Differential Modulation of Biomass Productivity and Fatty Acid Composition in *Dunaliella salina* by Salinity and Nutrient Stress

Mohammad Roozitalab[1], Gilan Attaran-Fariman*[1], Hasan Zadabbas Shahabadi[1,2]

Received: 2025-05-23 Accepted: 2025-08-21

Abstract

Dunaliella salina, a microalga renowned for its production of bioactive compounds, holds significant potential for biofuel generation. This study investigated the interactive effects of salinity and nutrient availability on the growth kinetics, biomass yield, total lipid content, and fatty acid composition of a *D. salina* strain isolated from the southern coast of Iran. The microalga was cultivated under three distinct salinity levels (35, 70, and 105 g/L), each supplemented with varying concentrations of nitrate (100%, 50%, 25%) and glucose (1, 2, and 3 g/L). The highest biomass yield (1449 mg/L) was achieved at the lowest salinity (35 g/L) when supplemented with 3 g/L glucose. Notably, the average biomass production across various nutrient treatments at 70 g/L salinity surpassed that observed at the other salinities. While alterations in nutrient concentrations did not significantly impact the overall lipid content ($P \geq 0.05$), the highest lipid accumulation was observed at the highest salinity (105 g/L). However, the lipid productivity at 35 g/L with 3 g/L glucose was superior due to the substantially higher biomass yield. Saturated Fatty Acids (SFAs) dominated the fatty acid profiles, ranging from 41% to 73% of the total fatty acids, whereas Polyunsaturated Fatty Acids (PUFAs) varied between 2% and 40%. Palmitic acid (C16:0) consistently represented the most abundant individual fatty acid (13-44%) across all treatments. The maximum accumulation of SFAs was observed at 70 g/L salinity. The findings of this study demonstrate the significant influence of salinity and nutrient regimes on the biomass and lipid characteristics of the Iranian *D. salina* isolate, suggesting its potential as a promising feedstock for biofuel production.

Keywords: *Dunaliella salina,* Biomass Production, Biofuel Feedstock, Fatty acids, Salinity Stress, Nutrients, Glucose Supplementation

Introduction

Microalgae represent a promising and abundant source of diverse biogenic materials, attracting significant attention across various biotechnology research domains. The inceasing global production of carbon dioxide, as represent by the Intergovernmental Panel on Climate Change

1-Marine Biology Department, Faculty of Marine Sciences, Chabahar Maritime University, Daneshgah Avenue, 99717-56499, Chabahar, Iran.
2-Agricultural Biotechnology Research Institute of Iran (ABRII), Isfahan Branch
*Corresponding author email address: g.attaran@cmu.ac.ir
Doi: 10.48308/pae.2025.241096.1120

(IPCC) indicated that fossil fuel and industrial CO_2 emissions averaged approximately 36 ± 2.9 $GtCO_2$ per year from 2010 to 2019, reaching in around 38 ± 3 $GtCO_2$ in 2019 (IPCC, 2022). More recent estimates from the International Energy Agency (IEA) indicate that energy-related CO_2 emissions reached a record 37.4 $GtCO_2$ in 2023 and approximately 37.8 $GtCO_2$ in 2024 (IEA, 2024a; IEA, 2025), posing a critical environmental challenge. Furthermore, the absorption of approximately 30% of anthropogenic carbon dioxide by ocean waters leads to detrimental ocean acidification, with devastating consequences for marine ecosystems, including coral reef degradation and biodiversity loss (Mata et al., 2010). In this context, biofuels derived from renewable sources offer a compelling alternative to fossil fuels due to their lower environmental impact (Scragg et al., 2003; Deora et al., 2023). Microalgae have the potential to absorb approximately 513 tons of CO_2 per hectare per year, corresponding to an annual yield of around 280 tons of dry biomass per hectare, under optimal solar conditions as reported by Bhola et al. (2014). Given that flue gases contain CO_2 concentrations ranging from 3% to 30%, a critical factor for successful biofixation is the precise selection of algae species capable of thriving and efficiently absorbing CO_2 at such elevated levels (Iglina et al., 2022).

The key factor governing the economic viability of biodiesel production from microalgae is the efficiency of lipid accumulation (Pacheco et al., 2017). While certain microalgal species exhibit high intracellular lipid content, their slow growth rates can result in overall low lipid productivity (Chu, 2012). Notably, the growth rate, biomass yield, and the qualitative and quantitative composition of fatty acids in microalgae are species-specific and are also significantly modulated by prevailing environmental conditions (Mata et al., 2013; Rios et al., 2016; Pacheco et al., 2017; Hopkins et al., 2019; Morales et al., 2020). Some microalgal taxa are rich in polyunsaturated fatty acids (PUFAs), valuable for various applications, while others accumulate significant amounts of triglycerides, readily convertible to biofuel (Bougaran et al., 2012). Enhancing the overall efficiency of fatty acid production necessitates optimizing both biomass accumulation and cellular lipid content (Mairet et al., 2011; Shokravi et al., 2020).

The genus *Dunaliella* comprises halotolerant microalgae capable of thriving in high salinity environments through sophisticated physiological mechanisms involving glycerol production and ion regulation (Thompson Jr., 1996). Within this genus, species exhibit considerable variability in lipid content and biomass production in response to diverse environmental cues (Hopkins et al., 2019). Specifically, *Dunaliella salina* (Dunal) Teodoresco is recognized as a promising source for the production of various bioactive compounds (Truc et al., 2017).

This study aimed to elucidate the combined effects of varying salinity levels and nitrate concentrations, as well as glucose supplementation (to induce mixotrophic growth), on the growth rate, biomass yield, total lipid content, and fatty acid profile of *Dunaliella salina* over a defined cultivation period.

The findings of this research contribute to a better understanding of the physiological responses of this microalgal species to key environmental factors relevant for optimizing its potential in biofuel and other biotechnological applications.

Material and methods

Microalgal strain and culture maintenance

The microalgal strain *Dunaliella salina (=Dunaliella bardawil*) used in this study was obtained from the culture collection of Chabahar Maritime University, Iran. This isolate was originally sourced from the northern coast of the Oman Sea (Iranian coast, Lipar region; pink lagoon) and identified through gene sequencing, with its sequence data deposited in GenBank under the accession number JX524863. Stock cultures were maintained axenically in f/2 culture medium (Guillard and Ryther, 1962) under controlled laboratory conditions, including a temperature of 25 ± 1 °C, a light intensity of 100 µmol photons m^{-2} s^{-1} with a photoperiod of 12:12 h light: dark, and continuous aeration with filter-sterilized air.

Experimental design and culture conditions

This study employed a factorial experimental design to investigate the individual and interactive effects of three salinity levels, three nitrate concentrations, and three glucose concentrations on the growth, biomass yield, total lipid content, and fatty acid composition of *D. salina*. Each treatment combination was conducted in triplicate over a 14-day cultivation period.

Three salinity levels were established by adjusting the f/2 culture medium to 35 g/L (representing standard seawater salinity), 70 g/L (two-fold seawater salinity), and 105 g/L (three-fold seawater salinity). Natural sea salt sourced from the Oman Sea was utilized to elevate the salinity of the base f/2 medium. To examine the effects of nitrate availability, sodium nitrate ($NaNO_3$) in the f/2 culture medium was adjusted to 25%, 50%, and 100% of the standard protocol, and these treatments were applied under all salinity conditions. For the mixotrophic growth conditions, glucose ($C_6H_{12}O_6$) was supplemented at concentrations of 1, 2, and 3 g/L to the culture medium at each salinity level and nitrate concentration.

All microalgal cultures were maintained in 250 mL Erlenmeyer flasks under controlled *in vitro* conditions, including a 12:12 h light: dark photoperiod, a constant temperature of 24 ± 1 °C, and continuous aeration with filter-sterilized air.

Statistical analyses were performed using SPSS software. The normality of the data was assessed using the Shapiro-Wilk test. Significant differences between treatment groups were determined using one-way and two-way Analysis of Variance (ANOVA), followed by Duncan's post-hoc test for pairwise comparisons. A significance level of $P < 0.05$ was used for all statistical analyses.

Assessment of Growth

Microalgal growth was monitored every 48 hours throughout the 14-day cultivation period. This 14-day duration was chosen because the growth curve observed in the preliminary assessment suggested that growth would plateau after this point. For the experimental setups, cultures were initiated by inoculating the stock culture at a 1:10 dilution. This dilution ratio was adjusted according to

the initial cell density of each treatment.

