Effect of Co_2 on Growth Parameters and Lipid Production in *Dunaliella* sp. ABRIINW-I_1 (Chlorophyceae) Isolated from Urmia Lake (West Azerbaijan, Iran)

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Abstract

Today, using CO_2 in microalgae cultures has been increasing for different purposes. Microalgae have the potential to produce high-value products along with CO_2 fixation. *Dunaliella* is a two-flagellate green microalga. The relatively good quality protein and fatty acid, besides lacking an indigestible cell wall make this alga an exceptional food in aquaculture and poultry fostering. In addition, there are many indigenous strains of algae with the advantage of adaptation to the regional climate condition. The main objective of this study was to evaluate the CO_2 effect on the growth pattern and biochemical composition of *Dunaliella* sp. ABRIINW-I_1 is native to Urmia Lake. Results showed that using CO_2 in the culture not only affects the biomass concentration (1.06 g/l AFDW vs 0.54 g/l in the control experiment) and growth period (reaching the stationary phase in 7 days rather than 14 days in the control experiment); but also influences the chemical composition. It seems that during the cultivation time, the lipid content increased in the cost of carbohydrates (33.1%DW). Fatty acid analysis revealed an optimal combination of saturated and unsaturated acids with the dominance of C16 and C18 fatty acids. It seems that CO_2 injection had no significant effect on the type of FA. The nutritional values of the studied strain were validated in this study, particularly when treated with CO_2. The results demonstrated that utilizing CO_2 in an algal culture could lead to decreased cost and energy requirements.

Keywords: Biomass, Fatty acid, Green alga, Growth period, Photobioreactor

Introduction

Dunaliella (Chlorophyceae, Chlorophyta), is a two-flagellate green microalga which morphologically characterized by lacking a rigid cell wall (Ben-Amotz and Avron, 1987). Various species of this genus can be isolated from marine and Salt Lake environments, ranging in salinity from 0.5% to the saturation extent (approximately 35 %). This remarkable adaptability to high salt concentration makes it the most tolerant eukaryote known (Avron,

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1992; Shariati and Lilai, 1994). In addition to the use of *Dunaliella* in mass production of pigment, factors like the relatively good quality of protein and fatty acid, lack of an indigestible cell wall, presence of high levels of β carotene (vitamin A), and being healthy, making it an exceptional food in aquaculture and poultry fostering (Hoseini Tafreshi and Shariati, 2009).

CO_2 is the major atmospheric gas that contributes to Global warming (Bilanovic et al., 2009), which is emitted from anthropogenic activities. Several technologies have been employed to remove the additional CO_2 from the atmosphere to decrease the troubles of CO_2 acquisition. Abiotic techniques such as physiochemical absorbents, and injection into deep oceans and geological formations expose critical challenges; whereas, biological mitigation proves to be both economically feasible and environmentally sustainable in the long term (Kumar et al. 2010). Studies confirmed the microalgal potential for CO_2 sequestration. (Bilanovic et al., 2009; Kassim and Meng, 2017; Nayak et al., 2016; Raeesossadati et al., 2014; Razzak et al., 2013; Wang et al., 2008; Zeng et al., 2011).

Microalgae have the potential to produce high-value products such as biofuels along with CO_2 fixation. The utilization of CO_2 in microalgae cultures for efficiency enhancement has been increased (Anjos et al., 2013; Bilanovic et al., 2009; De Morais and Costa, 2007; Knudsen et al., 2009; Lam et al., 2012; Li et al., 2011; Nakanishi et al., 2014; Rinanti et al., 2014; Yeh and Chang, 2011; You et al., 2010). Furthermore, carbon dioxide (CO_2) can be utilized to effectively regulate and maintain the pH levels in the culture, eliminating the requirement for costly buffers (Qiu et al., 2017). In this regard, studies on CO_2 utilization in *Dunaliella* cultures showed that CO_2 application influences the quantity along with the quality of various products (Moghimifam et al., 2020a). On the other hand, various strains of *Dunaliella* exhibited varying capabilities in product accumulation (Hosseinzadeh Gharajeh et al., 2020; Xu et al., 2018); their responses to the CO_2 level were also different (Moghimifam et al., 2020b). Numerous indigenous strains exist as a result of diverse salt lakes and marshes. These native strains possess the advantage of adaptation to the local climate condition; nevertheless, the growth potential and yield production of the majority of them remain uncertain.

Due to the high lipid content, microalgae are one of the possible sources for biodiesel production (Benhima et al., 2018). Lipid production in microalgae is affected by growth conditions (Chavoshi and Shariati, 2019; Schenk et al., 2009) like carbon sources (De Swaaf et al., 2003) and salt concentrations (Hopkins et al., 2019; Rizwan et al., 2017; Takagi et al., 2006). Studies conducted on *Dunaliella* have revealed a relatively high growth rate and a substantial lipid content, making it a promising candidate for the production of biofuel (Rasoul-Amini et al., 2014; Tang et al., 2011). Shuping et al. (2010) represented the bio-oil product of *D. tertiolecta* as a possible eco-friendly green biofuel. Furthermore, *Dunaliella* spp. possesses the advantage of growing in brackish to saltwater and wastewater environments, exhibiting remarkable salt

tolerance and effortless lipid extraction (Tang et al., 2011). In their study, Rismani and Shariati (2017) found that the optimum growth rate of *D. salina* occurred at a 1.5 M concentration of NaCl. They suggested this species could be utilized for biodiesel and Omega-3 productions, as they observed an increase in total lipid and omega-3 fatty acids production under salt stress conditions. In comparison with the other two indigenous isolates, Hosseinzadeh Gharajeh et al. (2020) noted that *Dunaliella* sp. ABRIINW-I_1 exhibited a significant increase in a uniform culture containing 1M NaCl.

