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Lipid Induction in *Dunaliella salina* Culture Aerated with Various Levels CO_2 and Its Biodiesel Production

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Abstract

In this work, the effect of various levels of CO_2 (0.01, 0.03, 3.0, 9.0 and 12.0%) aeration on the biomass production, lipid accumulation and its fatty acid profile as well as biodiesel properties of marine microalgae *Dunaliella salina* were investigated. The results show that the maximal biomass and lipid productivity (in parenthesis) in cultures aerated with different levels of 0.01, 0.03, 3.0, 9.0 and 12.0% CO_2 were 255 (5.36), 412 (15.10), 781 (25.32), 1451 (41.96) and 951 mg/L (59.23 mg $L^{-1}d^{-1}$), respectively. Whereas, the lipid contents in cells were 2.33, 5.62, 10.28, 28.36 and 40.65%, respectively. Moreover, the levels of CO_2 in culture medium had significant effect on fatty acid composition of *D. salina*. Linolenic and palmitic acids were identified as the major fatty acids in *D. salina* cells grown at different levels of CO_2. The quality of biodiesel produced from algal lipid by a transesterification reaction was located between the limit imposed by the European Standards (EU 14214) and ASTM (US D6751). Based on the results obtained, *D. salina* could be used for mass-cultured in outdoor ponds, as a promising alternative to current CO_2 mitigation strategy and as a suitable feedstock for biodiesel production.

Keywords: *Dunaliella salina*; Biodiesel; CO_2 mitigation; Fatty acids; Transesterification

Introduction

Nowadays, a number of researches and developmental efforts have been directed at reducing CO_2 emissions. That, the CO_2 is the main greenhouse gas (GHG). GHG contributes not only to global warming, but also to other impact on the environment and human life. Therefore, many attempts including physical, chemical and biological mitigation have been made to recover CO_2 from atmosphere [1]. In general, the strategies of CO_2 mitigation can be classified into three different processes: (1) chemical reaction-based approaches, (2) direct injection to underground or to the ocean and (3) biological CO_2 mitigation, with CO_2 being biologically converted to organic matters [2]. Biological CO_2 fixation is currently achieved through the photosynthesis of all terrestrial plants and microorganisms. Terrestrial plants are expected to contribute to only 3.0 – 6.0 % reduction in the global CO_2 emissions [3]. Microalgae have been identified as fast growing species whose CO_2 fixation rates are much faster than those of terrestrial plants, and their CO_2-fixation efficiency is about 10–50 times greater [4]. For instant, 1 kg of dry algae biomass require ca. 1.83 kg of CO_2 which can readily be obtained from the industrial flow gases via bio-fixation [5]. However, as a novel "green technology" microalgae have the same potential in the CO_2 fixation from three different sources, namely, CO_2 from atmosphere, CO_2 from discharge gases from heavy metal industry, and CO_2 from soluble carbonate. Moreover, microalgae can tolerate and utilize high level of some pollutants such as nitrogen and phosphorus from the wastewater [6]. Thus, microalgae CO_2 fixation is environmentally sustainable when combined with other environmental-protecting processes, such as wastewater treatment or heavy metal removal [7]. However, the microalgae could be cultivated to help in the reduction of atmospheric CO_2 and as a promising source for biofuel production and other valuable added products [8]. In addition, comparing with plants, microalgae have a relatively high lipid content (can exceed 80% by weight of dry biomass), high growth rate (biomass can be doubled within 3.5 h), via cultivation on non-arable land and with non-potable water, and there are no seasonal culture limitations, they contain valuable compounds, and can be easily harvested daily [9]. Also, microalgae can grow far away from the farm lands and forests such as ponds, fermentation units and even wastewater, thus minimizing the damages affecting the food-chain systems and can produce 30-100 times more energy per hectare [5,10].

