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Biodiesel Production, Characterization and Biochemical Variability by Microalga *Nannochloropsis oculata* under Stressed Culture Conditions

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ABSTRACT

Microalgae are recognized as a promising source for biofuel production, which is known as a renewable source of energy. This study was conducted to optimize the biodiesel production quantitatively and qualitatively by the microalga, *Nannochloropsis oculata* upon culturing on two different culture media (F/2 and Boussiba media) as a comparative study through stress in the main culture compositions by 1) Treating with different salinity concentrations (20, 40 and 60psu), 2) Depleting from phosphorus (P) and nitrogen (N) sources for the two media. After different treatments, the growth rate, protein, carbohydrate, lipid, and fatty acids contents were determined. The algal analysis cleared that increasing salinity to 60psu in F/2 medium and 40psu on the Boussiba medium led to increasing the number of *N. oculata* cells with the maximum protein and carbohydrate contents at late exponential phase. At lower salinity, there were no significant differences in total protein content while carbohydrate contents showed slight variations, and its minimum values were obtained at (N) starved medium. Total lipid and total fatty acids content were higher in *N. oculata* cultured on F/2 medium than on Boussiba medium at different treatments and achieved its maximum when cultured on N-starved > P-depleted > Salt-stressed 60psu, respectively. The highest physical properties of the produced biodiesel represented in the degree of unsaturation (SD), iodine values (IV), Cetane number (CN), and oxidation stability were recorded in *N. oculata* cultured on higher salinities and N-depleted conditions in the two media. Using higher salinities up to 40psu (available in the natural seawater), N-depletion or P-depletion in F/2 medium for achieving highly economical results in the production of *N. oculata* microalga. Biodiesel produced from *N. oculata* oil can be considered as a potential candidate for commercial sources.

INTRODUCTION

Biofuels are considered promising alternative energy source that could contribute to fuel supplies in the form of biodiesel **(Chisti, 2007)**. Biofuels, generated from biomass, are promising sources of fossil-derived fuels due to their distinct advantages such as carbon

neutrality, reduced emissions of gaseous pollutants, continuous availability of biomass feedstocks, and their safety of production by farming **(Li *et al*., 2011; Saifuddin and Boyce, 2016)**. Having such these sources can be an interesting of reducing greenhouse gases **(Mata *et al*., 2010)**. Some studies have been conducted to test the capability of different microorganisms as a source of biofuel to replace the conventional fuel **(Prince and Kheshgi, 2005)**. Microalgae particularly are rich in oil with rapid growth rates which make them an effective candidate for biofuel production **(Chisti, 2007)**.

Although there are numerous advantages to microalgal biodiesel production, the technology and our knowledge are limited. The energy efficiency of microalgae will also reduce the amount of fertilizer and nutrients needed, thus reducing pollution **(Schenk *et al*., 2008)**. Under nutrient stress, cells favor to accumulate lipid particularly triacyl glycerol (TAG) as the dominant ingredient **(Sibi *et al*., 2016)**, and depletion of N and P direct fixing carbon to lipids or carbohydrates synthesis. Therefore, N and P starvation is important for lipids metabolism **(Kamalanathan *et al*., 2016)**.

The utilization of saline water in non-arable areas will open up economic opportunities to arid areas. In general, the production of lipids is inversely related to the algae growth because algae tend to produce more oil when they are under stressful conditions. Salinity showed considerable effect on protein produced by microalgae **(Moisander *et al*., 2002)**. Several factors influences the lipid content of microalgae, such as phosphate limitation **(Reitan *et al*., 1994)**, salinity **(Rao *et al*., 2007; El-Sheekh et al. 2013, 2017, 2019)**, and iron content of the medium also affect the algal growth **(Liu *et al*., 2008) Johansen *et al.* (1990)** reported that all except one strain of *Chaetoceros muelleri* studied exhibited increases in lipid content under nutrient stress and that in most cases nitrogen stress, in particular, led to a higher lipid content.