In this research, the growth pattern and lipid production of *Dunaliella* sp. ABRIINW-I_1, native to Urmia Lake (northwest Iran) was investigated. This study aimed to investigate the effects of CO_2 on biomass yield, pigment content, and fatty acid profile of native *Dunaliella* strain at 0.5 M NaCl.

Material and methods

Isolate and growth conditions

The native isolate, *Dunaliella* sp. ABRIINW-I_1 was obtained from the Agricultural Biotechnology Research Institute of Iran (ABRII), Northwest Branch. This isolate was originally derived from Urmia Lake, in northwest Iran. Urmia Lake is the world's second hypersaline lake, with a salinity range between 140-220 g/L, exceeding 380 g/L in recent years due to the drastic reduction of surface area (Sharifi et al., 2018).

Flask cultivation

Dunaliella sp. ABRIINW-I_1 was cultured in 250 ml Erlenmeyer flasks using a sterile medium. Modified Johnson medium with 1.5 M NaCl (Hejazi and Wijffels, 2003) was used for culture. The cultures were kept in

Fig. 1. A photobioreactor, with a volume of 10 liters, illuminated by four artificial light sources and continuously aerated with filtered air

a phytotron at a temperature of 25 °C, 100 µmol photons/s/m, and a 16:8 h light: dark photoperiod.

PBR Cultivation

Dunaliella sp. ABRIINW-I_1 which has been cultured for 15 days, inoculated into a water jacket glass vessel photobioreactor (PBR) (Fermentor bioreactor- BioFlo 110) with a working volume of 10 L. The vessel was illuminated by four artificial light sources that remained constant. The culture is continuously aerated by filtered air (Castilla Casadiego et al., 2016). CO_2 was injected discontinuously using a mass flow controller (MFC) to regulate the flow rate of CO_2 and controlled by a controller to maintain the culture pH at 7.5. The experimental setup is shown in Figure 1. The initial optical density (OD) was approximately 0.2 at 730 nm and the culture growth was monitored daily. Optical density and pigments were measured throughout the cultivation period while sampling for additional biochemical analysis was conducted every week.

A control experiment was also run where CO_2 injection was not performed. The pH level was maintained at 7.5 by initially adding of Tris-Base (11.12 $g.L^{-1}$).

Biomass concentration

The optical density was measured at 730 nm using a UV/VIS spectrophotometer daily (Perkin Elmer, Lambda 35). The relationship between OD_{730} and biomass was determined experimentally. The biomass concentration was measured in dry weight (DW) and as ash-free dry weight (AFDW) according to Hosseinzadeh Gharajeh et al. (2020) and calculated through the following equations:

$$DW(g.L^{-1}) = OD_{730} \times 0.8, R^2 = 0.95 \quad (1)$$

$$AFDW(g.L^{-1}) = OD_{730} \times 0.41, R^2 = 0.95 \quad (2)$$

Pigment contents

Chlorophylls and carotenoid content of the culture were measured by spectrophotometer every day. The algal sample (3 to 5 ml) was centrifuged at 5000 rpm for 5 minutes. After removal of the supernatant, 3 to 5 ml of 100% pure acetone was added and homogenized using a vortex mixer. Then, the mixer recentrifuged at 5000 rpm for 5 minutes. The supernatant was used to scan the absorbance by spectrophotometer (Perkin Elmer, Lambda 35-UV-VIS), and the content of the pigment was calculated using the following formula (Lichtenthaler and Buschmann, 2001):

$$Chl_a\ (\mu g.mL^{-1}) = 11.24 \times A_{662} - 2.04 \times A_{645}$$

$$Chl_b\ (\mu g.mL^{-1}) = 20.13 \times A_{645} - 4.19 \times A_{662}$$

$$Cart\ (\mu g.mL^{-1}) = (1000 \times A_{470} - 1.9 \times Chl_a - 63.14 \times Chl_b) / 214$$

$$TChl\ (\mu g.mL^{-1}) = Chl_a + Chl_b$$

where A is the absorption value in the specific wavelength, Chl_a, Chl_b, and Cart are chlorophyll a, chlorophyll b, and carotenoids respectively. Pigment contents were expressed as %DW.

Carbohydrate content

Carbohydrates were determined by the phenol-sulphuric acid method (Hosseinzadeh Gharajeh et al., 2020). Measurements were carried out by spectrophotometer (Perkin Elmer, Lambda 35-UV-VIS) at 485 nm using glucose as standard.