Many microalgae species were used for CO_2 mitigation such as *Chlorella* sp. [11], *Nannochloropsis* sp. [12], *Scenedesmus* sp. [4], *Chlamydomonas* sp. [13] and *Spirulina* sp. [14]. Moreover, these species can be grown well under the natural light-dark cycle and are suitable for large scale outdoor cultivation systems [15]. Since, industrial exhaust gases containing 10.0–20.0% CO_2, these algae species are not inhibited by CO_2 with 50 ppm, but it can be inhibited by CO_2 when nitrogen oxides (NOx) is present [16]. However, few microalgae species grow well and achieve high CO_2 fixation ability such as *Chlorella* sp. (6240 mg $L^{-1}d^{-1}$) and *Anabaena* sp. (1450 mg.$L^{-1}d^{-1}$), under a relative high tolerance either for temperature or CO_2 level [17]. These species also can remove sulfur dioxides, nitrogen oxides and volatile organic compounds based on different culture or experimental conditions, such as CO_2 level, temperature, cultural medium and light intensity [18]. Therefore, they could be used to develop strategies for CO_2 emissions mitigation and it is interesting to be used as a replacement feedstock for the biofuel industry. The economic feasibility of microalgae mass culture for biofuel production greatly depends on the high biomass productivity and appreciable lipid yield [19].

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Dunaliella salina is one of a few microalgae that are commercially cultured on a relatively large scale to produce (the orange/ or red pigment) β-carotene. In this study, the effect of various CO_2 (as carbon source) levels in airstream on the biomass production and lipid accumulation in *D. salina* cultures was investigated. Also, the quantity and characterization of resultant fatty acid methyl esters as biodiesel product was tested according to European norm EN 4214.

Materials and Methods

Alga sources

Dunaliella salina was obtained from the Culture Collection of Alga at the University of Texas (Austin, TX, USA) and maintained in Natural Research Centre (NRC) from more than ten years ago.

Growth conditions

Dunaliella salina was cultured in a 4 liter flask, with 3 liters of culture medium containing 8% NaCl and 5 mM nitrogen (in KNO_3 form), pH 8.5 during spring season in NRC. All glass and plastic ware were washed with 1% HNO_3 and rinsed several times with distilled water. The cultures were gassed with 1.5% volume CO_2 in air. The cultures were continuously illuminated with 10 cool white fluorescent lamps (Philips 40 W) and light intensity level was approximately 200 Wm^{-2}. The algal masses were gravimetrically determined from triplicate experiments at 27 ± 3°C for 15 days [8].

Assays of CO_2 levels

In this experiment, *D. salina* was cultivated in 4 liter Erlenmeyer flask with 3 liter working volume of medium. Different cultures were aerated with different flow rates of CO_2 mixed with ambient air to prepare varied CO_2 levels of 0.01, 0.03, 3.0, 9.0 and 12.0%. The initial pH of cultures was 8.5 and regularly determined with Hanna pH meter.

Growth measurements

D. salina dry weight ($g.L^{-1}$) was measured regularly every two days using spectrophotometer method at a wavelength of 682 nm and dry weight method [8].

Harvesting

Under the above mentioned experimental conditions, the algal cells were harvested by centrifugation (6000 xg) at 4°C for 15 min and stored at -20°C.

Determination of lipid content

Lipid extraction: After appropriate cell growth (30 days), the cells were harvested from culture by centrifugation at 10,000 xg for 10 min. Cell pellets were frozen at -20°C for overnight and then 10 g of wet algae were disrupted with glass beads (Sigma grad) in a vortex mixer for 10 min. The lipids were extracted with chloroform/methanol mixture (2:1, v/v), then separated into chloroform (bottom layer) and aqueous methanol layers by adding methanol and water to give a final solvent ratio of about chloroform: methanol: water of 1:1:1 (v/v/v). The upper layer (methanol/water layer) was removed and the chloroform layer including lipids were washed several times with 10% NaCl solution, and evaporated to dryness under reducing pressure to yield the algal lipids. The total lipids was gravimetrically determined and stored at -20°C under nitrogen to prevent lipid auto-oxidation or used directly for subsequent analysis.