Kilham *et al.* (1997) found that P and N-limitation reduced protein composition and increased lipid composition in *Ankistrodesmus facatus*. **Reitan *et al*. (1994**) cultured the microalgae *Phaeodactylum tricornutum, Chaetoceros* sp., *Isochrysis galbana, Nannochloropsis atomus, Tetraselmis* sp. and *Gymnodinum* sp., they reported the increase of lipid contents at different nitrogen-starved media. When sufficient nutrients are available, proteins are synthesized; however, when nutrients are limited, cell division is suppressed and a greater amount of carbon is available for lipid storage **(Sukenik and Wahnon, 1991)**. The current study aimed to optimize the culture conditions mainly salinity and essential nutrients starvation (N and P) for biodiesel production by *Nannochloropsis oculata* through a comparative study for two commonly used culture media (F/2 and Boussiba media) with commercial ingredients constitution. Since the two media consituents are quantitatively different, Boussiba medium contain extremely higher and costly than F2 medium.

MATERIALS AND METHODS

Organism and growth conditions

The culture of marine microalga *Nannochloropsis oculata* was kindly provided from the culture collection maintained in the algal unit presented in the marine hatchery, National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt. The microalga was cultured on two different culture media named F/2 and Boussiba medium **(Guillard, 1975; Boussiba *et al*., 1987)** at different salinities (20, 40 and 60 psu) and without nitrogen and phosphorus. The cultures were grown in 1000 ml Erlenmeyer flasks

with 500 mL medium. The cultures were performed in a temperature-controlled incubator at 25 °C providing 24 h fluorescent illumination (40-watt, white tube light). To avoid sticking, the cultures were shaken 2-3 times every day. All the glassware and media were sterilized prior to inoculation and culture.

Growth measurements and biochemical analysis of *N. oculata*

A direct microscopic count of *N. oculata* was performed by hemocytometer, and the mean number of cells/ml was obtained. Cell numbers were applied triplicate every 2 days.

Total protein content was determined by the Folin-phenol method **(Lowry *et al.*, 1951)**. Total carbohydrate content was determined according to the phenol-sulfuric acid method using glucose as standards **(Dubois *et al.*, 1956)**. Total lipid was analyzed gravimetrically after extraction with chloroform-methanol (2:1) using the modified Folch method **(Bligh and Dyer, 1959)**. Fatty acid methyl esters (FAMEs) were prepared **(Vogel, 1975)**, and the analysis was performed in a gas liquid chromatograph (Pye Unicam Series 304 Gas Chromatograph) equipped with dual flame ionization detector and dual channel recorder. FAMEs were identified by comparing the retention times of experimental samples to those of known standards.

Evaluation of biodiesel properties

The Average Degree of Unsaturation (ADU%), Iodine Value (IV, g I_2.100 g^{-1} oil), Cetane Number (CN), Kinematic viscosity (υi, mm^2 s^{-1}), density (ρ), Higher Heating Value (HHV), Saponification Value (SV, mg KOH g^{-1}), Long Chain Saturation Factor (LCSF, wt%) and Cold Filter Plugging Point (CFPP, °C) and kinematic viscosity (υi, mm 2 s −1) were calculated as described in **Ashour *et al.* (2019)**; **Ramirez-Verduzco *et al.* (2012) and Mitra *et al.* (2016)** in the *N. oculata* cultured on F/2 and Boussiba media treated with different concentrations of salts, phosphorous (P) and nitrogen (N) depletion as shown in equations from 1 to 6:

$$DU = \Sigma [MUFA + (2 \times PUFA)] \quad \textbf{(Pan } \textit{et al.}\textbf{, 2017)} \tag{1}$$

Where, MUFA: monounsaturated and PUFA: polyunsaturated fatty acids percentages.

$$SV=\Sigma [(560\times N\%) / M] \quad \textbf{(Zhu } \textit{et al.}\textbf{, 2017)} \tag{2}$$

$$IV=\Sigma [(254\times N\%\times D) / M] \quad \textbf{(Rawat } \textit{et al.}\textbf{, 2011)} \tag{3}$$

$$CN = 46.3 + (5458 / SV) – (0.225 \times IV) \quad \textbf{(Nguyen } \textit{et al.}\textbf{, 2016)} \tag{4}$$

where, N%: the percentage of each fatty acid; M: the molecular mass of the fatty acid; and D: the number of carbon-carbon double bonds.