Lipid content and fatty acids profile

The modified method of Bligh and Dyer (Yang et al., 2014) was used for the extraction of lipid content. The lipid fatty acid methyl esters (FAME) were prepared by the esterification method to analyze the

fatty acid composition (Cheng et al., 2015). The preparation containing FAME and hexane was analyzed using GC analysis. The analysis was conducted on a Bruker capillary gas chromatograph (model Scion 456 GC). For the analysis, an Rt-2330 polar fused silica capillary column (with dimensions of 105 m X 0.250 mm and a film thickness of 0.20 µm) was utilized. The temperature profile was as follows: the oven temperature was set at 100°C and then raised to 140°C at a rate of 3°C per minute. Subsequently, it further increased to 170°C at a rate of 0.5°C per minute. Finally, it was raised to 220°C at a rate of 4°C per minute for 30 minutes. The carrier gas used was helium, flowing at a rate of 1 ml/m. The injector temperature was set at 250°C, and the detector which was an FID was also set at 250°C.

Statistical Analysis

Statistical analyses were performed using SPSS 16. Drawing graphs was performed by Microsoft Excel 2016.

Results

Biomass concentration

The influence of CO_2 on the biomass concentration and growth rate of native *Dunaliella* sp. ABRIINW-I_1 was studied. Higher dry weight (2.07 g/l) accounted for treatment with CO_2 after 6- days of cultivation, whereas in the control experiment, maximum dry weight was observed after 11 days of cultivation and the value was also low (1.05 g/l). The maximum biomass concentration (AFDW: 1.06 g/l) was also obtained in CO_2 treatment on the 6th day, while in the control experiment the maximum biomass concentration was 0.54 g/l (AFDW), which was obtained on the 11th day (Fig. 2). Biomass Productivity in treatment with CO_2 was calculated 0.17 g/l/day while in the control experiment was 0.04 g/l/day.

Pigments content

the content of chlorophylls (a, b, total) and total carotenoids as % DW are

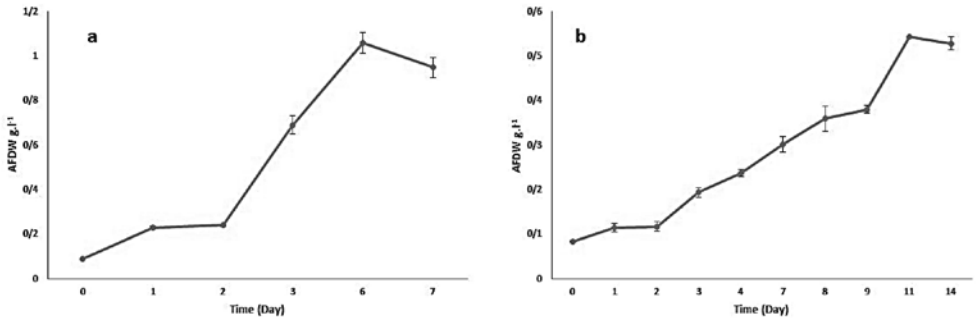

Fig. 2. Biomass concentration of Dunaliella sp. ABRIINW-I1 as AFDW measured in cultures with CO_2 injection (a) and the control experiment (b).

Table 1. Pigment contents (% DW) of *Dunaliella* sp. ABRIINW-I1 cultured in medium with and without CO_2 injection

	Chl_a	Chl_b	T Chl	Cart	T Pig	Chl_a/Chl_b	$Cart/Chl_a$	$Cart/Chl_b$
CO_2 treatment	1.45	0.4	1.86	0.43	2.29	0.19	0.015	0.056
Control experiment	1.33	1.3	2.63	0.34	2.97	0.09	0.025	0.025

shown in Table 1. In total, the maximum pigment content (sum of chlorophylls and carotenoids) was obtained at day 14 in the control experiment (2.97 % DW), whereas, in the presence of CO_2, this amount was 2.29 % DW at the end of the growth period (7^{th} day). This difference is primarily due to the higher biomass concentration in CO_2 treatment. Results of the t-test showed significant differences in chlorophyll a and total chlorophyll in biomass between two treatments ($p< 0.05$) and the amounts of chlorophyll a and total chlorophyll in biomass were significantly higher in CO_2 treatment. However, chlorophyll b and carotenoid content in biomass showed no significant difference between the two experiments ($p> 0.05$).

Lipid and carbohydrate contents

The *Dunaliella* sp. ABRIINW-I_1 biomass was harvested at weekly intervals to determine the total lipid and carbohydrate contents. The growth rate in the CO_2 treatment decreased after 6 days, leading to the termination of the experiment in the first week. On the other hand, the control experiment continued for two weeks. After 7 days of cultivation in the CO_2 treatment, the lipid and carbohydrate contents were 33.1% and 1.4% (DW) respectively, while in the control experiment, the lipid content was 21.45% (DW) and carbohydrate was 2.6% (DW) after 14 days of cultivation (Figure 3). The carbohydrate content showed a significant difference between the two treatments ($p < 0.05$), with the control experiment having a higher content due to the longer growth period.

Fatty acid profile

Table 2 shows the fatty acid composition of *Dunaliella* sp. ABRIINW-I_1 in two experiments. Fatty acid profile mainly composed of palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2), and Alpha linolenic acid (C18:3), which were dominant in both experiments.

