16:0 and C14:0 in biodiesel obtained from alga grown in modified medium rather than those obtained

from basal medium. Finally, the results of methyl ester profiles of modified medium considered suitable conditions for lipid production with highly SFA in *D.salina.*

Table (2): Gas Liquid chromatography (GLC) analysis of biodiesel extracted from basal and modified medium of *D. salina.*

Fatty acid	C-Number of fatty acids	Control (Basal medium)	Modified medium
Lauric	**C12:0**	**0.37**	**11.25**
Myristic	**C14:0**	**3.60**	**23.28**
Pentadecanoic	**C15:0**	**2.70**	**-**
Palmitic	**C16:0**	**45.41**	**54.47**
Palmitoleic	**C16:1**	**11.50**	**-**
Margaric	**C17:0**	**4.50**	**-**
Stearic	**C18:0**	**6.00**	**3.20**
Oleic	**C18:1**	**12.7**	**5.20**
Linoleic	**C18:2**	**10.12**	**2.60**
Arachidic	**C20:0**	**3.10**	**-**
Saturated fatty acid (SFA)	**---**	**65.68**	**92.2**
UnSaturated fatty acid (USFA)	**---**	**34.32**	**7.8**
Ratio of SFA/USFA	**---**	**1.9**	**11.8**
Total fatty acid		**100.0**	**100.0**

Physical and chemical characteristics of lipid extracted from *D.salina* grown in modified medium and basal one.

Information regarding that the physical and chemical properties of lipid are located in table (3). **Iodine value**: is the measure of degree of unsaturation of oil, fat and wax. The saturated one has no iodine value so the value is an expression about the level of unsaturation (C=C or C≡C). Its value from both basal and modified medium was (2.837, 2.75 gI_2 /100g oil) respectively. The two values were cope perfectly with American standard specification ASTM value (<12) meaning that algal oil is convey to be fuel oil. Also, acid value was (37.3 & 33 mg KOH/g oil) of both basal and modified medium respectively Moreover,

the percent of FFA represent as half the amount of acid value (18.8& 16.9 %) for oil extracted from basal and modified medium respectively..

Saponification value**:** it called the alkaline hydrolysis of fat or oil or the number of milligrams of KOH required to saponify completely the fatty acid present and also, to neutralize the free fatty acids present in one gram of fat or oil. Its value (187 &210 mg KOH/g oil) of both basal and modified medium respectively. Ester value: It is a measure of actually how much amount of glyceride is present in sample of oil which is saponifiable. Its value was equal (149.7&177) of both basal and modified medium respectively.

Table (3): Physical and chemical characteristics of lipid extracted from *D.salina* grown in modified medium and its corresponding basal medium.

Parameter	Unit	Control sample (Basal medium)	Treated sample (Modifid medium)	ASTM Diesel Standards
I_2 value	(g I_2/100g oil)	2.837	2.753	<12
Saponification	(mg KOH/g oil)	187	210	170-190
Acid value	(mg KOH/g oil) %	37.3	33	Max.2%
FFA	%	18.8	16.9	Max.0.5-0.8%
Ester value		149.7	177	-
%glycerin	%	8.18	9.14	-

Physical properties of B_{20} biodiesel of *D.salina* grown in modified medium.

The result in table (4) showed physical properties of B_{20} biodiesel which consisted of 20 % blend of biodiesel and 80 % of petro-diesel. These properties include, specific gravity (0.8513 g/cm3), acid value (0.78 mg KOH/g biodiesel) and kinematic viscosity at 50 ^{0}C (2.4 cSt) were coped with ASTM standard. Also, the flash point is the lowest temperature at which a fuel ignites its value (129 ^{0}C). The pour point (-6) is acceptable. The Cetane Number is a measure of the ignition quality of diesel fuel. The higher of the cetane number is , the easier to be standard (direct injection) diesel engine its value was equal 50.5. There were other properties such as total sulfur (0.54), carbon residue (0.002) and copper corrosion were also cope perfectly with american biodiesel standard ASTM. This meaning that the biodiesel extracted from *D.salina* grown in modified medium is high quality biodiesel according to ASTM .