$$LCSF = (0.1\times C16{:}0) + (0.5\times C18{:}0) + (1\times C20{:}0) + (1.5\times C22{:}0) + (2 \times C24{:}0)$$ **(Abomohra *et al.*, 2018)**. (5)

$$CFPP = (3.1417\times LCSF) − 16.477 \quad \textbf{(Shao } \textit{et al.}\textbf{, 2018)} \tag{6}$$

Where, C16:0, C18:0, C20:0, C22:0 and C24:0 represent the weight percentage of the corresponding fatty acids.

Statistical analysis

Each measurement was done in triplicates and the mean and SD of the experimental results was calculated using MS-Excel. Two-way ANOVA was used to test the effects of salinities, N and P depletion on the growth, protein, carbohydrate, lipid and fatty acids. When differences were found in the two-way ANOVA, Tukey's multiple comparison test (HSD) of one-way ANOVA was used to compare the mean differences **(Zar, 1984)** by the SPSS statistical package (Version 12.0, SPSS, Chicago, IL). Differences were considered significant at $p \leq 0.05$.

RESULTS

1. Effect of salinity on *Nannachloropsis oculata* growth post 8 days of culture

The growth represented as cell number of *N. oculata* on F/2 media was greatest (doubling) than Boussiba media (Figure 1 and 2, respectively) at different salinities, N and P starved. The results showed that *N. oculata* cultured on F/2 medium gives the maximum cell number at salinity 60psu (Figure 1), ANOVA test confirms that cell numbers of *N. oculata* cultured on F/2 medium was significantly different at the various treatments ($p \leq 0.05$).

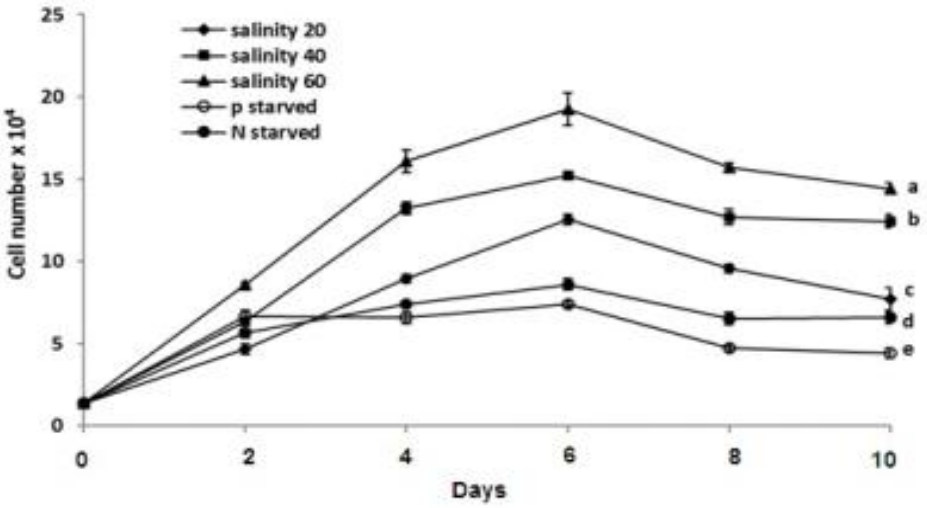

Fig. 1. Impact of different salinity concentrations, P and N starvation on the cell number of *N. oculata* cultured on F/2 medium.