Discussion

In this study, the effects of CO_2 on growth parameters, pigment and carbohydrate contents, lipid content, and FA profile of *Dunaliella* sp. ABRIINW-I_1 native to Urmia

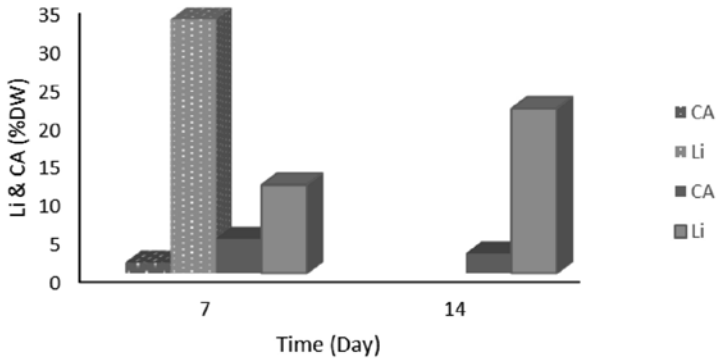

Fig. 3. Biochemical composition of *Dunaliella* sp. ABRIINW-I1 in two treatments. The dotted columns are the results of CO_2 treatment. Li: lipid, CA: Carbohydrate

Lake was evaluated. Results revealed that CO_2 injection could increase the growth and biomass of the studied isolate in addition to decreasing the growth duration. The biomass production obtained in CO_2 treatment was 4.25 fold as much for the control experiment. Other researchers that had studied the CO_2 effect achieved optimum growth of *Dunaliella* taxa in the concentration of 1-6% CO_2 (Kim et al., 2012; Suzuki et al., 1995; Tang et al., 2011).

Algae are characterized by a wide range of pigments such as chlorophylls, and carotenoids. Chlorophyll a; type of chlorophyll, plays a key role in photosynthesis. Other pigments are accessory pigments, acting as photoprotective agents and preventing harmful radicals. In total, chlorophyll amount was reported approximately 0.5-1.5% dry weight in microalgae (Becker, 1994). In both experiments, it was anticipated that the amount of chlorophyll a would be higher than that of chlorophyll b, and the results confirmed this expectation. Hosseinzadeh Gharajeh et al. (2020) reported similar results. One of the significant characteristics of *Dunaliella salina* is the high potential to accumulate enhanced levels of carotenoids particularly β carotene when subjected to specific stressors such as nutrient limitation, high salinity, and light stress (Saha et al., 2018). However, in our experiments, where these stressors were absent, the carotenoid content did not exhibit a significant increase. Carbohydrates are the primary

Table 2. Fatty acid profile of *Dunaliella* sp. ABRIINW-I1 (DW%) in two treatments

	FA	CO_2 treatment Day 7	Control experiment Day 7	Control experiment Day 14
1	14:0 Myristatic acid	0.83 ± 0.091	1.32±0.61	0.79 ± 0.049
2	16:0 Palmitic acid	26.28 ± 0.55	29.82±2.15	23.95±1.25
3	16:1 9C Palmitoleic acid	1.27 ± 0.14	2.64±0.26	3.04±0.21
4	18:0 Stearic acid	15.38 ± 2.46	22.13±4.58	13.37±1.9
5	18:1 9C Oleic acid	4.43± 0.42	4.5±0.55	4.15±0.1
6	18:1 11C Vaccenic acid	1.65± 0.15	1.77±0.16	2.35±0.12
7	19:0 Nanodecanoic acid	0.54± 0.042	0.3±0.43	0.67±0.07
8	18:2 9C,12C Linoleic acid (n6)	15.6 ±0.75	13.1±2.22	14.92±0.5
9	18:3 6C,9C,12C Gamma Linolenic acid (n6)	3.86±0.14	3.12±0.6	4.59±0.26
10	18:3 9C,12C,15C Alpha linolenic acid (n3)	29.63±0.17	20.6 ±4.37	31.75±1.85
11	20:0 Arachidic Acid	0.39±0.17	0.56±0	0.38±0.041
12	ΣSFA	43.53± 1.83	54.25± 7.07	39.17±3.05
13	ΣMUFA	7.35± 0.72	8.92± 0.12	9.55±0.44
14	ΣPUFA	49.11± 1.09	41.24±13.46	51.26±2.62
15	Σn3 PUFA	29.63±0.17	20.6 ±4.37	31.75±1.85
16	Σn6 PUFA	19.46± 0.89	16.22± 2.83	19.51±0.77
17	n3:n6	1.52	1.27	1.62
18	n6:n3	0.65	0.78	0.61
19	UFA: SFA	1.29	0.84	1.55

organic products during the process of photosynthesis. It appears that during the cultivation progresses, there is an increase in lipid content at the expense of carbohydrates. According to Hosseinzadeh Gharajeh et al. (2020), after comparing three isolates of *Dunaliella* it was determined that the cells tend to reserve the carbon and energy in the form of lipids rather than carbohydrates. On the other hand, the utilization of CO_2 resulted in the high lipid content within a brief period. In this regard, the daily lipid productivity exhibited approximately a five-fold surge when treated with CO_2. Some researchers have linked the heightened lipid content in the presence of CO_2 to the mechanism of lipid synthesis, thereby enhancing the lipid content of microalgae in the presence of inorganic carbon (Kassim and Meng 2017; White et al. 2013).