Cultivation of *D. salina* at large scale: *D. salina* was cultured in a 150 liter aquarium with 140 liters of culture medium containing 8.0% NaCl and 5 mM nitrogen (in KNO_3 form). The cultures were aerated with 12.0% CO_2 level to accumulated high quantity of algae lipid be used for production of biodiesel. The pH of culture was 8.5 and regularly determined with Hanna pH meter.

Preparation of biodiesel: Biodiesel from algal lipids was derived by acid catalyzed transesterification method. Lipids were mixed with methanol with 1:56 molar ratio (weight ratio) and the reaction was carried out at 35°C for 4 h in the presence of sulfuric acid as catalyst with 1:1 weight ratio of catalyst to lipid [20].

Fatty acid analysis: The all algal lipids (except biodiesel product), fatty acids of *D. salina* lipids were converted into methyl esters (FAMEs) by direct trans-methylation using methanol sulphuric acid [21]. Then, FAMEs of algal lipids/ biodiesel were analyzed by using Agilent Technologies 6890 N Network GC-system (USA) equipped with a flame ionization detector (FID) and a HP-5% phenyl methyl silixane capillary column (30 m×0.32 mm i.d., 0.25 μm film thickness). Nitrogen was used as carrier gas at a flow rate of 2.0 ml/min. The oven temperature was set at 70°C with a 2 min hold, raised to 230°C at 8°C min^{-1} and held at 230°C for 20 min. Injector and detector temperatures were 250 and 280°C, respectively. Individual FAMEs was identified by comparing their retention times with that of FAMEs authentic standards (Aldrich-Sigma, purity > 99% by GC-FID).

Measurement of physiochemical properties of biodiesel: The physiochemical properties of algal biodiesel were analyzed according to the standard procedures as follows: acid value (AV, expressed as mg KOH required neutralizing the acidity of 1g oil) was determined by titration with alcoholic KOH solution according to AOAC (Ca 5a-40) method [22]. Iodine value (IV, expressed as the mass of iodine in grams (g) that is consumed by 100 grams oil) and peroxide value (PV, reported as milliequivalents of O_2/ kg oil, meq/kg) were estimated according to AOAC methods Cd 1-25 and Cd 8-53, respectively [22]. The viscosity was measured with capillary viscosimeter in constant temperature bath at 40°C (UNE- ISO 3104). The oxidation rates and unsaturation degree were calculated based on GC analysis of FAME.

Statistical analysis: Statistical analyses were performed using a one-way analysis of variance ANOVA, and the significance ($P<0.05$) of the difference between means was determined by Duncan's multiple range tests. The data were presented as mean values ± SD (standard deviations).

Results and Discussion

Growth of *D. salina* aerated with different CO_2 levels

The various growth parameters (respect to time) of marine microalgae, *D. salina* grown on a culture exposed to various CO_2 levels of 0.01, 0.03, 3.0, 9.0 and 12.0% in air, include maximum cell density (X_{max}) and overall biomass yield ($P_{overall}$) were recorded in Table 1. As shown in Figure 1, among all algae cultures, the typical growth curves include initial and logarithmic phases were observed. In initial stage (as early as the fifth days of cultivation), the changes in X_{max} (average $4.5x10^6$ cells/ml) were not significant among all cultures. After 5 days of culture, significant increase in algae cell density associated with increasing CO_2 levels of aeration in *D. salina* cultures throughout the cultivation periods (30 days) were recorded. At end of cultures (30 days), the X_{max} values of cultures aerated with 0.01, 0.03, 3.0, 9.0 and 12.0 % CO_2 levels were $1.6×10^6$, $2.0×10^6$, $2.3×10^6$, $2.6×10^6$, and $3.5×10^6$ (cells/ml), respectively. Thus, the growth of *D. salina* culture aerated