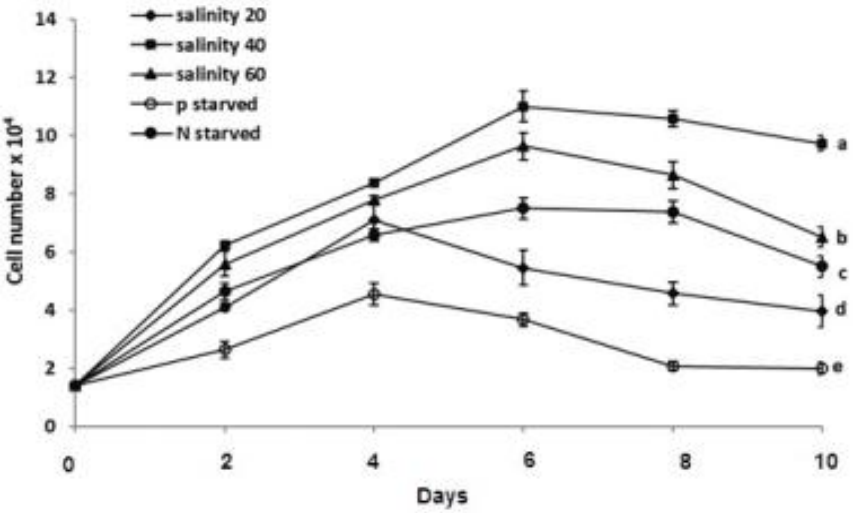

Fig. 2. Impact of different salinity concentrations, P and N starvation on the cell number of *N. oculata* cultured on Boussiba medium during the culture period.

2. Effect of salinity on total protein and carbohydrate contents of *Nannachloropsis oculata* post 8 days of culture

As shown in Figure 3, there were no significant differences in total protein content in *N. oculata* grown on the two-culture media (F/2 and Boussiba) at different salinity concentrations. Carbohydrate content showed slight variations when *N. oculata* cultured on F/2 and Boussiba media and attained its maximum at salinity 60psu, its minimum was at (N) starved medium (Figure 4).

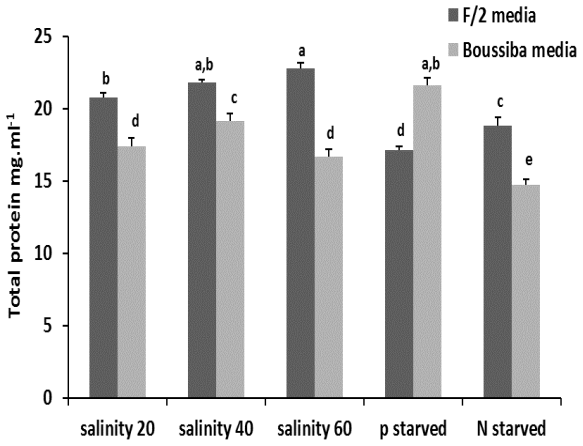

Fig. 3. Total protein content of *N. oculata* cultured on F/2 and Boussiba media.

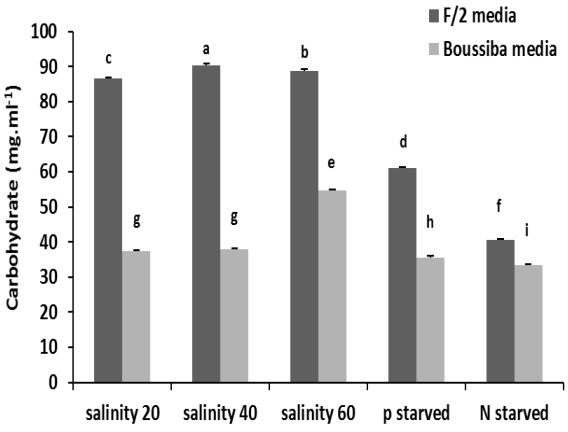

Fig. 4. Total carbohydrate content of *N. oculata* cultured on F/2 and Boussiba media.

3. Effect of salinity on total lipid and fatty acids content of *Nannachloropsis oculata* post 8 days of culture

The total lipid content was higher in *N. oculata* cultured on F/2 medium than on Boussiba medium at different treatments (Figure 5) and attained its maximum when cultured on N-starved F/2 medium. The total fatty acids were increased upon culturing *N. oculata* on F/2 media treated with 60psu (518 µg/L). Starvation of (P) from F/2 led to an increase in the total fatty acid contents (656.71 µg/L), when compared with all different treatments.