At each sampling time, aliquots were aseptically withdrawn from the culture flasks and initially assessed microscopically (Nikon ECLIPSE 50i light microscope) to verify cell viability and overall culture health. Subsequently, the samples were fixed with a 2% (w/v) Lugol's iodine solution to immobilize the cells for enumeration. Cell density was determined using a Neubauer counting chamber (Marienfeld GMBH and Co., Germany) following standard hemocytometry procedures. Cell counts were performed in triplicate for each culture flask at each time point, and the average cell density (cells/mL) was calculated. During the exponential growth phase an increase in cell density, from N_0 to N_1, can be utilized to determine the specific growth rate (μ). The specific growth rate is defined as below.

$$\mu\ (day^{-1}) = Ln\ N_1 - Ln\ N_0 / t_1 - t_0$$

N_0 represents the initial cell density at time t_0, and N_1 represents the cell density at a later time (Moheimani et al., 2013). Growth curves were then generated by plotting cell density against time.

Evaluation of biomass, total lipid content, and fatty acid composition

Biomass determination

At the termination of the 14-day cultivation period, biomass production was determined gravimetrically. Aliquots of the microalgal cultures were transferred to pre-weighed centrifuge tubes (W_1) and centrifuged at 4000 rpm for 15 minutes to pellet the cells. The resulting microalgal pellets were washed twice with distilled water to remove residual salts from the culture medium. The centrifuge tubes containing the washed biomass were then transferred to a freeze dryer (JFD 2L, JAL TEB, Iran) and lyophilized at -30 °C for 24 hours to ensure complete removal of moisture (Talebi et al., 2013). Following lyophilization, the tubes were re-weighed (W_2), and the dry weight biomass concentration (mg/L) was calculated by subtracting the initial weight of the tube (W_1) from the final weight (W_2) and normalizing to the volume of the initial culture sample.

Total lipid extraction

Total lipids were extracted from the freeze-dried algal biomass using a modified Bligh and Dyer method (Bligh and Dyer, 1959; Nigam et al., 2011). Briefly, approximately 100 mg of lyophilized algal biomass was transferred to a glass vial, and 3 mL of a 1:2 (v/v) methanol/chloroform solution was added. The mixture was then agitated on a magnetic stirrer at 25 °C for 18 hours to facilitate lipid solubilization. Subsequently, the mixture was centrifuged at 2000 rpm for 3 minutes to separate the phases. The chloroform layer, which contains the extracted lipids, was carefully transferred to a pre-weighed glass test tube. The solvent was then evaporated under a gentle stream of nitrogen gas within a fume hood, and the sample was dried in an oven at 80 °C for 2 hours to ensure complete solvent removal. After drying, the test tubes containing the total lipids were re-weighed, and the total lipid content was expressed as a percentage of the dry weight biomass.

Fatty acid methyl ester (FAME) preparation and analysis

The fatty acid composition of the *D. salina* biomass was determined using a direct trans-esterification method to yield fatty

acid methyl esters (FAMEs) (Talebi et al., 2013). Briefly, approximately 8 mg of lyophilized algal biomass was placed in a glass vial, and 300 µl of extract buffer was added. The mixture was then incubated at 75 °C for 2 hours to facilitate the trans-esterification reaction. Following incubation, 300 µL of a 0.9% (w/v) sodium chloride (NaCl) aqueous solution and 300 µL of n-hexane were added to the mixture, and the resulting solution was vigorously vortexed. The samples were then centrifuged at 5000 rpm at 20 °C for 3 minutes to achieve phase separation. The upper hexane layer, containing the extracted FAMEs, was carefully transferred to a clean gas chromatography (GC) vial for analysis. FAME analysis was performed using a Shimadzu GC-2014 gas chromatograph equipped with a flame ionization detector (FID) and a BPX70 capillary column (25 m × 0.22 mm internal diameter, 0.25 µm film thickness). Nitrogen was used as the carrier gas at a constant flow rate of 1.0 mL/min. Fatty acid methyl esters were identified by comparing their retention times to those of a commercially available fatty acid methyl ester standard (FAMQ-005, Accu Standard, USA). The relative abundance of each fatty acid was expressed as a percentage of the total identified fatty acids.

Results

Growth rate analysis

Figure 1 illustrates the growth curves of *Dunaliella salina* cultivated at three salinity levels (35, 70, and 105 g/L) under varying nitrate and glucose concentrations over a 14-day culture period. At the lowest salinity of 35 g/L (Fig. 1A), the highest cell density (6.42×10^6 cells/mL) was observed in cultures supplemented with 3 g/L glucose, surpassing those with lower glucose concentrations. A notable increase in cell density was observed after 4 days of cultivation across all nutrient treatments at this salinity. At 70 g/L salinity (Fig. 1B), the maximum cell density achieved was 6.49×10^6 cells/mL in the culture containing 2 g/L glucose on day 8. Furthermore, at this salinity, no statistically significant differences ($P \geq 0.05$) were observed in the maximum cell densities achieved across the different nitrate concentrations when glucose was held constant. In contrast, growth was significantly inhibited at the highest salinity of 105 g/L (Figure 1C). The maximum cell density observed at this salinity level (1.54×10^6 cells/mL) was recorded in the treatment that included 1 mg/L nitrate. Notably, at a concentration of 105 g/L, the addition of glucose did not significantly alter cell density when compared to cultures with varying nitrate concentrations. Moreover, microalgal growth was minimal during the initial 4 days of cultivation at this high salinity, followed by a gradual increase in cell density.

Biomass production

The highest biomass yield (1449 mg/L) across all treatments was observed at a salinity of 35 g/L in the mixotrophic cultures supplemented with 3 g/L glucose. However, the average biomass production across all nutrient treatments was higher at a salinity of 70 g/L compared to both 35 g/L and 105 g/L (Fig. 2). At 70 g/L, the maximum biomass yield (1150 mg/L) was achieved with 1 g/L glucose supplementation. In contrast, biomass production was significantly re-

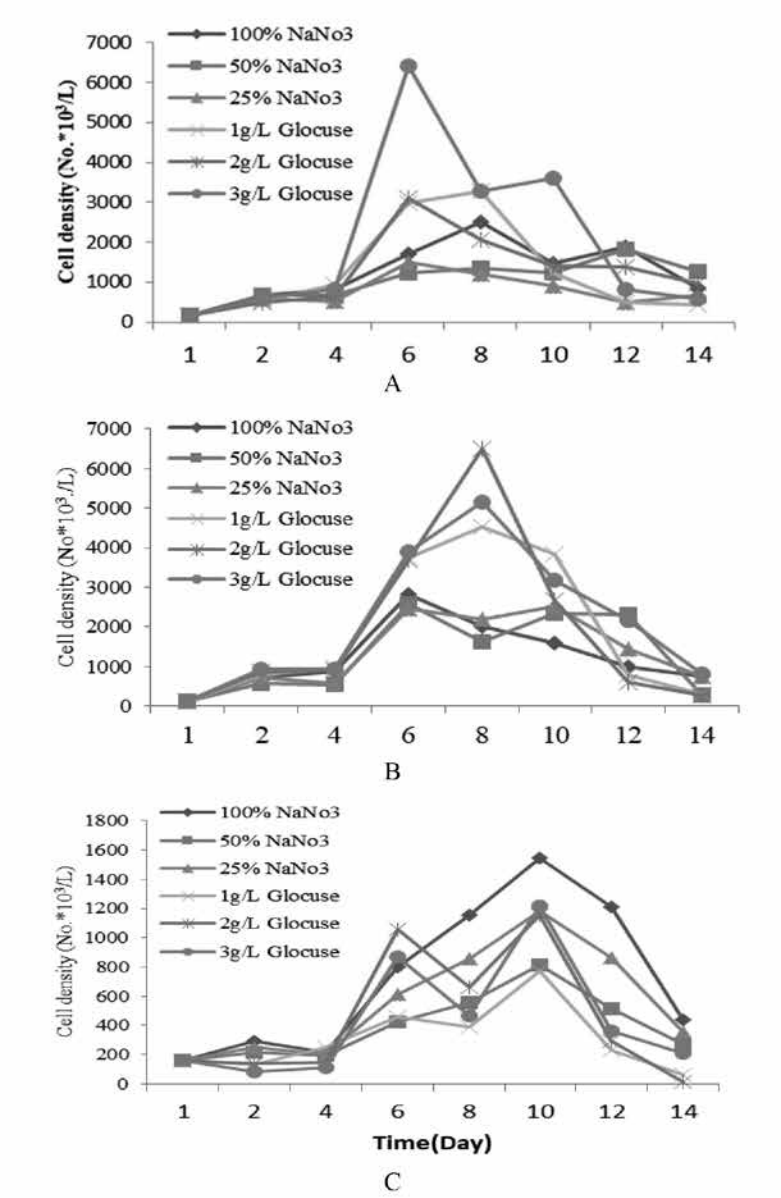

Fig. 1 Growth rate of *D. salina* at salinities; (A) 35 g/L, (B) 70 g/L, and (C) 105 g/l affected by nutrients