Table (4): physical properties of B_{20} biodiesel of *D.salina* grown in modified medium.

Parameter	Unit	B_{20}(modified medium)	Biodiesel blends ASTM D-7467
Specific gravity	g/cm3	0.8513	0.820 - 0.870
Acid value	(mg KOH/g oil) %	0.78	Max.2%
Kinematic viscosity at 50 ^{0}C	CSt	2.40	1.9 - 4.1
Pour point	C°	-6	-
Cloud point	C°	0	-
Flash point	C°	129	>52
Total sulphur	Wt %	0.54	-
Carbon residue	Wt %	0.002	< 0.35
Copper corrosion	-	Low	-
Cetane index	-	50.5	>40

3.2 Discussion

Gas chromatography analysis of fatty acid methyl esters of *D. salina* from both basal medium and modified medium which contain the optimum concentration of different nutrients for lipid production shows variability in the resulted lipid profile. Measurement of FAMEs in algae biomass is a desirable procedure to indicate amount of suitable lipids which can be converted to biodiesel (**Yecong *et al*., 2011)**. Several studies have reported the total oil content of *D. salina* as well as the fatty acid content **(Herrero *et al.,* 2006; Lamers *et al*., 2010 and Diaz-Palma *et al.,* 2012)**. The results indicate that there are different types of fatty acids are present in *D. salina* from Lauric (C12:0) to Arachidic (C20:0) fatty acids most of these fatty acids are saturated. It was found that treating *D. salina* with modified medium resulted in an obvious increase in the relative percentages of saturated fatty acid (SFA) more than its corresponding control (basal medium). The percentage of saturated fatty acid (cetane number) of biodiesel obtained from *D. salina* grown in modified medium reach (92.2%) whereas its value obtained from the alga collected from basal medium was (65.68)%. These results manifested that the ratio of saturated fatty acid to the unsaturated

one of modified and basal medium was 11.8 and 1.9 respectively. The increase in SFA is due to increase in the content of Myristic acid (C14:0) Palmitic acid (C16:0) and Stearic acid (C14:0) while, there was a decrease in unsaturated fatty acid (USFA). Other interesting observation include that there was a decrease in saturated fatty acid in the basal medium. These results are in agreement with those obtained by **(Yecong *et al*., 2011)** who reported that culturing conditions, growth phase, and environmental factors are the criteria that can affect both lipid content and fatty acid profile. **Deng *et al.,* (2009)** showed that the proper percentage of saturated and unsaturated fatty acid is very important to microalgae as a biodiesel feedstock. **Rasoul-Amini *et al*., (2011)** mentioned that the high saturated fatty acids of *D. salina* give an excellent cetane number and oxidative stability to biodiesel. **Mandal and Mallick, (2011) also,** reported that increasing palmitic acid is desirable for good quality biodiesel. **Herrero *et al*., (2006) and Lamers *et al*., (2010)** also studied that the fatty acid profile of *D. salina* identified high expression of palmitic (C16: 0), alpha-linolenic (C18: 3) and oleic acid (C18:1). Finally, the results of methyle ester profiles of modified medium considered asuitable conditions for lipid production with highly SFA in *Dunaliella salina.* ASTM International, known until 2001 as the American Society for Testing and Materials (ASTM), is an international agency that develops and publishes voluntary consensus technical **standards** for a wide range of materials. It put the ideality of characteristics of lipid and biodiesel all over the world. These properties such as iodine value, is the measure of degree of unsaturation of oil, fat and wax. The saturation one has no iodine value so the value is an expression about the level of unsaturation. The previous definition was coincident with the value of iodine in both kind of oil (oil take up from basal or modified medium) the ratios were (2.837, 2.75 gI_2 /100g oil) respectively. The two values were coped perfectly with American standard specification ASTM value (<12) **(European Standard EN 14111, 2003)** meaning that algal oil is convey to be fuel oil. Acid value: This value expressed as the amount of KOH in mg necessary to neutralize to the free fatty acid in g. The largest amount of acid value, the free fatty acid in the oil. These mean that it could be saponifable and removed by washing before transestrification in this case acid value equal (37.3 & 33 mg KOH/g oil) of both basal and modified medium respectively. Moreover, the percent of FFA represent as half the amount of acid value (18.8&16.9 %) for oil extracted from basal and modified medium respectively.