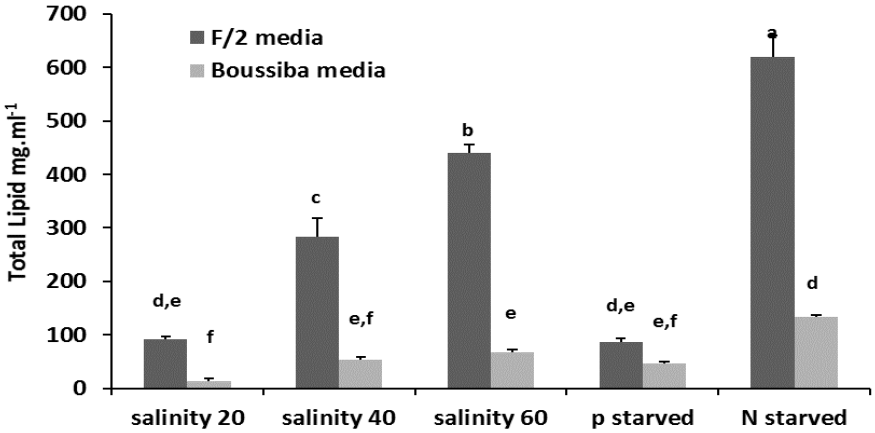

Fig. 5. Total lipid content of *N. oculata* cultured on F/2 and Boussiba media.

Table 1. Fatty acids analysis of *Nannochloropsis oculata* cultured on F/2 medium treated with different concentrations of salinities, P and N starvation.