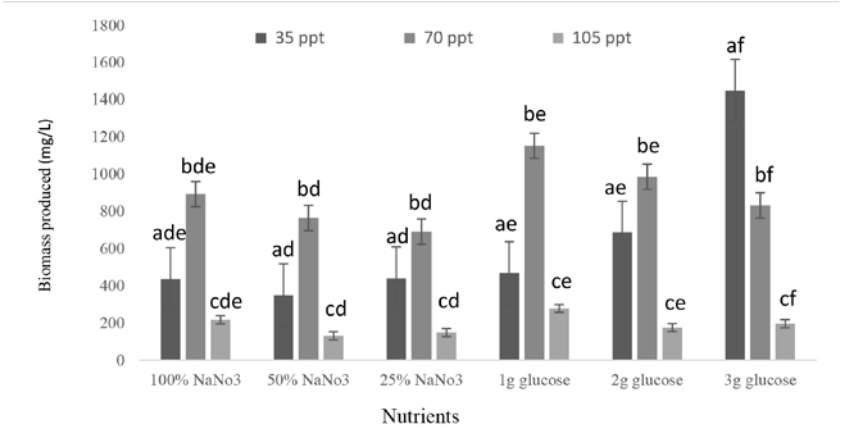

Fig. 2. Biomass yield (mg/L) of *D. salina* after 14 days of cultivation under varying salinity and nutrient concentrations; (Similar letters indicate no significant difference. (Duncan 0.05); a,b,c: represents the salinity effect; d,e,f: represents the effect of nutrients)

duced at the highest salinity of 105 g/L.

Two-way ANOVA revealed a statistically significant effect of both nutrient composition and salinity on the biomass yield of *D. salina*. Furthermore, a significant interaction between nutrient composition and salinity was observed, indicating that the effect of nutrients on biomass yield varied depending on the salinity level (Supp. Table 1)

The average biomass yield at 70 g/L (912.5 mg/L) was notably higher than at 35 g/L (638 mg/L) and 105 g/L (191 mg/L). While a slight decrease in biomass was observed with decreasing nitrate concentrations (Fig. 3), this trend was not statistically significant ($P \geq 0.05$). Conversely, in cultures supplemented with glucose, biomass yield increased from an average of 616 mg/L at 1 g/L glucose to 825 mg/L at 3 g/L glucose. This increase was statistically significant only at the 3 g/L glucose concentration ($P \leq 0.05$).

One-way ANOVA further elucidated the effect of glucose at each salinity level. At 35 g/L, biomass production varied significantly with different glucose concentrations ($P = 0$), with the highest average biomass (1449 mg/L) observed at 3 g/L glucose (supp. Table 1). At 70 g/L, the average biomass produced in glucose-supplemented treatments (1044 mg/L) did not differ significantly ($P \geq 0.05$) from the average biomass produced in treatments with varying nitrate concentrations (785 mg/L), suggesting that nutrient changes had a minimal impact on biomass yield at this salinity. At 105 g/L, a significant difference in biomass production was observed between nitrate and glucose treatments ($P = 0$). The highest biomass yield (278 mg/L) at this salinity was achieved with 1 g/L glucose. Furthermore, when comparing only the glucose-supplemented treatments at 105 g/L, significant differences in mean biomass production were found ($P \leq 0.05$), indicating that glucose concentration did influence biomass yield at this high salinity.

Total Lipid Content

The percentage of total lipid content based on the dry weight of *D. salina* biomass under different salinity and nutrient treatments is presented in Table 1. . The highest rate of total lipid (26.17%) was observed at a salinity of 70 g/L in cultures supplemented with 3 g/L glucose. Conversely, the lowest lipid percentage (16.77%) was recorded at a salinity of 35 g/L with 50% nitrate. Notably, the total lipid content was not determined for glucose-supplemented cultures at the highest salinity (105 g/L) due to the limited biomass yield obtained under these condi-

Table 1. Average lipid level (%) of *D. salina* in different culture conditions

Salinity	100% $NaNO_3$	50% $NaNO_3$	25% $NaNO_3$	1g glucose	2g glucose	3g glucose
35(ppt)[a]	2.56±19.03	2.53±16.73	3.2±20.4	2.7±18.07	1.4±17.63	3.8±22.67
70(ppt)[b]	3.19±22.67	1.9±26	3±19.93	3.4±24.4	2.9±24.27	1.6±26.17
105(ppt)[b]	0.6±24.43	2±25	2.09±22.07	-	-	-

a, b indicates a significant difference in different salinities (Duncan test)

tions.

The results of two-way ANOVA indicated a statistically significant effect of salinity on the percentage of lipid dry weight ($P< 0.001$). In contrast, the impact of different nutrient concentrations on the mean lipid percentages was not statistically significant ($P \geq 0.05$), suggesting that the tested nutrient variations did not significantly alter the overall percentage of lipid within the biomass. However, the total lipid produced per liter of culture medium exhibited considerable variation across different nutrient and salinity treatments, as represented in Figure 3.

Two-way ANOVA revealed a statistically significant effect of both salinity and nutrient levels on the total lipid produced per liter of culture medium ($P \leq 0.05$). Duncan's post-hoc analysis further indicated significant differences in the mean total lipid production across all three tested salinity levels. Conversely, no statistically significant differences were observed in the mean total lipid production in response to varying nitrate concentrations ($P \geq 0.05$). A statistically significant interaction between salinity and nutrient levels ($P < 0.001$) suggests that the effect of nutrients on total lipid production was dependent on the salinity. Furthermore, treatments supplemented with different glucose concentrations showed significant differences in mean total lipid production ($P \leq 0.05$), specifically between the 3 g/L glucose treatment and the 1 g/L and 2 g/L glucose treatments.

Fatty acid profile

Gas chromatography-flame ionization detector (GC-FID) analysis identified 17 distinct fatty acids in the *Dunaliella salina* biomass across the various treatment conditions. The relative abundance of these fatty acids varied significantly with both salinity and nutrient availability. Notably, the proportion of stearidonic acid (SDA, C18:4n-3) at a salinity of 35 g/L was substantially higher compared to the levels observed at 70 g/L and 105 g/L. A significant inverse correlation was found between salinity and SDA content, with increasing salinity leading to a marked decrease in SDA. Furthermore, at 35 g/L salinity, the addition of glucose to the culture

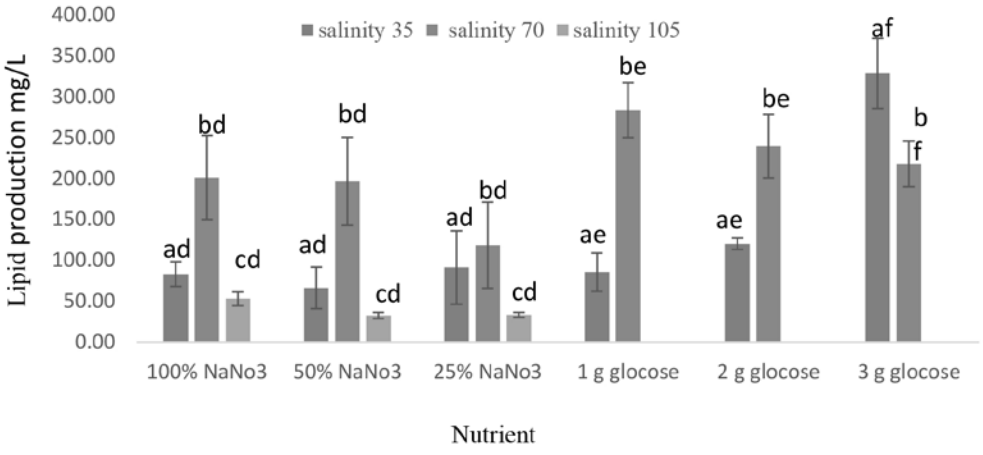

Fig. 3. Lipid production (mg/L) in *D. salina* culture; a, b, c represents the effect of salinity; d, e, f represents the effect of nutrients

medium resulted in a significant reduction in the relative abundance of SDA within the total fatty acid pool.

The specific composition and relative quantities of each identified fatty acid at salinities of 35 g/L, 70 g/L, and 105 g/L are presented in Tables 2, 3, and 4, respectively. These Tables provide a comprehensive overview of the impact that various nutrient regimes (nitrate and glucose concentrations) influenced the fatty acid profiles at each designated salinity level.