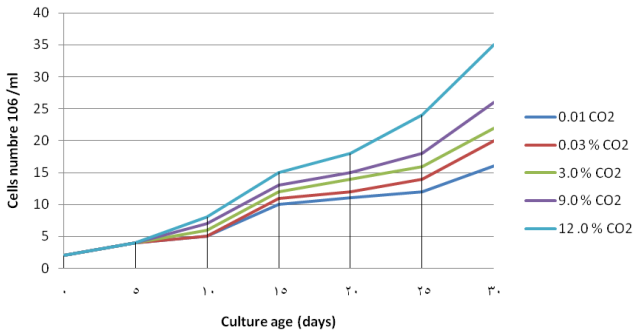

Figure 1: Effect of CO_2 levels on growth of *Dunaliella salina* cells.

with high CO_2 levels, ranged from 3.0 to 12.0% was enhanced compared with those in culture aerated with low CO_2 (0.01 or 0.03% CO_2) levels. These results were confirmed by that data obtained by Oat et al. (2009) and Chiu et al. (2009). They observed that microalga, *D. salina* and *N. oculata* cultures grew well in an enriched CO_2 aeration compared with cultures aerated with 0.30 % CO_2, and similar growth curves were obtained at the CO_2 concentration ranges of 2.0 to 10.0 %. As show in Table 1, the yield of culture biomasses ($P_{overall}$ mg/ml) were significantly increased by increasing of CO_2 level ranges of 0.03% to 9.0% (412 ± 110 to 1451 ± 282 mg/L), and was decreased at 12% (551 ± 95 mg/L). Thus, among all algal cultures, the culture aerated with 9.0 % CO_2 showed the greatest biomass yield, compared with those cultures aerated with other CO_2 levels (0.03, 3.0 and 12.0%). However, it has been known that CO_2 is an essential substrate for microalgae growth, and the CO_2 level in ambient air (0.03%) is suboptimal for higher plants and algal growth. Although, most of plants can tolerate up to 0.1 CO_2%, many microalgae species could tolerate to high CO_2 level up to 12.0% [12]. However, the algae growth can be affected by high CO_2 levels and the concentration of dissolved oxygen evolved by photosynthesis pathway [23]. In this study, among all *D. salina* cultures, the high growth rate was observed in cultures aerated with either 3.0 or 9.0% CO_2. These findings results are consistent with that results reported in several reports, that the *D. tertiolecta* could be grown well in the cultures aerated with CO_2 ranges from 2 to 10% [24,25]. Cheng et al. [26] reported that the high biomass of *Chlorella vulgaris* was obtained in cultures aerated with 2% CO_2 level. In general, the most of microalgae can be grown well in CO_2 level ranging from 1% to 5% [23,27]. Thus, it is important to note that certain algae strains have the ability to adapt to the high CO_2 concentrations [24].

Lipid CO_2 content of *D. salina* aerated with different CO_2 levels

Microalgae, as biological system for CO_2-mitigation, have received considerable attention due to their high CO_2 fixation efficiency and high lipid yield [1]. The overall total lipid content (TL) and lipid productivity (TLP) of *D. salina* cultures aerated with five different CO_2 levels (0.01, 0.03, 3.0, 9.0 and 12.0% CO_2) were determined after 30 days (Table 1). The results revealed that high TL levels were 18.65% and 28.36% in *D. salina* cultures aerated with 12.0% and 9.0% CO_2, respectively. Hence, this high TL content was about 8 to 6 times that obtained from cultures aerated with 0.03% CO_2 (ambient air). Therefore, under high CO_2 concentration, biosynthesis of lipid compounds may be increased over than other components, mainly proteins compounds or from way of fixed more carbon, as a reserve of energy for the algal growth. This result also is confirmed by the other reports that the *D. salina* showed the elevation of metabolic of CO_2 and correlated with increasing of lipid biosynthesis [24,28]. Also, it is possible that the high lipid content found in *D. salina* is as a result of an adaptive strategy for mitigation of CO_2 in its natural environment. The lipid productivities (TLP mg $L^{-1}d^{-1}$) in *D. salina* culture aerated with 0.01, 0.03, 3.0, 9.0 and 12.0% CO_2 levels for 30-day cultivation were 5.36, 15.10, 25.32, 41.96 and 59.23 mg $L^{-1}d^{-1}$, respectively (Table 1). The values of TLP obtained in this work were laid between most of the TLP values reported in the literature for some microalgae species grew under the same conditions. For instance, TLP was found to be 39 mg $L^{-1}d^{-1}$ for *Scenedesmus* sp. strain KCTC [29], 52.4 mg $L^{-1}d^{-1}$ for *Scenedesmus* sp strain JPCC [30], and 58.5 mg L^{-1} d^{-1} for *S. obliquus* [31]. From the perspective of engineering applications, the lipid productivity seems to be more important performance index than lipid content for production of biodiesel from microalgal lipids. Moreover, the lipid productivity of 78.73 mg $L^{-1}d^{-1}$ produced from *S. obliquus* CNW-N ranked in the top 20 species for its use as a potential source of high quality biodiesel [32].