Fatty acid	Treatment F/2 medium					Treatment Boussiba medium				
	Salin. 20psu	Salin. 40psu	Salin. 60psu	p starved	N starved	Salin. 20psu	Salin. 40psu	Salin. 60psu	p starved	N starved
Saturates (SFA)										
C 6:0	0.4	0	3.82	0	0	0	0	0	0.21	0.18
C 8:0	1.22	0.8	27.59	0.17	4.4	0	0	0	0.11	0.39
C 10:0	0.97	0.85	1.62	0	1.46	0	0	0	0.13	0
C 11:0	1.81	0	1.44	0	1.58	0	0.59	0.25	2.17	1.29
C 12:0	9.8	1	2.3	0.46	1.35	0.17	0.67	0.37	1.03	0.33
C 13:0	4.54	5.09	1.95	3.28	4.47	0.53	6.39	0.94	1.13	3.13
C 14:0	13.07	16.98	13.29	35.83	1.85	0.43	10.29	5.17	7.86	25.58
C 15:0	1.5	1.72	0.69	2.01	1.43	0.42	2	0.59	0.56	1.45
C 16:0	**50.23 (14.20%)**	**44.51 (33.65%)**	**47.66 (9.19%)**	**333.14 (50.73%)**	**363.96 (44.93%)**	**2.39 (22.87%)**	**19.65 (29.77%)**	**15.39 (36.66%)**	**70.41 (47.08%)**	**168.57 (37.39%)**
C 17:0	0.62	0.79	1.38	0.6	1.42	0	0	0	0.23	0.84
C 18:0	24.69	6.44	23.12	7.02	19.28	0.55	1.87	2.69	1.35	17.83
C 20:0	6.76	1.93	8.83	0	1.3	0	0	0	0	1.1
C 21:0	37.95	11.46	6.92	0	1.85	0.2	1.05	1.73	0	1.48
C 22:0						0	0	0	0	0.93
C 23:0	2.92	0	0	0	0	0	1.85	2.07	0	0
Sum	**156.48**	**91.57**	**140.61**	**382.51**	**404.35**	**4.69**	**44.36**	**29.2**	**85.19**	**223.1**
% to total FA	**44.22%**	**69.23%**	**27.12%**	**58.25%**	**49.92%**	**44.88%**	**67.21%**	**69.56%**	**56.96%**	**49.49%**
Monounsaturates (MUFA)										
C 14:1	1.68	1.91	0.98	1.31	2.32	0.46	2.64	0.81	0.59	1.46
C 15:1	1.54	1.65	1.41	1.06	1.27	0.25	0.81	0.51	0.39	1.04
C 16:1	**5.51 (1.56%)**	**9.26 (7.00%)**	**18.04 (3.48%)**	**161.31 (24.56%)**	**220.27 (27.19%)**	**0.2 (1.91%)**	**1.54 (2.33%)**	**0.17 (0.40%)**	**21.2 (14.17%)**	**94.72 (21.01%)**
C 17:1	2.57	5.12	2.62	0.6	2.44	0.34	1.21	1.12	0.31	1.8
C 18:1	26.79	2.54	0	0	0	0	1.22	0	0	0
C 20:1	2.51	2.49	26.88	0	0	0	0	2.14	2.22	4.77
C 22:1	5.83	0	7.61	0	4.94	0.71	1.16	3.87	1.04	3.16
Sum	**46.43**	**22.97**	**57.54**	**164.28**	**231.24**	**1.96**	**8.58**	**8.62**	**25.75**	**106.95**
% to total FA	**13.12%**	**17.37%**	**11.10%**	**25.02%**	**28.55%**	**18.76%**	**13.00%**	**20.53%**	**17.22%**	**23.72%**
Polyunsaturates (PUFA)										
C 18:2	**116.33 (32.88%)**	**2.91 (2.20%)**	**149.29 (28.80%)**	**74.65 (11.37%)**	**166.7 (20.58%)**	0.0	0.48	0	7.39	**83.28**
C 18:3	15.33	1.19	1.42	0.68	0	0.43	0	0.23	0.25	1.18
C 20:2	1.06	0.35	47.92	0	5.63	0	0	0	1.19	0
C 20:3	0	0	22.31	0	0	0.24	0.56	0	0	1.89
C 20:4	1.28	1	44.07	11.33	2.12	0	0	0	0.81	4.47
C 20:5	0	0	31.55	3.16	0	0	0	0	0.37	2.28
C 22:2	4.26	0	0	0	0					
C 22:6	12.66	12.27	23.7	20.1	0	**3.13 (29.95%)**	**12.02 (18.21%)**	**3.93 (9.36%)**	**28.61 (19.13%)**	**27.69 (6.14%)**
Sum	**150.92**	**17.72**	**320.26**	**109.92**	**174.45**	**3.8**	**13.06**	**4.16**	**38.62**	**120.79**
% to total FA	**42.65%**	**13.40%**	**61.78%**	**16.74%**	**21.54%**	**36.36%**	**19.79%**	**9.91%**	**25.82%**	**26.79%**
Total fatty acids (ug/l)	**353.83**	**132.26**	**518.41**	**656.71**	**810.04**	**10.45**	**66**	**41.98**	**149.56**	**450.84**

The dominant fatty acid was C16:0 which resulted in high saturated fatty acid content of 58.25% of total fatty acids (Table 1). However, polyunsaturated fatty acids represented 16.74% of total fatty acids. Upon culturing cells on F/2 media starved from (N), the total fatty acids recorded the highest concentrations among all treatments (810.04 µg/L). While, the use of Boussiba as a culturing media for *N. oculate*, the total fatty acids were increased in the 60psu salinity (66 µg/L). Starvation of N in this media led to increase in the total fatty acids up to 450 µg/L and P depletion (149.56 µg/L) (Table 1).

4. Biodiesel properties of *Nannachloropsis oculata* cultured on F/2 and Boussiba media treated with different salinity, P and N starvation

As shown in Table 3, the results showed that the average degree of unsaturation and iodine values were the highest value in *N. oculata* cultured on F/2 medium at salinity 60psu and on Boussiba medium at salinity 40psu. The long chain saturation factor (LCSF) was in the range 6 on Boussiba medium at salinity 60psu and N starved condition. The best cetane number (CN) was obtained upon culturing *N. oculata* on F/2 medium at salinity 40psu and Boussiba medium at salinity 20psu. The oxidation stability was increase at salinity 40psu of both media.