The Shapiro-Wilk test confirmed the normality of the saturated fatty acid (SFA) data across the 15 treatment groups and 45 replicates ($P \geq 0.05$). Duncan's post-hoc analysis revealed a statistically significant difference in the mean SFA content at a salinity of 70 g/L compared to both 35 g/L and 105 g/L ($P \leq 0.05$). Specifically, the proportion of SFAs was significantly higher at 70 g/L salinity than at the other two salinity levels, while no significant difference in SFA content was observed between the 35 g/L and 105 g/L salinity conditions. Regarding glucose supplementation, the treatments containing 1 g/L and 2 g/L glucose exhibited a higher percentage of SFAs. However, increasing the glucose concentration to 3 g/L resulted in a statistically significant reduction in the

Table 2. Fatty acid composition of *D. salina* cultivated at a salinity of 35 under various nutrient conditions

Fatty acids	100% $NaNO_3$	50% $NaNO_3$	25% $NaNO_3$	1g /L glucose	2g /L glucose	3g/L glucose
Dodecanoic (C12:0)	1.06±0.58	1.89±0.78	1.31±0.38	0.94±0.39	2.68±0.38	0.60±0.19
Tetradecanoic (C14:0)	0.94±0.53	1.33±0.84	1.43±0.81	8.61±0.66	15.79±1.0	4.2±4.94
pentadecanoic (C15)	1.18±0.39	1.75±0.70	1.53±0.79	0.85±0.59	3.54±4.16	0.43±0.18
Hexadecanoic (C16:0)	25.99±1.30	26.44±1.1	29.5±.54	26.6±5.79	34±2.60	26.33±3.45
Palmitoleic (C16:1)	4.44±0.50	6.27±0.30	4.42±2.76	2.96±0.88	10.3±1.34	6.92±0.27
Octadecanoic (C18:0)	17.49±2.30	23.23±1.0	20.85±1.5	23.07±6.5	12.49±0.7	9.76±0.98
Octadecenoic (C18:1)	5.25±2.90	1.13±1.01	2.09±0.22	2.82±1.53	4.29±0.51	9.70±8.42
Linoleic (C18:2)	12.04±0.75	7.81±0.02	5.23±0.05	8.51±0.76	5.03±0.25	10.58±3.46
Linolenic (C18:3)	0.31±0.54	0.22±0.20	0.22±0.20	.64±0.35	6.10±5.58	16.77±5.42
Stearidonic (C18:4)	22.99±1.42	23.77±0.01	22.78±0.4	16.00±1.2	0.24±0.24	0
Eicosanoic (C20:0)	0.10±0.09	0.05±0	0.04±0.04	0.79±1.38	1.20±1.24	0.24±0.35
Eicosenoic (C20:1)	3.23±1.05	3.23±0.34	2.43±0.35	1.92±1.72	1.10±0.11	1.20±0.29
Eicosatrienoic (C20:4)	1.54±0.18	0	1.01±0.90	1.67±1.42	0	9.10±6.62
Ecisoapentanoic(C20:5	0	0.78±0.69	0	0	0	0
Behenic (C22:0)	0.08±0.07	0	0.11±0.10	0	0	0.08±0.15
Docosanoic (C22:1)	3.19±0.35	1.99±0.77	6.85±0.46	4.53±1.12	3.12±0.51	3.11±0.81
Docosatetraenoic(C22:4)	0.15±0.13	0.11±0.10	0.15±0.13	0.07±0.11	0	1.72±0.99
SFA	46.76	55.47	54.73	73.52	69.80	41.56
USFA	53.24	44.53	45.27	39.11	30.20	58.44
MUFA	23.02	10.63	9.04	7.69	15.71	17.90
PUFA	40.21	33.90	36.23	31.42	14.49	40.54
SFA/USFA ratio	0.88	1.25	1.21	1.56	2.31	0.71
Degree of unsaturation (DU)	94.45	78.43	81.51	70.54	44.69	98.98

Table 3. *D. salina* fatty acid composition at salinity 70g/L and different nutrients

Fatty acids	100% NaNO3	50% NaNO3	25% NaNO3	1g /L glucose	2g /L glucose	3g /L glucose
Dodecanoic (C12:0)	1.48±0.39	1.2±0.14	40.1±0.30	0.71±0.32	0.94±0.32	1.77±0.40
Tetradecanoic (C14:0)	11.31±2.54	10.39±1.69	8.64±1.99	20.31±4.21	23.3±12.91	5.01±3.12
pentadecanoic (C15)	0.97±0.15	1.96±0.21	89.1±0.20	1.06±0.15	0.72±0.25	1.29±0.37
Hexadecanoic (C16:0)	44.25±0.43	27.54±0.05	27.74±5.18	24.38±0.76	13.34±3.37	36.16±5.28
Palmitoleic (C16:1)	1.04±0.62	2.83±1.08	3.06±0.62	2.08±0.98	3.25±2.73	6.17±1
Octadecanoic (C18:0)	22.63±6.95	19.62±1.21	18.74±5.38	20.86±6.94	18.36±4.17	12.25±11
Octadecenoic (C18:1)	5.87±4.01	3.41±0.26	6.67±4.22	2.75±0.11	2.57±1.90	2.17±2.07
Linoleic (C18:2)	11.12±2.48	12.14±0.45	13.83±4.23	4.89±0.03	6.19±2.78	9.23±3.47
Linolenic (C18:3)	11.16±9.72	17.62±1.06	13.04±4.53	6.68±4.81	6.07±3.91	20.13±0.45
Stearidonic (C18:4)	0	0.11±0.11	0	0	0	0.16±0.05
Eicosanoic (C20:0)	0	0.7±0.7	0.1±0.97	0	0.77±0.83	1.01±0.97
Eicosenoic (C20:1)	1.08±0.98	0.98±0.04	0.87±0.2	2.2±1.66	1.11±0.38	1.51±0.11
Eicosatrienoic (C20:4)	0.8±0.75	0	0	0	0.1±0.09	0
Ecisoapentanoic(C20:5	0	0	0	0	0	0
Behenic (C22:0)	0	0	0	0	0	0.05±0.05
Docosanoic (C22:1)	3.27±0.05	1.87±1.21	2.75±0.15	3.2±0.81	4.81±1.51	2.42±2.39
Docosatetraenoic(C22:4)	0.11±0.12	0	0.26±0.25	0	0.15±0.14	0.22±0.04
SFA	64.83	49.42	68.27	68.27	40.66	54.44
USFA	35.17	45.82	19.22	19.93	19.83	42.06
MUFA	8.71	39.31	28.44	12.09	43.26	9.91
PUFA	26.46	11.27	2.2	8.81	5.4	32.15
SFA/USFA ratio	1.84	1.08	3.55	3.47	2.05	1.29
(DU)	61.64	61.85	32.83	29.71	54.07	74.21

Table 4. *D. salina* fatty acid composition at salinity 105 g/L and different nutrients

Fatty acids	100% $NaNO_3$	50% $NaNO_3$	25% $NaNO_3$
Dodecanoic (C12:0)	1.92±0.54	1.29±0.53	1.04±0.85
Tetradecanoic (C14:0)	6.59±3.73	1.81±0.24	2.54±1.37
pentadecanoic (C15)	2±0.47	0.96±0.42	0.70±0.44
Hexadecanoic (C16:0)	44.83±8.90	35.31±0.66	33.66±5.21
Palmitoleic (C16:1)	4.34±1.32	4.16±0.05	3.91±0.44
Octadecanoic (C18:0)	10.56±1.28	9.38±1.80	7.61±3.73
Octadecenoic (C18:1)	3.75±3.86	4.22±0.34	14.31±8.47
Linoleic (C18:2)	11.82±9.28	28.36±2.45	25.13±3.13
Linolenic (C18:3)	6.70±6.39	6.44±7.33	6.08±5.06
Stearidonic (C18:4)	0	0.08±0.01	0.13±0.04
Eicosanoic (C20:0)	0	0.26±0.30	0.02±0.02
Eicosenoic (C20:1)	2.50±0.94	2.09±0.27	2.07±0.07
Eicosatrienoic (C20:4)	0.07±0.07	0	0.09±0.08
Ecisoapentanoic(C20:5	0.09±0.09	0	0.080.05د
Behenic (C22:0)	0	0.08±0.07	0.13±0.03
Docosanoic (C22:1)	4.69±2.22	5.31±0.89	2.26±1.8
Docosatetraenoic(C22:4)	0.15±0.15	0.26±0.01	0.24±0.07
SFA	65.98	49	45.65
USFA	31.51	51	54.35
MUFA	10.59	10.55	20.42
PUFA	33.93	40.45	33.93
SFA/USFA ratio	2.09	0.96	0.84
(DU)	78.45	91.45	88.28

percentage of SFAs ($P \leq 0.05$). The mean percentage of monounsaturated fatty acids (MUFA) exhibited statistically significant differences across the tested salinity levels ($P \leq 0.05$). However, no significant differences in the mean MUFA percentage were observed in response to varying nutrient levels ($P \geq 0.05$). In contrast to MUFA, the mean percentage of polyunsaturated fatty acids (PUFA) did not show statistically significant differences across all salinity levels and nutrient conditions ($P \geq 0.05$). While the average percentage of PUFA fatty acids was not significantly different across the three salinity levels ($P \geq 0.05$), changes in nutrient availability did appear to influence PUFA content (Figure 4). Generally, the proportion of unsaturated fatty acids (USFA), including both MUFA and PUFA, decreased in glucose-supplemented cultures at all tested salinities.