Analysis of the FA showed that the *Dunaliella* sp. ABRIINW-I_1 mostly accumulated the C16 and C18 fatty acids, which generally are dominant in algal cells (Elsey et al. 2007; Lang et al. 2011; Talebi et al. 2013). There was no significant difference in the type of FAs between the two treatments. The main components of the FA profile were n3-ALA, saturated FAs (C16:0 and C18:0), and n6- linoleic acid. Similar results were observed by Hosseinzadeh Gharajeh et al. (2020). Herrero and colleagues (2006) identified palmitic acid and ALA as the primary fatty acids in *D. salina*. In contrast to their findings, oleic acid was not found in high concentrations in this particular study. Previous studies have linked the increase of oleic acid in various algal cells to the deficiency in N and P in culture medium (Ho et al., 2010; Hu and Gao, 2006; Ji et al., 2013; Msanne et al., 2012). In our work, these nutrients were sufficiently added to the culture as KNO_3 and KH_2PO_4, and the nutrient defect was not the case.

Yecong and colleagues (2011) revealed that the growth phase could also affect the lipid content and FAMEs. The findings from the control experiment indicated that during the initial week, the amount of the SAFAs was high. However, as the growth progressed, the SAFAs decreased, while the amount of the USFAs increased. Consequently, at the stationary phase, the fatty acid profiles in both treatment groups exhibited no significant difference. Additionally, lipid content altered throughout the growth phase. Among the PUFAs, n3 and n6 fatty acids are essential for humans and all other mammals. The n6/n3 balance plays a crucial role in decreasing the risk of coronary heart disease (Simopoulos, 2008). Although in our study this ratio did not match the international standards (Ma et al., 2016), the n3/n6 ratio, which is a valuable indicator for assessing exceeded nutritional value of oily foods, exceeded certain fish species. This was supported by Cakmak et al. (2014) in their work on the biochemical composition of *Dunaliella salina*, where they found that the extracted oils had a higher nutritional value compared to some fishes. Studies on FA composition in some fishes characterized the ratio of 1.03-2.8 for the n3/n6 ratio (Cakmak et al., 2012; Donmez, 2009; Guler et al., 2007).

In total, various parameters including salinity, light intensity, duration, temperature, medium type, and nitrate

concentration could influence the growth, pigment, lipid, and carbohydrate contents of algae (Al-Adali et al., 2012). The halotolerant *Dunaliella salina* is the known commercial microalgae with various strains of *Dunaliella* growing in saltwater ecosystems of Iran. The main aim of this work was to investigate the effects of CO_2 injection on the growth and biochemistry of native *Dunaliella* sp. ABRIINW-I_1. Results revealed that utilizing CO_2 in a culture not only affects the biomass concentration and growth period but also affects the chemical composition. Furthermore, fatty acid analysis revealed an optimal combination of saturated and unsaturated acids with the dominance of C16 and C18 fatty acids. Fatty acid methyl esters play a crucial role in the biodiesel properties of algal biomass. In addition, lipids have great importance at various growth stages of many aquaculture animals. Our results confirmed the nutritional values of the studied strain particularly when treated with CO_2. The introduction of CO_2 had no significant impact on the type of fatty acids. The results demonstrated that throughout the microalgae cultivation of microalgae, CO_2 sequestration, biofuel, and food production could be combined. Additionally, the utilization of CO_2 in algal cultivation could lead to a decrease in costs and energy requirements.

References

Anjos M, Fernandes BD, Vicente AA, Teixeira JA, Dragone G. (2013). Optimization of CO_2 bio-mitigation by *Chlorella vulgaris*. Bioresource Technology. 139: 149-154. Doi:10.1016/j.biortech.2013.04.032.

Al-Adali K, Ahmed E, Kumar P, Ayaril N. (2012). Effect of salinity, temperature, nutrients and CO_2 on growth of two species of microalgae from Red Sea, Saudi Arabia. Journal of King Abdulaziz University: Marine Sciences. 23 (1): 57-73. Doi: 10.4197/Mar. 23-1.5.

Avron M. (1992). Osmoregulation In: Dunaliella: Physiology, Biochemistry, and Biotechnology. (Ed. by M. Avron, A. Ben- Amotz A), CRC Press, Boca Raton. pp. 135–159.

Becker EW. (1994). Microalgae: Biotechnology and microbiology. Cambridge University Press, Cambridge.

Ben-Amotz A and Avron M. (1987). On the mechanism of osmoregulation in *Dunaliella* In Energetic and Structure of Halophilic Microorganism, Caplan SR and Gizburg M (Ed). Elsevier, Amsterdam, North-Holland. pp. 529–541.

Benhima R, El Arroussi H, Kadmiri IM, El Mernissi N, Bennis I, Smouni AA, Bendaou N. (2018). Nitrate Reductase Inhibition Induces Lipid Enhancement of *Dunaliella tertiolecta* for Biodiesel Production. The Scientific World Journal. 2018: 1-8. Doi:10.1155/2018/6834725.

Bilanovic D, Andargatchew A, Kroeger T, Shelef G. (2009). Freshwater and marine microalgae sequestering of CO_2 at different C and N concentrations – Response surface methodology analysis. Energy Conservation and Management. 50: 262-267. Doi:10.1016/j.enconman.2008.09.024.

Castilla Casadiego D, Albis Arrieta A, Angulo Mercado E, Cervera Cahuana S, Baquero

Noriega, K, Suárez Escobar A, Moralez Avendano, ED. (2016). Evaluation of Culture Conditions to Obtain Fatty Acids from Saline Microalgae Species: *Dunaliella salina*, Sinecosyfis sp. and Chroomonas sp. BioMed Res Int. 2016: 7 pp. Doi:10.1155/2016/5081653.