Effect of CO_2 level on fatty acid composition

Effect of CO_2 levels on the fatty acids profile (FAs) of *D. salina* cultures was illustrated by the GC-FID analysis (Table 2). No significant differences in quality of FAs compositions were recorded among all *D. salina* cultures aerated with various CO_2 concentrations. The most abundant fatty acids were palmitic acid (C16:0, 24.85% - 27.26%), oleic (C18:1, 1.81- 3.81%), linoleic (C18:2, 9.34- 6.47%) and linolenic acid (C18:3, 4.79- 6.65% of the total fatty acids). Also, other fatty acids, e.g., myristic (14:0, 4.11 – 10.12 %) and stearic (18:0, 1.05 - 1.81% of the total fatty acids) were identified as minor FAs. Thus, *D. salina* contained high quantity (85%) of saturated (Cn:0) fatty acids than that (16.41%) of unsaturated fatty acids (C18: 1,2,3,4) (Table 4). However, the composition of fatty acids of *D. salina* was similar to those reported for *D. tertiolecta* grew under the similar conditions [27], where the C16:0 and C18:3 were identified as the most abundant FAs. The present FAs profiles are similar to those reported in *D. tertiolecta* by Gouveia and Oliveira [33], they identified C18:2, C18:3 and C16:0 as major fatty acids followed by small quantity of C18:0 and C16:1. In general, the fatty acid composition in microalgae varies according to culture conditions, with predominating of special fatty acids [8, 28].

Biodiesel from *Dunelliela salina*

The chain length, degree of unsaturation, and proportion of total lipids made up by triglycerides are important algal lipid characteristics for biodiesel production and may affect the quality of biodiesel [32]. In this study, *D.salina* biodiesel was characterized by high content of methyl ester fatty acids of C16:0, followed by C18:3 and C18:2. However, the higher content of saturated chains (such as C16:0) in *D. salina* biodiesel may be expected to produce a more stable fuel without considering the effect of the naturally occurring antioxidants in *D. salina* lipids. In general, the present results revealed that the FAME in *D. salina* contained the saturated and unsaturated carbon chain lengths from C16 to C18 (Tables 3 and 4). The relative high proportion of saturated (85%) fatty acids in *D. salina* algae is considered an optimal composition from a fuel quality standpoint. This profile is like that present in *Dunaliella* sp. and lipids found in other microalgae species that could be considered as potential organism for biodiesel production [24,27].

Physiochemical properties of *D. salina* biodiesel

Recently, many current researches are focused on microalgae

to produce biodiesel from algal lipids, that algal biodiesel is not significantly different from biodiesel produced from vegetable oils. The production of biodiesel from *Dunaliella* marine microalgae have been reported in literatures [24,27]. In this study, density, viscosity, acid value (AV), peroxide value (PV) and iodine value (IV) as biodiesel properties were determined in *Dunaliella* biodiesel. Also, oxidative rates and unsaturation degree were calculated based on GC analysis. The values of physiochemical items of *Dunaliella* biodiesel were comparable with those levels for biodiesel fuel standards recorded in ASTM D 6751 (American Society for Testing Material) and the European standard EN 14214 (Table 4). The results also indicate that the *Dunaliella* biodiesel properties were comparable to those of biodiesel produced from several microalgel and vegetable oils tested according to the EN 14214 and B 100 specifications established by ASTM D6751-02 requirements [20,34,35]. For instance, acid value of *Dunaliella* biodiesel (0.38 mg KOH/ g FAME) was found to be lower than the maximum value (<0.5 mg mg KOH/g FAME) recorded in the European Norm (EN 14214). This indicates that the transesterification process was approximately complete. Chinnasamy et al. [36] reported that the completion of transesterification reaction was achieved by the