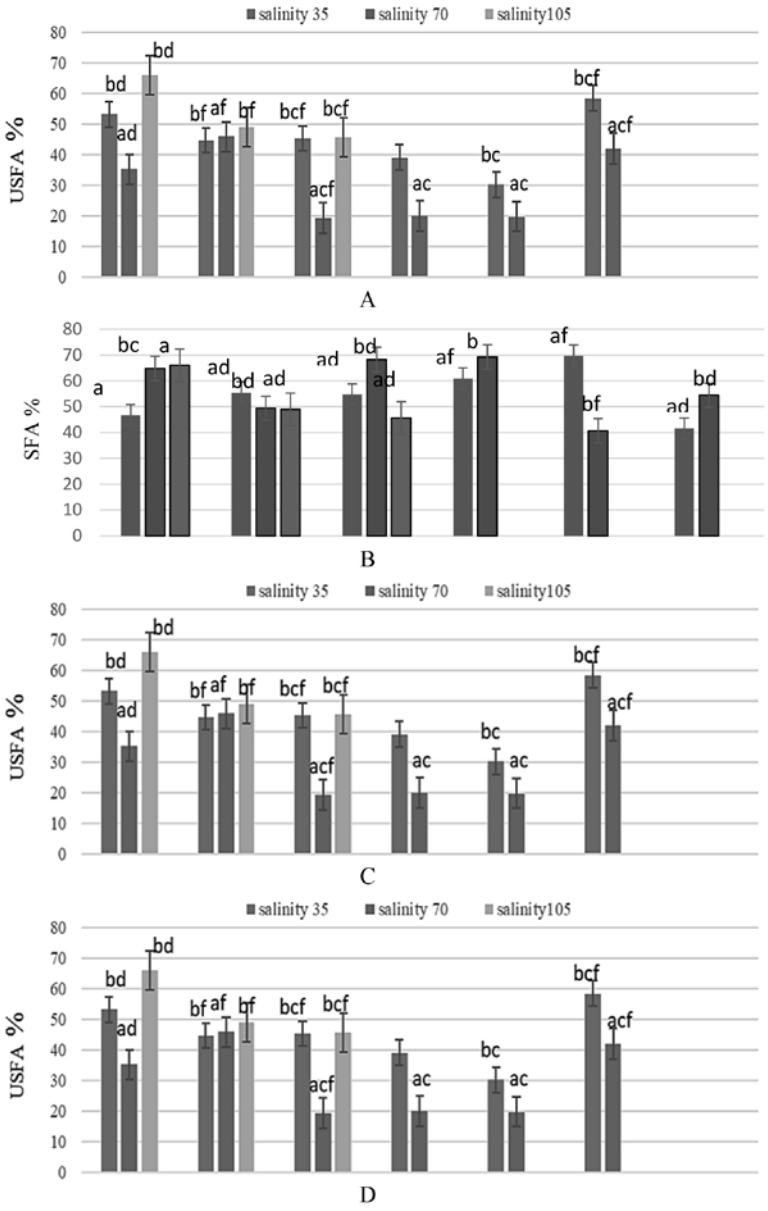

Fig. 4. USFA, SFA, MUFA, PUFA percentage of total fatty acids in *D. salina* under different treatment conditions, a, b. show the effect of salinity and c, d, f; Nutrient. Similar letters indicate no significant difference in mean percentage (Duncana)

Discussion

Effect of salinity on growth and biomass production

The response of microalgae to salinity fluctuations is species-specific, and within the genus *Dunaliella*, varying growth rates have been documented across a range of salinities (Borowitzka and Siva, 2007). Notably, *Dunaliella* species are generally recognized for their capacity to tolerate high salinity environments (Figure 1). In this study, the highest average biomass yield was observed at a salinity of 70 g/L ($P \leq 0.05$). However, the absolute maximum biomass (1449 mg/L) was achieved at 35 g/L in the treatment supplemented with 3 g/L glucose (Fig. 2). Numerous studies have investigated the influence of salinity on the growth rate and biomass accumulation of *Dunaliella* species (Chen et al., 2009; Takagi and Yoshida, 2006; Vo and Tran, 2014; Vo et al., 2017). Chen et al. (2009) reported an optimal salinity range of 1-2 M NaCl for the growth of *D. salina*, with significant growth inhibition at higher salinities. Conversely, Abu-Rezq et al. (2010) observed increased growth in *D. salina* with salinity elevation from 25 ppt to 45 ppt. Takagi and Yoshida (2006) found that *D. tertiolecta* experienced a sharp decline in biomass when salinity increased from 1 M to 2 M, but a less pronounced reduction occurred between 0.5 M and 1 M. Can et al. (2016) demonstrated that the optimal growth and biomass of *D. salina* were achieved at approximately 2 M NaCl, with a substantial decrease beyond this concentration. The high salinity tolerance of *Dunaliella* is a well-established characteristic (Borowitzka et al., 1977; Peeler et al., 1989). However, further increases beyond an optimum can significantly impede biomass accumulation (Takagi and Yoshida, 2006).

In the present study, the increase in mean biomass production of *D. salina* when salinity was elevated from 35 g/L to 70 g/L aligns with the salt-tolerant nature of this microalga (Abu-Rezq et al., 2010; Peeler et al., 1989). This suggests that for the specific *D. salina* isolate used, 70 g/L may represent a more favorable salinity range for overall biomass production compared to standard seawater salinity. However, the highest individual biomass yield at 35 g/L under mixotrophic conditions indicates a potential interaction between salinity and carbon source availability in maximizing biomass.

Effect of nitrate and glucose on growth andbbiomass production

Nitrogen is a crucial macronutrient for the growth of all photosynthetic organisms, including microalgae. In this study, nitrate was employed as the nitrogen source at three concentrations: 100%, 50%, and 25% of the standard f/2 medium. Our findings indicated that varying nitrate concentrations did not exert a statistically significant effect on the final biomass yield of *D. salina*. This suggests that within the tested range, nitrogen availability may not have been the primary limiting factor for growth. Jiménez and Niell (1991) proposed that at certain concentrations, increased nitrogen availability might not directly translate to accelerated cell division due to limitations in the algal nitrogen uptake rate. Similarly, our results imply that the *D. salina* strain under these conditions may not have exhibited a proportional

increase in nitrogen uptake with increasing nitrate concentrations.

Gao et al. (2013) reported that nitrogen deficiency in the culture medium led to reduced biomass in *Chlorella muelleri* and *D. salina,* showing a less pronounced response to nitrogen limitation compared to *C. muelleri*. Given the lack of significant difference in biomass production across the tested nitrate concentrations in our study, it can be inferred that a concentration as low as 25% nitrate may adequately meet the nitrogen requirements of this Iranian *D. salina* strain over a 14-day cultivation period. This observation has significant implications for cost-effective nutrient management in large-scale microalgal cultivation.

In addition to inorganic nutrients, the role of carbon sources is critical for microalgal growth. While autotrophic growth relies on CO_2 as the primary carbon source, numerous studies, including Liu (2014), have demonstrated that incorporating of glucose as an organic carbon source can significantly enhance biomass and lipid production through mixotrophic or heterotrophic metabolism. Microalgae utilize light energy for growth under phototrophic conditions, and suboptimal lighting can limit growth rates. Under heterotrophic conditions (absence of light), a suitable organic carbon source like glucose becomes essential for achieving high biomass yields (Isleten-Hosoglu et al., 2012; Perez-Garcia et al., 2011). Glucose has been frequently identified as a preferred organic carbon source for various microalgae (Isleten-Hosoglu et al., 2012; Perez-Garcia et al., 2011).