Saponification value called the alkaline hydrolysis of fat or oil to neutralize the free fatty acids present in one gram of fat or oil. Long chain fatty acids found in fats have low saponification value because they have relatively fewer numbers of carboxylic functional groups per unit mass of the fats, as compared to short chain fatty acid its value equal (187 &210 mg KOH/g oil) of both basal and modified medium respectively. Ester value is a measure of actually how much amount of glyceride is present in sample of oil, which is saponifiable. It was estimated from the difference between saponification value and acid value its value equal (149.7 &177) of both basal and modified medium respectively. Results showed that increasing its value due to that modified medium contain more glyceride than basal medium.

All these results confirmed that oil extracted from modified medium is better than basal medium due to, its iodine value, acid value, FFA, saponification value and ester value were coped perfectly with ASTM. Therefore, the oil extracted from modified medium was converted to biodiesel via transestrification process through which sodium hydroxide uesd as a catalyst and occasionally analysed in Egyptian Petroleum Research Institute and compared with ASTM. physical properties of B_{20} biodiesel which consisted of 20% blend of biodiesel and 80% of petro-diesel which include density, kinematic viscosity, acid value, flash point, pour point, cloud point, total sulphur, carbon residue, copper corrosion and cetane index. Density influences the performance of the oil in the injectors its value equal (0.8513 g/cm3); this value is cope perfectly with American standard specification ASTM value (0.820-0.870 g/cm3). Viscosity is a significant fuel property with respect to in-use performance of biodiesel since it influenced the operation of the fuel injection equipment. Viscosity increases with increasing fatty acid chain length and degree of saturation. A higher kinematic viscosity would create engine problems like engine deposits **Hoekman *et al*., 2012 and Balat, 2008).** Transesterification lead to a decrease in the viscosity of the oil.

This process transforms the triglycerides (TG) contained in the oil into a mixture of alkyl-esters (biodiesel); being the latest the more feasible alternative to diesel fuels **(Tabernero *et al.,* 2011).** Viscosity value at 50 ^{0}C is (2.4 cSt) and it cope with ASTM which its value is (1.60-6.50 cSt). Acid value of biodiesel is (0.78 mg KOH/g biodiesel) while acid value of oil is (2.753 mg KOH/ 100g oil) so, transesterification processes reduce the acid value. Cold flow properties are important parameters for biodiesel production for northern countries like Canada and could be measured by cloud and pour points. The decrease of temperature could lead to the formation of visible crystals ($d \geq 0.5$ µm) in the biodiesel at a limit called cloud point **(Knothe, 2005).** Usually, cloud and pour points increase as a function of the molar ratio of biodiesel in diesel fuel from 0 to100% **(National Renewable Energy Laboratory, 2009)**. A higher level of polyunsaturated compounds in microalgae biodiesel could be a benefit in terms of cold properties (cloud and pour points) for a blend microalgae biodiesel/petrodiesel in cold climates. the flash point is the lowest temperature at which a fuel ignites its value (129 ^{0}C) and ASTM value is equal (< 52) . **Johnson and Wen, (2009)** reported that the flash point of microalgae ranging from (115-204). The Cetane Number is a measure of the ignition quality of diesel fuel .The higher the cetane number, the easier it is to start a standard (direct injection) diesel engine is (50.5). **Stansell *et al.*, (2011)** studied that the cetane numbers of many species based on their FAME content and found cetane numbers ranging from 39 to 54 while cetane number of petro diesel fuel are at least between 47 and 51 **(ASTM Standard D6751-10, 2010; Knothe, 2006).**