CO_2 Aeration %	Biomass (Cells dry weight $mg^{-1}L$)	Total lipid Productivity ($mgL^{-1}d^{-1}$)	Lipid content %
0.01	255 ± 75[a]	**5.36**	2.33
0.3	412 ±110[b]	25.1	5.62
3.0	781 ± 213[c]	45.32	10.28
9.0	1451 ±282[d]	51.96	22.36
12.0	551 ± 95[e]	69.23	40.65

Each value represents the mean of three replicates and based on dry weight
All values are significant at (P< 0.5)

Table 1: The levels of lipid production and growth rate of *Dunaliella salina* cultivated in media supplemented with different CO_2 levels.

Fatty acids[a]	Relative content (%)[b]				
	0.01 CO_2	0.03 % CO_2	3.0 % CO_2	9.0 % CO_2	12 .0 % CO_2
$C_{12:0}$	0.23	0.20	0.10	1.24	1.52
$C_{14:0}$	4.11	6.25	8.57	9.01	10.12
$C_{16:0}$	24.85	25.32	26.51	26.60	27.26
$C_{18:0}$	1.81	1.24	0.67	1.05	1.30
$C_{18:1}$	1.81	3.63	2.42	3.70	3.81
$C_{18:2}$	9.34	6.05	6.47	4.22	2.11
$gC_{18:3}$	-	4.81	5.20	6.65	4.79
$C_{18:4}$	3.11	1.55	2.17	1.84	1.45
C_{18}	49.68	46.04	44.26	41.41	40.16
$C_{20:0}$	2.13	1.80	1.81	1.48	1.66
$C_{22:0}$	2.19	1.15	1.08	1.21	0.98

[a]: Fatty acid was identified based on the retention time of standard fatty acids
[b]: The amount of the fatty acid wasevaluated through the peak area

Table 2: Fatty acids composition of *Dunaliella salina* culture aerated with various CO_2 levels.

Lipid criteria	0.0 CO_2	0.03 % CO_2	3.0 % CO_2	9.0 % CO_2	12 .0 % CO_2
Total saturated fatty acids	85.0	82.0	82.0	82.0	83.0
Total monounsaturated fatty acid	1.81	3.63	2.42	3.7	3.81
Total polyunsaturated fatty acids	12.45	12.41	13.84	12.71	8.35
Total unsaturated fatty acids	14.26	16.04	16.26	16.41	12.16
TU/TS	1.48	1.38	1.28	1.19	1.13
DU	0.328	0.363	0.396	0.394	0.282
RO	2.69	2.74	3.18	3.12	2.21

TU/TS: Total unsaturated / Total saturated
DU: Degree of Unsaturated
TMSFA/100 + 2 [Tdi= FA/100] + 3 [T Tri= FA /100] + 4 [Tetra= FA /100]
Rate of oxidation = [%UFA 1 = x1 /100] + [%UFA 2 = x 12 /100] + [%UFA 3 = x 25 /100] + [%UFA4 = x 50 /100]

Table 3: Evaluation criteria of *Dunaliella salina* lipids cultivated on nutrient media supplement with different CO_2 levels.