Mixotrophic cultivation, which enables microalgae to simultaneously utilize light and organic carbon sources, is often more efficient in terms of biomass production compared to purely phototrophic or heterotrophic modes (Heredia-Arroyo et al., 2011). The ATP generated during photosynthetic processes can also enhance glucose metabolism and be reused in CO_2 fixation, leading to synergistic effects (Heredia-Arroyo et al., 2011; Li et al., 2014; Xu et al., 2004). Wan et al. (2011) observed the highest biomass and growth rate in *D. salina* under mixotrophic conditions with 15 g/L glucose supplementation. Similarly, Chandra et al. (2014) reported increased biomass production in a mixed microalgal culture with glucose addition. Our study also demonstrated that *D. salina* at different salinities exhibited varying responses to glucose supplementation (Fig. 4). At 35 g/L salinity, increasing glucose concentrations led to a significant increase in biomass, with the highest biomass yield (almost a three-fold increase) observed at 3 g/L glucose. However, this positive effect of glucose was less pronounced or absent at higher salinities (70 g/L and 105 g/L). This differential response could be attributed to the physiological adaptations of *D. salina* to increasing salinity, potentially affecting its ability to efficiently utilize exogenous glucose under high salt stress.

Lipid production

Microalgal lipid content exhibits significant variability, ranging from 1% to 85% of their dry weight, and is known to be influenced by environmental conditions (Chisti, 2007). Our findings demonstrate that in vitro manipulation of salinity and nutrient availability significantly impacted both the total lipid

content and the lipid production efficiency of *D. salina* (Fig. 3). The total lipid content in *D. salina* biomass across all treatments in this study ranged from 16.7% to 26.1%, which aligns with a previously reported value of 21.26% for this strain (Attaran Fariman, 2014). These values are within the broader range reported for other microalgae, such as 30% for some species (Fried et al., 1982) and 8% for others (Vanitha et al., 2007).

Studies by Azachi et al. (2002) and Takagi and Yoshida (2006) on *D. salina* indicated that increasing salinity from 0.5 M to 3.5 M led to an increase in total lipid content. In the present study, while elevated salinity generally increased the lipid percentage in *D. salina*, lipid biodegradation efficiency appeared to decline at salinities exceeding 70 g/L. Notably, changes in nutrient concentrations (glucose and nitrogen) did not significantly affect the overall percentage of lipid in *D. salina* biomass. The highest lipid percentage (26.17%) was observed at 70 g/L salinity with 3 g/L glucose, while the lowest (16%) occurred at 35 g/L with 0.05 g/L nitrate. This observation contrasts with Gu et al. (2012), who reported that while increased salinity enhanced the lipid percentage in *Nannochloropsis oculata*, the optimal oil production efficiency was achieved at lower salinity due to higher biomass yields. Consistent with Gu et al.'s (2012) findings, our study also showed that despite higher lipid percentages at higher salinities, the best lipid production efficiency was obtained at 35 g/L with 3 g/L glucose due to the significantly higher biomass accumulation under these conditions.

Nitrogen deficiency is known to inhibit protein synthesis in microalgae (Quigg and Beardall, 2003), while photosynthetic activity may persist. Consequently, the energy generated from photosynthesis can be redirected towards the synthesis of carbohydrates or lipids, depending on the algal species (Gao et al., 2013). In our study, reducing the nitrate level in the culture medium did not significantly increase the total lipid percentage (Table 4). This is consistent

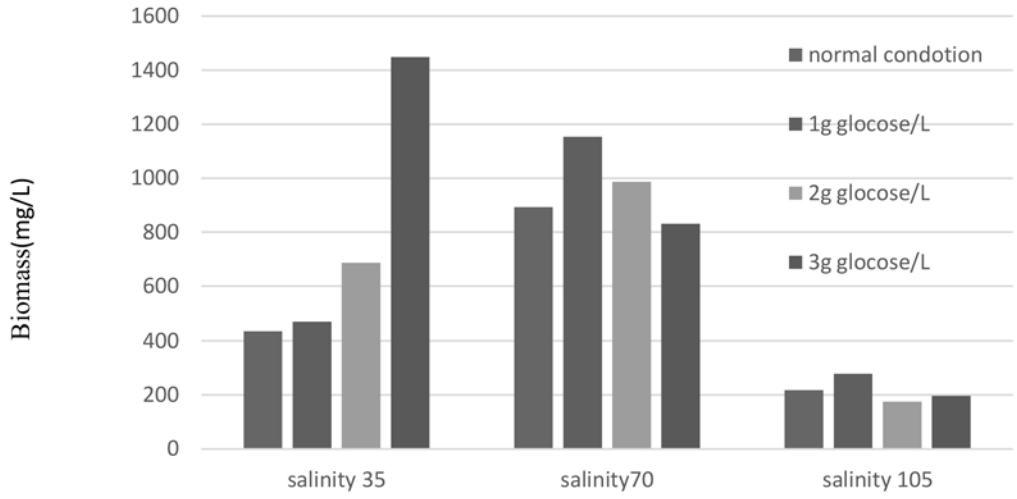

Fig. 5. The effect of different concentrations of glucose on the biomass of *D.salina*

with some research indicating no significant change in lipid percentage in certain *Dunaliella* species, such as *D. primolecta* (Uriarte et al., 1993) and *D. tertiolecta* (Lombardi and Wangersky, 1995), under nitrogen-limited conditions. Gordillo et al. (1998) found that *Dunaliella viridis* only increased lipid content during nitrogen deficiency when supplemented with 1% CO_2, suggesting the importance of the carbon to nitrogen ratio in lipid accumulation under nitrogen stress.

While some studies suggest that mixotrophic conditions can enhance both biomass and lipid content (Xu et al., 2004), others report increased lipid percentage under phototrophic conditions (Cheirsilp and Torpee, 2012), or no significant difference in intracellular lipid content across different trophic modes (Heredia-Arroyo et al., 2010). In our study, different glucose concentrations did not significantly affect the lipid percentage in *D. salina*, but the overall lipid production was enhanced due to increased biomass at higher glucose concentrations (especially at 35 g/L salinity). This aligns with reports of increased lipid production in *D. salina* under mixotrophic conditions (Wan et al., 2011). Tables 1-4 provide further comparative data on lipid production in different microalgae, including *D. salina*, under mixotrophic conditions.

Fatty acid composition and bBiofuel potential

The fatty acid composition of microalgae is influenced by various factors, including species, temperature, growth phase, light intensity, and nutrient availability. In this study, palmitic acid (C16:0) was the most abundant fatty acid in *D. salina* across all three tested salinities (Tables 2-4). Increasing salinity led to an increase in the proportion of palmitic acid, as well as the unsaturated fatty acids linoleic acid (C18:2) and linolenic acid (C18:3). This observation is partially consistent with Takagi and Yoshida (2006), who noted that most fatty acids in *Dunaliella* are composed of unsaturated C18 and saturated C16 fatty acids. However, Vanitha et al. (2007) reported C22 and C18:2 as the most abundant fatty acids in *D. salina* variants, highlighting potential strain-specific variations.

For biodiesel production, a higher content of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs), and a lower content of polyunsaturated fatty acids (PUFAs), is generally preferred for better fuel quality, particularly oxidative stability (Li et al., 2013). Deyab (2021) found increased SFAs in *D. salina* under nitrogen deficiency, while Chen et al. (2011) reported similar SFA and USFA profiles in *D. tertiolecta* with and without nitrate. Our study showed that changes in nitrate levels did influence the proportion of saturated and unsaturated fatty acids, with reduced nitrate leading to a decrease in the percentage of SFAs. However, very low nitrate concentrations (50% and 25%) resulted in similar SFA levels.

Overall, the *D. salina* strain isolated from Iran's southeast coast, with its total lipid content and significant proportions of SFAs (43.03% to 59.62%) and PUFAs (32.5% to 40.76%), demonstrates considerable potential for biofuel production. Notably, the observed changes in the proportions of saturated and unsaturated fatty acids in response

to varying salinity concentrations suggest that the lipid profile of this microalga can be tailored to suit different climatic conditions, with higher SFA content potentially advantageous in hot climates and higher USFA content in cold regions due to their lower freezing points.

Conclusion

This study demonstrated that salinity significantly affects the growth and lipid accumulation of an Iranian *D. salina* isolate, with optimal average biomass at 70 g/L but maximum yield under glucose supplementation at 35 g/L. While nitrate levels were not limiting, glucose enhanced biomass at lower salinity, highlighting the benefit of mixotrophic cultivation. Higher salinity generally increased lipid content, but maximum lipid production was linked to higher biomass at 35 g/L with glucose. Palmitic acid was the dominant fatty acid, and salinity influenced the proportions of saturated and unsaturated fatty acids, suggesting potential for biofuel production with a modifiable lipid profile. This research highlights the importance of optimizing salinity levels and exploring mixotrophic approaches to maximize biomass and lipid production in this *D. salina* isolate for biotechnological applications, particularly in biofuel production. Future work should focus on scaling up and assessing economic feasibility.