Table 2. Properties of biodiesel from *Nannochloropsis oculata* cultured on F/2 and Boussiba media treated with different concentrations of salts, P and N starvation.

	DU	LCSF	CFPP	IV (gI_2100g^{-1} fat)	SV (mg $KOHg^{-1}$)	CN	ΣFAs	ΣSFAs	ΣMUFAs	ΣPUFAs	Kinematic viscosity (υ) ($mm^2 s^{-1}$)	Density (ρ) ($g\ cm^{-3}$)	HHV ($MJ\ kg^{-1}$)	C18:3 (wt%)	Db ≥ 4(wt%)	Oxidation stability (h)
Biodiesel Standard EN (14214)			-	≤5/≤−20	≤120	-	≥51	≥51	-	-	-	-	3.5–5.0	0.86–0.9	NA	≤12
Biodiesel Standard ASTM D6751−02		-	-	NA	NA	-	≥47	≥47	-	-	-	-	1.9–6.0	NA	NA	-
min/max		max	max	max	max	max	min	min	min	min	max	max	max	max	min	max
Threshold value		-	-	5	120	-	47	47	-	-	-	-	-	0.9	-	12
F2 Media																
salin 20	98.43	8.06	8.84	90.63	194.13	61.18	353.83	156.5	46.43	150.92	4.60	0.90	41.28	4.33	34.38	5.76
salin 40	44.29	7.28	6.40	45.73	202.94	70.91	131.87	91.18	22.97	17.72	4.64	0.94	42.66	0.90	2.47	40.52
salin 60	134.6	4.85	1.23	146.22	197.89	46.85	518.41	140.6	57.54	320.26	3.94	0.90	40.70	0.27	38.04	6.65
p starved	58.49	5.61	1.14	57.82	204.96	63.83	656.71	382.5	164.3	109.92	4.12	0.89	40.46	0.10	11.37	12.87
N starved	71.62	5.84	1.88	64.33	204.43	61.05	810.04	404.4	231.3	174.45	4.02	0.87	39.45	0.00	21.27	8.32
BM Media																
salin 20	91.48	4.92	1.02	103.31	193.34	75.14	10.45	4.69	1.96	3.80	5.62	1.16	53.07	0.00	10.91	19.95
salin 40	52.58	4.39	2.67	61.37	206.67	71.19	66.00	44.36	8.58	13.06	4.58	1.01	45.92	0.73	1.76	50.05
salin 60	40.35	6.87	5.11	52.20	201.97	69.83	41.98	29.20	8.62	4.16	4.61	0.94	42.73	0.00	9.77	15.38
p starved	68.96	5.17	0.25	76.05	199.35	71.75	149.35	84.98	25.75	38.62	4.94	1.03	47.00	4.95	1.11	23.48
N starved	77.31	6.27	3.22	90.00	199.31	60.43	450.84	223.1	23.72	26.79	4.30	0.91	41.82	18.47	1.47	8.74

DISCUSSION

Economical production of microalgae lipid in large scales is conditioned by increasing the lipid content of potential strains **(Hariskos and Posten, 2014)**. The results obtained from the present study showed that culturing microalgae on the media

containing salinity 60psu increased their cell numbers. This finding was consistent with the results reported by **Abu-Rezq *et al.* (1999)**, they found that *N. salina* growth rate changed with different salinity concentrations. Further, they found that *N. salina* grown better upon treatment with 20 and 40ppt salinity. The lipid percentage of cell dry weight was reported as 37.7% for *N. oculata* studied earlier for biodiesel production **(Ashour *et al.*, 2019)**.

Changes in salinity affect the biochemical composition of microalgae **(Moisander *et al.*, 2002)**. The obtained data indicated the increase in protein contents of *N. oculata* with salinity stress. This result in accordance with **Rai *et al.* (2015)**, they reported that protein synthesis in most microalgae cells influenced by high salinities. The present study showed an increase in the carbohydrate's contents of *N. oculata* cultured on the two different media with different salinity concentrations. This was consistent with the previous study, which reported that carbohydrate contents in microalgae increased to adapt salinity stress conditions **(Mohy El Din, 2015)**.