Cakmak YS, Kaya M, Asan-Ozusaglam M. (2014). Biochemical composition and bioactivity screening of various extracts from *Dunaliella salina*, a green microalga. EXCLI Journal. 13: 679-690. PMID: 26417292; PMCID: PMC4464408.

Cakmak YS, Zengin G, Guler GO, Aktusek A, Ozparlak H. (2012). Fatty acid composition and ω3/ω6 ratios of muscle lipids of six fish species in Sugla Lake, Turkey. Archives of Biological Sciences. 64: 471-477. Doi: 10.2298/ABS1202471C.

Chavoshi ZZ and Shariati M. (2019). Lipid production in *Dunaliella bardawil* under autotrophic, heterotrophic, and mixotrophic conditions. Brazilian Journal of Oceanography. 67: 1-8. Doi: 10.1590/s1679-87592019024906709.

Cheng YS, Labavitch J, Vander Gheynst J. (2015). Elevated CO_2 concentration impacts the cell wall polysaccharide composition of green microalgae of the genus *Chlorella*. Letters in Applied Microbiology. 60: 1-7. Doi:10.1111/lam.12320.

De Morais MG and Costa JAV. (2007). Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. Journal of Biotechnology. 129 (3): 439-445. Doi: 10.1016/j.jbiotec.2007.01.009.

De Swaaf ME, Sijtsma L, Pronk JT. (2003). Highcell-density fed-batch cultivation of the docosahexaenoic acid-producing marine alga *Crypthecodinium cohnii*. Biotechnology and Bioengineering. 81: 666-672. Doi: 10.1002/bit.10513.

Donmez M. (2009). Determination of fatty acid compositions and cholesterol levels of some freshwater fish living in Porsuk Dam, Turkey. Chemistry of Natural Compounds. 45: 14-7. Doi: 10.1007/s10600-009-9219-z.

Elsey D, Jameson D, Raleigh B, Cooney MJ. (2007). Fluorescent measurement of microalgal neutral lipids. Journal of Microbiological Methods. 68: 639-642. Doi: 10.1016/j.mimet.2006.11.008.

Guler GO, Aktumsek A, Citil OB, Arslan A, Torlak E. (2007). Seasonal variations on the total fatty acid composition of fillets of zander (Sander lucioperca) in Beysehir Lake (Turkey). Food Chemistry. 103: 1241-1246. Doi: 10.1016/j.foodchem.2006.10.029.

Hejazi M and Wijffels R. (2003). Effect of light intensity on β-carotene production and extraction by *Dunaliella salina* in two-phase bioreactors. Biomolecules Engineering. 20: 171-175. Doi: 10.1016/S1389-0344(03)00046-7.

Herrero M, Ibanez E, Cifuentes A, Reglero G, Santoyo S. (2006). *Dunaliella salina* microalga pressurized liquid extracts as potential antimicrobials. Journal of Food Protection. 69: 2471-2477. Doi: 10.4315/0362-028X-69.10.2471.

Ho SH, Chen CY, Chang JS. (2010).

Scenedesmus obliquus CNW-N is a potential candidate for CO_2 mitigation and biodiesel production. Bioresource Technology. 101: 8725–8730. Doi:10.1016/j.biortech.2010.06.112.

Hosseieni Tafreshi A and Shariati M. (2009). *Dunaliella* biotechnology: methods and applications. Journal of Applied Microbiology. 107: 14–35. DOI: 10.1111/j.1365-2672.2009.04153.x.

Hopkins TC, Sullivan Graham EJ, Schuler AJ. (2019). Biomass and lipid productivity of *Dunaliella tertiolecta* in a produced water-based medium over a range of salinities. Journal of Applied Phycology. 31: 3349-3358. Doi: 10.1007/s10811-019-01836-3.

Hosseinzadeh Gharajeh N, Valizadeh M, Dorani E, Hejazi MA. (2020). Biochemical profiling of three indigenous *Dunaliella* isolates with a main focus on fatty acid composition toward potential biotechnological application. Biotechnology Reports. 26: 1-9. Doi: 10.1016/j.btre.2020.e00479.

Hu H and Gao K. (2006). Response of growth and fatty acid compositions of *Nannochloropsis* sp. to environmental factors under elevated CO_2 concentration. Biotechnology Letters. 28: 987–992. Doi: 10.1007/s10529-006-9026-6.

Kassim MA and Meng TK. (2017). Carbon dioxide (CO_2) biofixation by microalgae and its potential for biorefinery and biofuel production. Science of the Total Environment. 584-585: 1121-1129. Doi: 10.1016/j.scitotenv.2017.01.172.

Kim W, Park LM, Gim GH, Jeong SH, Kang CM, Kim DJ, Kim SW. (2012). Optimization of culture conditions and comparison of biomass productivity of three green algae. Bioprocess and Biosystem Engineering. 35: 10-27. Doi: 10.1007/s00449-011-0612-1.

Knudsen JN, Jensen JN, Vilhelmsen PJ, Biede O. (2009). Experience with CO_2 capture from coal flue gas in pilot-scale: Testing of different amine solvents. Energy Procedia. 1 (1): 783-790. Doi: 10.1016/j.egypro.2009.01.104.