Acknowledgements

This research is from an MSc thesis at Chabahar Maritime University. The authors gratefully acknowledge the support and resources provided by the officials of the Chabahar Free Zone Quality Control Laboratory and the Chabahar Maritime and Marine Science Laboratory staff.

References

Abu-Rezq, T.S., Al-Hooti, S. and Jacob, D.A., 2010. Optimum culture conditions required for the locally isolated *Dunaliella salina*. *Journal of Algal Biomass Utilization*, 1(2), pp.12-19.

Attaran-Fariman, G., 2014. *Identification of native microalgae of the Oman Sea and their evaluation as live food in aquaculture*. Final report of Iranian Fisheries Institute, 166pp.

Azachi, M., Sadka, A., Fisher, M., Goldshlag, P., Gokhman, I. and Zamir, A., 2002. Salt induction of fatty acid elongase and membrane lipid modifications in the extreme halotolerant alga *Dunaliella salina*. *Plant Physiology*, 129(3), pp.1320-1329. DOI: https://doi.org/10.1104/pp.001909.

Bhola, V., Swalaha, F., Ranjith Kumar, R., Singh, M. and Bux, F., 2014. Overview of the potential of microalgae for CO_2 sequestration. *International Journal of Environmental Science and Technology*, 11, pp.2103-2118.

Bligh, E.G. and Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8), pp.911-917. DOI: https://doi.org/10.1139/o59-099.

Borowitzka LJ, Kessly DS, Brown AD. 1977. The salt relation of *Dunaliella*. Further observation on glycerol production and its regulation. *Arch. Microbiol.,* 13: 131–38. DOI: https://doi.org/10.1007/BF00428592.

Borowitzka, M.A. and Siva, C.J., 2007. The taxonomy of the genus *Dunaliella* (Chlorophyta, Dunaliellales) with emphasis on the marine and halophilic species. *Journal of Applied Phycology*, 19(5), pp.567-590. DOI: https://doi.org/10.1007/BF00428592.

Bougaran, G., Rouxel, C., Dubois, N., Kaas, R., Grouas, S. and Cadoret, J.P., 2012. Enhancement of neutral lipid productivity in the microalga *Isochrysis affinis Galbana* (T-Iso) by a mutation-selection procedure. *Biotechnology and Bioengineering*, 109(11), pp.2737-2745. DOI: https://doi.org/10.1002/bit.24560.

Can, S.S., Cirik, S., Koru, E., Turan, G., Tekoğul, H. and Subakan, T., 2016. Effects of salinity, light and nitrogen concentration on growth and lipid accumulation of the green algae *Dunaliella salina*. *Fresenius Environmental Bulletin*, 25(5), pp.1437-1447.

Chandra, R., Rohit, M.V., Swamy, Y.V. and Mohan, S.V., 2014. Regulatory function of organic carbon supplementation on biodiesel production during growth and nutrient stress phases of mixotrophic microalgae cultivation. *Bioresource Technology*, 165, pp.279-287. DOI: https://doi.org/10.1016/j.biortech.2014.02.102.

Cheirsilp, B. and Torpee, S., 2012. Enhanced growth and lipid production of microalgae under mixotrophic culture condition: effect of light intensity, glucose concentration and fed-batch cultivation. *Bioresource Technology*, 110, pp.510-516. DOI: https://doi.org/10.1016/j.biortech.2012.01.125.

Chen, H., Jiang, J.G. and Wu, G.H., 2009. Effects of salinity changes on the growth of *Dunaliella salina* and its isozyme activities of glycerol-3-phosphate dehydrogenase. *Journal of Agricultural and Food Chemistry*, 57(14), pp.6178-6182 DOI: https://doi.org/10.1021/jf900447r.

Chen, M., Tang, H., Ma, H., Holland, T.C., Ng, K.S. and Salley, S.O., 2011. Effect of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*. *Bioresource Technology*, 102(7), pp.1649-1655. DOI: https://doi.org/10.1016/j.biortech.2010.09.062

Chisti, Y., 2007. Biodiesel from microalgae. *Biotechnology Advances*, 25(3), pp.294-306. DOI: https://doi.org/10.1016/j.biotechadv.2007.02.001.

Chu, W.L., 2012. Biotechnological applications of microalgae. *International e-Journal of Science, Medicine and Education*, 6(1), pp. S24-S37.

Deora, P.S., Verma, Y., Muhal, R.A., Goswami, C. and Singh, T., 2022. Biofuels: An alternative to conventional fuel and energy source. *Materials Today: Proceedings*, 48, pp.1178-1184. DOI: https://doi.org/10.1016/j.matpr.2021.08.227.

Deyab, M.A., El-Sadany, A., Ghazal, M.A. and El-Adl, M., 2021. Nitrogen deficiency maximizes the production and accumulation of β-carotene via induction of different macromolecule derivatives in *Dunaliella salina* (Dunal) Teodoresco. *Egyptian Journal of Botany*, 61(2), pp.453-466. doi: 10.21608/ejbo.2021.40359.1542.

Fried, A., Tietz, A., Ben-Amotz, A. and Eichenberger, W., 1982. Lipid composition of the halotolerant alga, *Dunaliella*

salina. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism*, 713(2), pp.419-426. DOI: https://doi.org/10.1016/0005-2760(82)90261-2.

Gao, Y., Yang, M. and Wang, C., 2013. Nutrient deprivation enhances lipid content in marine microalgae. *Bioresource Technology*, 147, pp.484-491. DOI: https://doi.org/10.1016/j.biortech.2013.08.066

Gordillo, F.J., Goutx, M., Figueroa, F.L. and Niell, F.X., 1998. Effects of light intensity, CO_2 and nitrogen supply on lipid class composition of *Dunaliella viridis*. *Journal of Applied Phycology*, 10(2), pp.135-144. DOI: https://doi.org/10.1023/A:1008067022973.

Gu, N., Lin, Q., Li, G., Tan, Y., Huang, L. and Lin, J., 2012. Effect of salinity on growth, biochemical composition, and lipid productivity of *Nannochloropsis oculata* CS 179. *Engineering in Life Sciences*, 12(6), pp.631-637. DOI: https://doi.org/10.1002/elsc.201100204

Guillard, R.R. and Ryther, J.H., 1962. Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Canadian Journal of Microbiology*, 8(2), pp.229-239. DOI: https://doi.org/10.1139/m62-029

Heredia-Arroyo, T., Wei, W. and Hu, B., 2010. Oil accumulation via heterotrophic/mixotrophic *Chlorella protothecoides*. *Applied Biochemistry and Biotechnology*, 162(7), pp.1978-1995. DOI: https://doi.org/10.1007/s12010-010-8974-4

Heredia-Arroyo, T., Wei, W., Ruan, R. and Hu, B., 2011. Mixotrophic cultivation of *Chlorella vulgaris* and its potential application for the oil accumulation from non-sugar materials. *Biomass and Bioenergy*, 35(6), pp.2245-2253. DOI: https://doi.org/10.1016/j.biombioe.2011.02.036

Hopkins, T.C., Graham, E.J.S. and Schuler, A.J., 2019. Biomass and lipid productivity of *Dunaliella tertiolecta* in a produced water-based medium over a range of salinities. *Journal of Applied Phycology*, 31(5), pp.3349-3358. DOI: https://doi.org/10.1007/s10811-019-01836-3 .

Iglina, T., Iglin, P. and Pashchenko, D., 2022. Industrial CO_2 capture by algae: a review and recent advances. *Sustainability*, 14(7), p.3801. DOI: https://doi.org/10.3390/su14073801.

IPCC. (2022). *Climate Change 2022: Mitigation of Climate Change. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* [P.R. Shukla, J. Skea, R. Slade, et al. (eds.)]. Cambridge University Press. DOI: https://doi.org/10.1017/9781009157926 .

International Energy Agency (IEA). 2024. *CO_2 Emissions in 2023*, IEA, Paris. DOI: https://www.iea.org/reports/co2-emissions-in-2023.

International Energy Agency (IEA). 2025. *Global Energy Review 2025*, IEA, Paris. DOI: https://www.iea.org/reports/global-energy-review-2025.