Increases in salinity may produce a slight increase in total lipids in algae due to the accumulation of small molecules such as glycerol as a response to osmotic pressure **(Hu *et al.*, 2004)**. *Botryococcus braunii* growth rate was slowed by increasing salinity, but the effect on lipid concentration was minimal **(Vazquez-Duhalt *et al.*, 1991)**. In contrast, **Rao, *et al.* (2007)** found that there was an increase in *Botryococcus braunii* lipid production at higher salinity levels. **El-Sheekh *et al*. (2013)** reported that Increase of salinity enhanced both biomass and fatty acid productivity of *Scenedesmus obliquus*. Also **El-Sheekh *et al.* (2017)** concluded that the increase in NaCl concentration up to +100% caused an increase in growth, esterified fatty acid (EFA) content, and EFA productivity of *S.obliquus*.

Nutrient limitation alga are able to induce lipid production **(Rodolfi *et al.*, 2009)**, however, nutrient starvation led to halt in growth, and N-starvation is the effective approach to enhance lipid for biofuel production from *Chlorella pyrenoidosa* **(Nigam *et al*., 2011)**. This study showed that culturing microalga on the media containing salinity 60psu increased the lipids production. Our results were in agreement with **Hu and Gao (2005)** which indicated that salts stress increased the production of lipids from microalgae. Our data agreed with the studies indicated the increase in the lipid and fatty acid compositions in *C. vulgaris* and *A. obliquus* strains for biodiesel production **(Pandit *et al.*, 2017)**. *Nannochloropsis* spp. can produce a high lipid content in its cells 22.7-29.7 % of the dry weight **(Mata *et al*., 2010)**. The data obtained from the present study was in agreement with **Ito *et al.* (2013)**; they reported that under N stress conditions the lipids quantities in microalgal cells were increased. The quality of biodiesel, particularly the cetane number, iodine value and saponification value were evaluated among all treatments, which commensurate, with the ignition quality, oxidative stability, and SFAs/USFAs ratio. **El-Sheekh *et al*. (2017)** showed that biodiesel from *S. obliquus* characterized by iodine value was high (70 g iodine/100 g oil), but viscosity, all biodiesel characteristics are in accordance with the European standards for fuel diesel (EN 590:1999).

The present study reported high cetane number and low iodine number values for biodiesel production, these findings were consistent with previous studies **(Karpagam *et al.*, 2015; Yodsuwan *et al.*, 2017)**. In general, values of CN, IV, CFPP and υi are in accordance with those recommended by **International standards (2008)**. However, the

high content of fatty acids with double bonds equal and/or higher than 4 (Db≥4) results in biodiesel oxidation instability. Comparatively, *N. oceanica* NIOF15/001 showed comparable high b≥4 values **(Ashour *et al.*, 2019)**. This is related to the high level of USFAs, especially EPA, which offers an additional advantage of dual use of *N. oceanica* for essential PUFAs extraction followed by the production of biodiesel though refining process.

CONCLUSION

In the production of biodiesel by using microalgae, having high biomass and lipid for a techno-economically sustainable system are the major obstacles, which could be overcome by manipulating medium constituents as revealed by this study. These results concluded *Nannochloropsis oculata* as a promising candidate for biodiesel production. It was suggested that, in respect to cost effectiveness and commercial avaliability, F/2 was preferred as a suitable medium due to its lower chemical constituents better than Boussiba medium for commercial cultivation of *N. oculata* in a large scale biodiesel production. Also recommended using higher salinities up to 40psu (available in the natural sea water) or N-depletion and P-depletion in F/2 medium that is highly economic and significantly improved biomass and lipid production of *N. oculata* than Boussiba medium. However, further studies should be encouraged for achieving the developments to implement this approach on large scale microalgae-based lipid production can be increased by salinity alteration and nutrient deprivation.

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