Kumar A, Ergas S, Yuan X, Sahu A, Zhang Q, Dewulf J, Xavier Malcata F, Van Langenhove H. (2010). Enhanced CO_2 fixation and biofuel production via microalgae: recent developments and future directions. Trends in Biotechnology. 28 (7): 371-380. Doi: 10.1016/j.tibtech.2010.04.004.

Lam MK, Lee KT, Mohamed AR. (2012). Current status and challenges on microalgae-based carbon capture. International Journal of Greenhouse Gas Control. 10: 456-469. Doi: 10.1016/j.ijggc.2012.07.010.

Lang I, Hodac L, Friedl T, Feussner I. (2011). Fatty acids profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biology. 11: 124. Doi: 10.1186/1471-2229-11-124.

Li F-F, Yang Z-H, Zeng R, Yang G, Chang X, Yan J-B, Hou Y-L. (2011). Microalgae capture of CO_2 from actual flue gas discharged from a combustion chamber. Industrial & Engineering Chemistry Research. 50 (10): 6496-6502. Doi: 10.1021/ie200040q.

Lichtenhaler HK and Buschmann C. (2001). Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Current Protocol in Food Analytical Chemistry. 1: F4. 3.1-F4. 3.8. Doi: 10.1002/0471142913.faf0403s01.

Ma X, Jiang Z, Lai C. (2016). Significance of increasing n-3 PUFA content in Pork on human health. Critical Reviews in Food Science and Nutrition. 56:1-13. Doi 10.1080/10408398.2013.850059.

Moghimifam R, Niknam V, Ebrahimzadeh H, Hejazi MA. (2020a). CO_2 biofixation and fatty acid composition of two Indigenous *Dunaliella* sp. isolates (ABRIINW-CH2 and ABRIINW-SH33) in response to extremely high CO_2 levels. Bioprocess and Biosystems Engineering. 43: 1587-1597. Doi: 10.1007/s00449-020-02350-4.

Moghimifam R, Niknam V, Ebrahimzadeh H, Hejazi MA. (2020b). The influence of different CO_2 concentrations on the biochemical and molecular response of two isolates of *Dunaliella* sp. (ABRIINW-CH2 and ABRIINW-SH33). Journal of Applied Phycology. 32: 175-187. Doi: 10.1007/s10811-019-01914-6.

Msanne J, Xu D, Konda AR, Casas-Mollano JA, Awada T, Cahoon EB, Cerutti H. (2012). Metabolic and gene expression changes triggered by nitrogen deprivation in the photoautotrophically grown microalgae *Chlamydomonas reinhardtii* and *Coccomyxa* sp. C-169. Phytochemistry. 75: 50–59. Doi: 10.1016/j.phytochem.2011.12.007.

Nakanishi A, Aikawa S, Ho S, Chen C, Chang J, Hasunuma T, Kondo A. (2014). Development of lipid productivities under different CO_2 conditions of marine microalgae *Chlamydomonas* sp. JSC4. Bioresource Technology. 152: 247-252. Doi: 10.1016/j.biortech.2013.11.009.

Nayak M, Karemore A, Sen R. (2016). Performance evaluation of microalgae for concomitant wastewater bioremediation, CO_2 biofixation, and lipid biosynthesis for biodiesel application. Algal Research. 16: 216-223. Doi: 10.1016/j.algal.2016.03.020.

Qiu R, Gao S, Lopez PA, Ogden KL. (2017). Effects of pH on cell growth, lipid production and CO_2 addition of microalgae *Chlorella sorokiniana*. Algal Research. 28: 192-199. Doi: 10.1016/j.algal.2017.11.004.

Raeesossadati MJ, Ahmadzadeh H, McHenry MP, Moheimani NR. (2014). CO_2 bioremediation by microalgae in photobioreactors: Impacts of biomass and CO_2 concentrations, light, and temperature. Algal Research. 6: 78-85. Doi: 10.1016/j.algal.2014.09.007.

Rasoul-Amini S, Mousavi P, Montazeri-Najafabady N, Mobasher MA, Mousavi SB, Vosough F, Dabbagh F, Ghasemi Y. (2014). Biodiesel Properties of Native Strain of *Dunaliella Salina*. International Journal of Renewable Energy Research. 4(1): 39-41. Doi: 10.20508/ijrer.v4i1.989.g6241.

Razzak SA, Hossain MM, Lucky RA, Bassi AS, Lasa, H. (2013). Integrated CO_2 capture, wastewater treatment and biofuel production by microalgae culturing—A review.

Renewable and Sustainable Energy Review. 27: 622-653. Doi: 10.1016/j.rser.2013.05.063.

Rinanti A, Dewi K, Kardena E, Astuti DI. (2014). Biotechnology Carbon Capture and Storage (CCS) by Mix-culture Green Microalgae to Enhancing Carbon Uptake Rate and Carbon Dioxide Removal Efficiency with Variation Aeration Rates in Closed System Photobioreactor. Journal Teknology. 69 (6): 105-109. Doi: 10.11113/jt.v69.3317.

Rissmani S and Shariati M. (2017). Changes of the total lipid and Omega-3 fatty acid contents in two microalgae Dunaliella salina and *Chlorella vulgaris* under salt stress. Brazilian Archives of Biology and Technology. 60: 1-11. Doi: 10.1590/1678-4324-2017160555.