There were other properties such as total sulfur is equal (0.54) and carbon residue is a measure of the presence at high temperatures of some natural compounds with high molecular weight at high temperatures some compounds decompose giving rise to carbon residues with the value (0.002). Biodiesel has been found to be more corrosive to automotive materials than diesel **(Fazal *et al*., 2012)**

due to the presence of oxygen moieties, auto-oxidation, increased polarity of biodiesel and its hygroscopic nature. Copper corrosion has very limited value so, biodiesel is safe to use. The physical properties of biodiesel (B_{20}) cope perfectly with American biodiesel standard ASTM. Meaning that the biodiesel extracted from modified medium is high quality biodiesel according to ASTM.

4. Conclusion

Using modified medium which contain the optimization factors (2.25M salinity, pH=8.5, $0.5g^{-1}$ $MgCl_2$, $0.5g^{-1}$ KNO_3) are beneficially for elevation the lipid percentage in *D.salina* to 65.2%. It also, increases the percentage of saturated fatty acid by (92.2%) when compared with its corresponding control that equal (65.6%). The biodiesel extracted from *D.salina* grown in modified medium was perfectly coping with ASTM. Through which, measuring the flash point, pour point, cloud point and cetane number.

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الملخص العربى

إنتاج وقود حيوي عالي الجودة من ديونيلا ساالينا با ستخدام العوامل المثلي للتنمية طبقا ل ASTM

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يعتبر الوقود الحيوي هو البديل الفعلي لتلك الاونة الاخيرة للبترول الحفري وذلك لما قرره المتخصصون من اختفاء الوقود الحفري في عام ٢٠٤٠. ولابد من البحث عن بدائل لسد النقص المتوقع علي تلك السلعة الحيوية علي مستوي العالم. ولهذا اتجه الباحثون لسد تلك الفجوة لإيجاد مصادر متعددة لإنتاج الوقود الحيوي والذي يمكن انتاجه من المحاصيل الزيتية والزيوت المستعملة والدهون الحيوانية بشرط ألا يتعارض هذا مع احتياجات الانسان إلي الزيوت وحتى يمكن تلبية الطلب العالمي علي وقود النقل اتجه العلماء لاستخدام الطحالب كبديل للمصادر المختلفة لإنتاجه. ولكى يصبح الحلم واقع تتسابق الهيئات العلمية علي الحصول علي احد الأجناس الطحلبية التي يمكن استخلاص زيوتها بنسب مرتفعة. ومن هذا المنطلق جاءت فكرة البحث واختبار أحد الطحالب البحرية بمعدل نمو عال وإنتاج وفير من الزيوت والتي يسهل تحويلها إلي وقود حيوي. وتم اختيار طحلب ديونيلا ساالينا لتنميته معمليا وخارجيا في احواض أرضية تحت ظروف مختلفة يمكن اختيار الأنسب منها لزيادة النسبة المئوية للأحماض الدهنية المشبعة والتى وصلت إلي ٩٢.٢% بزيادة ٣٠% علي العينة القياسية من الطحالب . وتم إعاذ السبب في تلك الزيادة إلي زيادة معدلات إنتاج حمض المريستيك (C14:0) وحمض البالمتيك (C16:0) وحمض الأسترك (C18:0) المشبع علي حساب الأحماض غير المشبعة . وزيادة في التأكيد علي أن الطحالب تعتبر مصدرا أساسيا وبديل مثالي لإنتاج الوقود الحيوى ولقد تم دراسة الصفات الكيميائية والفيزيائية لذلك الوقود الحيوي في أحد المعاهد المتخصصة والتى أكدت تماثل الرقم السيتانى و نقطة الوميض و نقطة الانسكاب وكذلك نقطة التجمد ألي حد كبير من مثيلاتها القياسية الأمريكية ASTM.