Isleten-Hosoglu, M., Gultepe, I. and Elibol, M., 2012. Optimization of carbon and nitrogen sources for biomass and lipid production by *Chlorella saccharophila* under heterotrophic conditions and development of Nile red fluorescence-based method for quantification of its neutral lipid content. *Biochemical Engineering*

Journal, 61, pp.11-19. DOI: https://doi.org/10.1016/j.bej.2011.12.001

Jiménez, C. and Niell, F.X., 1991. Growth of *Dunaliella viridis* Teodoresco: effect of salinity, temperature and nitrogen concentration. *Journal of Applied Phycology*, 3(4), pp.319-327. DOI: https://doi.org/10.1007/BF02392885.

Li, T., Wan, L., Li, A. and Zhang, C., 2013. Responses in growth, lipid accumulation, and fatty acid composition of four oleaginous microalgae to different nitrogen sources and concentrations. *Chinese Journal of Oceanology and Limnology*, 31(6), pp.1306-1314. DOI: https://doi.org/10.1007/s00343-013-2316-7.

Li, T., Zheng, Y., Yu, L. and Chen, S., 2014. Mixotrophic cultivation of a *Chlorella sorokiniana* strain for enhanced biomass and lipid production. *Biomass and Bioenergy*, 66, pp.204-213. DOI: https://doi.org/10.1016/j.biombioe.2014.04.010.

Liu, J., 2014. Optimization of biomass and lipid production by adjusting the interspecific competition mode of *Dunaliella salina* and *Nannochloropsis gaditana* in mixed culture. *Journal of Applied Phycology*, 26(1), pp.163-171. DOI: https://doi.org/10.1007/s10811-013-0099-z.

Lombardi, A. and Wangersky, P.J., 1995. Particulate lipid class composition of three marine phytoplankters *Chaetoceros gracilis*, *Isochrysis galbana* (Tahiti), and *Dunaliella tertiolecta* grown in batch culture. *Hydrobiologia*, 306(1), pp.1-6. DOI: https://doi.org/10.1007/BF00007853.

Mairet, F., Bernard, O., Masci, P., Lacour, T. and Sciandra, A., 2011. Modelling neutral lipid production by the microalga *Isochrysis aff. galbana* under nitrogen limitation. *Bioresource Technology*, 102(1), pp.142-149. DOI: https://doi.org/10.1016/j.biortech.2010.06.138.

Mata, T.M., Almeidab, R. and Caetanoa, N.S., 2013. Effect of the culture nutrients on the biomass and lipid productivities of microalgae *Dunaliella tertiolecta*. *Chem Eng*, 32, p.973.

Moheimani, N.R., Borowitzka, M.A., Isdepsky, A. and Fon Sing, S., 2013. Standard methods for measuring growth of algae and their composition. In: Borowitzka, M.A. and Moheimani, N.R. (eds.) *Algae for Biofuels and Energy*. Dordrecht: Springer, pp.265-284. DOI: https://doi.org/10.1007/978-94-007-5479-9_16.

Morales, M., Aflalo, C. and Bernard, O., 2021. Microalgal lipids: A review of lipids potential and quantification for 95 phytoplankton species. *Biomass and Bioenergy*, 150, p.106108. DOI: https://doi.org/10.1016/j.biombioe.2021.106108.

Nigam, S., Rai, M.P. and Sharma, R., 2011. Effect of nitrogen on growth and lipid content of *Chlorella pyrenoidosa*. *American Journal of Biochemistry and Biotechnology*, 7(3), pp.124-129. http://thescipub.com/abstract/10.3844/ajbbsp.2011.124.129.

Pacheco, M.M., Hoeltz, M., de Souza, D., Benitez, L.B., Schneider, R.C. and Müller, M.V., 2017. Current approaches in producing oil and biodiesel from microalgal biomass. In: *Waste Biomass Management–A Holistic Approach*. Springer, pp.289-310. DOI: https://doi.org/10.1007/978-3-319-49595-8_13.

Peeler, T.C., Stephenson, M.B., Einspahr,

K.J. and Thompson, G.A., 1989. Lipid characterization of an enriched plasma membrane fraction of *Dunaliella salina* grown in media of varying salinity. *Plant Physiology*, 89(3), pp.970-976. DOI: https://doi.org/10.1104/pp.89.3.970.

Perez-Garcia, O., Bashan, Y. and Esther Puente, M., 2011. Organic carbon supplementation of sterilized municipal wastewater is essential for heterotrophic growth and removing ammonium by the microalga *Chlorella Vulgaris* 1. *Journal of Phycology*, 47(1), pp.190-199. DOI: https://doi.org/10.1111/j.1529-8817.2010.00934.x.

Quigg, A. and Beardall, J., 2003. Protein turnover in relation to maintenance metabolism at low photon flux in two marine microalgae. *Plant, Cell and Environment*, 26(5), pp.693-703. DOI: https://doi.org/10.1046/j.1365-3040.2003.01004.x.

Rios, L., Da Silva, C., Tasic, M., Wolf-Maciel, M. and Maciel Filho, R., 2016. Cultivation of three microalgae strains under mixotrophic conditions for biodiesel production. *Chemical Engineering Transactions*, 50, pp.409-414. http://dx.doi.org/10.3303/CET1650069.

Scragg, A., Morrison, J. and Shales, S., 2003. The use of a fuel containing *Chlorella vulgaris* in a diesel engine. *Enzyme and Microbial Technology*, 33(7), pp.884-889. DOI: https://doi.org/10.1016/j.enzmictec.2003.01.001.

Shokravi, Z., Shokravi, H., Chyuan, O.H., Lau, W.J., Koloor, S.S.R., Petrů, M. and Ismail, A.F., 2020. Improving 'lipid productivity'in microalgae by bilateral enhancement of biomass and lipid contents: A review. *Sustainability*, 12(21), p.9083. DOI: https://doi.org/10.3390/su12219083.

Takagi, M. and 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(3), pp.223-226. DOI: https://doi.org/10.1263/jbb.101.223.

Talebi, A.F., Mohtashami, S.K., Tabatabaei, M., Tohidfar, M., Bagheri, A. and Ghasemi, Y., 2013. Fatty acids profiling: a selective criterion for screening microalgae strains for biodiesel production. *Algal Research*, 2(3), pp.258-267. DOI: https://doi.org/10.1016/j.algal.2013.04.003.

Thompson Jr, G.A., 1996. Lipids and membrane function in green algae. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism*, 1302(1), pp.17-45. DOI: https://doi.org/10.1016/0005-2760(96)00045-8.

Truc, M.V., Phuc, N.H., Trung, V.P., Hieu, H.V. and Son, T.L., 2017. Accumulation of lipid in *Dunaliella salina* under nutrient starvation condition. *American Journal of Food and Nutrition*, 5(2), pp.58-61. DOI:10.12691/ajfn-5-2-2.

Uriarte, I., Farias, A., Hawkins, A.J.S. and Bayne, B.L., 1993. Cell characteristics and biochemical composition of *Dunaliella primolecta* butcher conditioned at different concentrations of dissolved nitrogen. *Journal of Applied Phycology*, 5(4), pp.447-453. DOI: https://doi.org/10.1007/BF02182737.

Vanitha, A., Narayan, M., Murthy, K. and Ravishankar, G.A., 2007. Comparative

study of lipid composition of two halotolerant algae, *Dunaliella salina* and *Dunaliella salina*. *International Journal of Food Sciences and Nutrition*, 58(5), pp.373-382. DOI: https://doi.org/10.1080/09637480701252252.

Vo, T.Q. and Tran, D.T., 2014. Effects of salinity and light on the growth of *Dunaliella* isolates. *Journal of Applied and Environmental Microbiology*, 2(6), pp.208-211.

Vo, T.Q., Tran, S.M., Nguyen, P.T. and Mai, T.T., 2017. Growth, carotenoid production, antioxidant capacity, and lipid accumulation of *Haematococcus* sp. under different light intensities. *American Journal of Plant Biology*, 2(4), pp.142-147.

Wan, M., Liu, P., Xia, J., Rosenberg, J.N., Oyler, G.A. and Betenbaugh, M.J., 2011. The effect of mixotrophy on microalgal growth, lipid content, and expression levels of three pathway genes in *Chlorella sorokiniana*. *Applied Microbiology and Biotechnology*, 91(3), pp.835-844. DOI: https://doi.org/10.1007/s00253-011-3399-8.

Xu, F., Hu, H.H., Cong, W., Cai, Z.L. and Ouyang, F., 2004. Growth characteristics and eicosapentaenoic acid production by *Nannochloropsis* sp. in mixotrophic conditions. *Biotechnology Letters*, 26(1), pp.51-53. DOI: https://doi.org/10.1023/B:BILE.0000009460.81267.cc.