Rizwan M, Mujtaba J, Rashid N, Lee K. (2017). Enhancing Lipid Production of Dunaliella tertiolecta by Manipulating the Interactive Effect of Salinity and Nitrogen. Chemistry and Biochemistry Engineering. Q 31 (3): 199–207. Doi: 10.15255/CABEQ.2017.1092.

Saha SK, Kazipet N, Murray P. (2018). The carotenogenic *Dunaliella salina* CCAP 19/20 produces enhanced levels of carotenoid under specific nutrient limitations. Biomed Research International. 2018: 7532897. 11pp. Doi: 10.1155/2018/7532897.

Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, Olaf K, Hankmer B. (2009). Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenergy Research. 1: 20-43. Doi: 10.1007/s12155-008-9008-8.

Shariati M. Lilley RMC. (1994). Loss of intracellular glycerol from *Dunaliella* by electroporation at constant osmotic pressure: subsequent restoration of glycerol content and associated volume changes. Plant Cell & Environment. 17: 1295–1304. Doi: 10.1111/j.1365-3040.1994.tb00531.x.

Sharifi A, Shah-Hosseini M, Pourmand A, Esfahaninejad M, Haeri-Ardakani O. (2018). The Vanishing of Urmia Lake: A Geolimnological Perspective on the Hydrological Imbalance of the World's Second Largest Hypersaline Lake In Nooran et al. (eds.), Lake Urmia: A Hypersaline waterbody in a drying climate. The Handbook of Environmental Chemistry. Springer Nature Switzerland AG. Doi: 10.1007/698_2018_359.

Shuping Z, Yulong W, Mingde Y, Kaleem I, Chun L, Tong J. (2010). Production and characterization of bio-oil from hydrothermal liquefaction of microalgae *Dunaliella tertiolecta* cake. Energy. 35: 5406-5411. Doi: 10.1016/j.energy.2010.07.013.

Simopoulos AP. (2008). The Importance of the Omega-6/Omega-3 Fatty Acid Ratio in Cardiovascular disease and other chronic diseases. Experimental Biology and Medicine. 233: 674-688. Doi: 10.3181/0711-MR-311.

Suzuki T, Matsuo T, Ohtaguchi K, Koide K. (1995). Gas-sparged bioreactors for CO_2 fixation by *Dunaliella tertiolecta*. Journal of Chemical Technology & Biotechnology. 62: 351-358. Doi: 10.1016/j.jcou.2017.09.013.

Takagi M, Karseno Y, Yoshida T. (2006). Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae *Dunaliella* cells. Journal of Bioscience and Bioengineering. 101: 223-226. Doi: 10.1263/jbb.101.223.

Talebi AF, Mohtashami SK, Tabatabaei M, Tohidfar M, Bagheri A, Zeinalabedini M, Mirzajanzadeh M, Shafaroudi SM, Bakhtiari S. (2013). Fatty acids profiling: a selective criterion for screening microalgae strains for biodiesel production. Algal Research. 2: 258-267. Doi: 10.1016/j.algal.2013.04.003.

Wang B, Li Y, Wu N, Lan CQ. (2008). CO_2 bio-mitigation using microalgae. Applied Microbiology and Biotechnology. 79:707-718. Doi: 10.1007/s00253-008-1518-y.

White DA, Pagarette A, Rooks P, Ali ST. (2013). The effect of sodium bicarbonate supplementation on growth and biochemical composition of marine microalgae cultures. Journal of Applied Phycology. 25: 153-165. Doi: 10.1007/s10811-012-9849-6.

Xu Y, Ibrahim IM, Wosu CI, Ben-Amotz A, Harvey PJ. (2018). Potential of New Isolates of *Dunaliella Salina* for Natural Carotene Production. Biology. 7 (14): 1-18. Doi: 10.3390/biology7010014.

Yang F, Xiang W, Sun X, Wu H, Li T, Long L. (2014). A novel lipid extraction method from wet microalga *Picochlorum* sp. at room temperature. Marine Drugs. 12: 1258–1270. Doi: 10.3390/md12031258.

Yecong L, Yi-Feng C, Paul C, Min M, Wenguang Z, Blanca M, Zhu J, Ruan R. (2011). Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. Bioresource Technology. 102: 5138-5144. Doi: 10.1016/j.biortech.2011.01.091.

Yeh K-L, and Chang J-S. (2011). Nitrogen starvation strategies and photobioreactor design for enhancing lipid content and lipid production of a newly isolated microalga *Chlorella vulgaris* ESP-31: implication for biofuel. Biotechnology Journal. 6: 1358-1366. Doi: 10.1002/biot.201000433.

Yoo C, Jun S-Y, Lee J-Y, Ahn C-Y, Oh H-M. (2010). Selection of microalgae for lipid production under high levels of carbon dioxide. Bioresource Technology. 101 (1): 71-74. Doi: 10.1016/j.biortech.2009.03.030.

Zeng X, Danquah MK, Dong Chen X, Lu Y. (2011). Microalgae bioengineering from CO_2 fixation to biofuel production. Renewable and Sustainable Energy Review. 15: 3252-3260. Doi: 10.1016/j.rser.2011.04.014.