Property	Units	Biodiesel of D. salina	Diesel[a] Fuel	ASTM[a] Biodiesel Standard
Density	($Kg^{-1}L$)	0.844	0.838	0.86 - 0.9
Viscosity	$mm^2 S^{-1}$, at 40 ºC	3.84	1.9 - 4.1	3.5 - 5.0
Acid value	mg KOH $^{-1}$ g	0.38	Max 0.5	Max 0.5
Peroxide number	meq/kg	ND		
Iodine value	g I_2/100 g	69	120	Max 120

a: The data about diesel and ASTM biodiesel standard were taken from published literature

Table 4: Comparison of physiochemical properties of *Dunaliella salina* biodiesel, diesel fuel and biodiesel standard.

disappearance of all free fatty acids, which was converted into methyl esters. The values of density at 15°C (kgm^3) and viscosity at 40 °C (mm^2/s) were 0.844 and 3.840, respectively in biodiesel produced from *D. salina* biodiesel. These values were within the ranges specified (Max. 900 kg/m^3 and 5.0 mm^2/s) in European biodiesel standards (UNE-EN 14214). However, low viscosity value of *Dunaliella* biodiesel could be due to the presence of low amounts of unsaturated FAs. According to [24], low viscosity and melting points of biodiesel produced from *S. obliquus* were associated with low quantity of unsaturated components and can lead to improvement of the quality of biodiesel. Thus, it seems that there is a relationship between FAME composition in *Dunaliella* biodiesel and the viscosity value, since C12-C18 saturated fatty acid have viscosity values in ranges of 2.43- 5.85 mm^2/s (at 40°C), whereas these values are 4.51, 3.65, and 4.14 mm^2/s for C18:1, C18:2 and C18:3, respectively [37]. According to those values and FA composition and their relative percentage in *Dunaliella* biodiesel one would expect to obtain low viscosity value for this biodeseil. The iodine value of *Dunaliella* biodiesel (IV, as a function for measuring the total unsaturated degree in biodiesel) was 98 g I_2/100g. This value was lower than those values recorded in both European biodiesel (120 g I_2/100g) standard (UNE-En 14214) and Spain Royal (61/2006) Decree (120 g I_2/100g) [34]. The low IV for biodiesel is necessary due to the fact that heating high unsaturated FA results in polymerization products that could lead to deterioration of the lubricating process [38]. Moreover, it is known that the degree of oxidation for unsaturated fatty compounds proceeds with different rates depending on the number and position of double bonds in the FA molecule. In this study, *Dunaliella* biodiesel contained moderate quantity of linoleic (C18:2) and linolenic (C18:3) acids, which tend to produce biodiesel with high oxidative stability. Regarding with the peroxide value (PV, 0.0 meq/kg biodiesel), it could be reported that no hydroperoxide compound are produced during transesterification process. In other words, the *Dunaliella* lipids do not contain or generate acyl radicals (R·) during the formation of biodiesel methyl esters. Based on the low values of rate of oxidation (RO, 2.74) and degree of unsaturation (DU, 0.396) of *Dunaliella* biodiesel, it could be estimated that high oxidative stability of biodiesel towards auto-oxidation chain reactions. It has been well documented in the literature that relative rate of oxidation (RO) for methyl esters of C18:1, C18:2 and C18:3 were 1, 41 and 98, respectively [39]. The degree of unsaturation, double bound orientation and chain length of fatty acid alkyl esters had high impact on oil stability index [40]. The values of stability index (ISO, at 110°C/ h) of methyl esters C12:0 to C18:0 series, C18:1, C18:2 and C18:3 were >40 h, 2.5 h, 1.0 h and 0.2 h, respectively [37]. By applying these values, the *Dunaliella* biodiesel exhibit superior oxidative stability due to the presence of relatively high levels of saturated (SFA, %) and lower high-polyunsaturated fatty acids (HPUFA) (Table 3). Since, high content of PUFAs is undesirable in biodiesel and could be responsible for the poor volatility, low oxidation stability, and for polymerization with gum formation. Therefore, biodiesel could be treated with antioxidant compound as a stabilizer to prevent auto-oxidation process [41]. Finally, methyl ester of C18:1 was considered to be much better than methyl esters of C18:2 or C18:3 for improving Cetin number and oxidative stability without any concomitant adverse effect on the cold properties of the diesel [42-44]. Thus, *D. salina* is suitable foodstock for production of high quality biodiesel due to high lipid content and relatively high contents of saturated fatty acids and oleic acid.

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