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Effect of Various Bicarbonate Supplements on Biodiesel Production and Valuable Biochemical Components of the Marine Eustigmatophyceae *Nannochloropsis oculata* (Droop)

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MICROBIAL breeding media must be cost-effective, enable high growth, meet exact requirements and be readily available. The effect of different levels of sodium bicarbonate (SB) [25, 50, 75 and 100%] in the growth medium on the biochemical constituents (protein, carbohydrates, lipids, fatty acids, and amino acids) of the *Nannochloropsis oculata* was assessed compared to the F/2 Guillard standard medium. The obtained results revealed that the chemical constituents of *N. oculata* were influenced by the used level of sodium bicarbonate. The highest total protein, carbohydrate contents, and the maximum percentage of essential amino acids (EAA) (59.68%) were obtained by using the B1 medium (25% SB) as compared to the control (100% F/2). The highest total lipid content was achieved by using the B4 medium (100% SB) producing (20.09 %). In accordance, the highest total saturated fatty acids percentage (TSFA) of *N. oculata* was recorded by B4 medium. However, the highest total unsaturated fatty acids percentage (USFA) was exhibited by the B1 medium. The EN 14214 and ASTM D-6751 analyzes of the production of biodiesel have shown that the produced biodiesel by B4 medium (100% SB) and the control media of high quality. In conclusion, the addition of sodium bicarbonate is an excellent policy to increase chemical composition and lipid accumulation. The present study recommended taming results for either biodiesel production or aquaculture feeding by using proposed B4 medium as a lipid promoter or B1 medium as a protein promoter.

Keywords: Amino acids, Biodiesel production, Fatty acids, *Nannochloropsis oculata*, Proximate composition.

Introduction

Microorganisms with very high growth rates in various cultural circumstances, like microalgae, have major chemical diversity applications in many areas, including biotechnology, food science and aquaculture (Templeton & Lauens, 2015). Because of their nature, microalgae are put as an essential future food for humans. Microalgae are the source of many exciting items not only in biomedicine and balanced foodstuffs but also in technology.

In addition to natural use in aquaculture, microalgae are used directly in formulated feeds for larval and juvenile fish (Sarker et al., 2016), providing a beneficial n-3 LC-PUFA supply to farmed fish. In marine hatcheries, *Nannochloropsis* is the leading algal species and has a significant importance in aquaculture (Bondioli et al., 2012). Further aspects are required in order to increase aquaculture production to find a new, higher-quality microalgae species and to apply a micro-algae species as feed sources (Hemaiswarya et al., 2011). The key amino acid requirements of juvenile shrimp have been

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analyzed in order to determine protein quality in dinoflagellate (Lim et al., 2018). Microalgae are helpful in improving traditional food nutritional value and to promote the growth and development of target products (Tokuşoglu & Ünal, 2003). Microalgae's chemical profile can vary with cultural conditions and age (Carvalho et al., 2009). Variated cultures affect a significant number of microalgae species that have been studied for the purpose of understanding their physiology and generating mass culture (Grobbelaar, 2010). EL-Mohsnawy et al. (2020) recorded that the characteristic nutritional regimes on *chlorella vulgaris* improved the yield of omega 3 and 9 fatty acids, which resulted in high food quality for human and aqua-creatures.

Suen et al. (1987) and Alonso et al. (2000) proposed that nitrogen limitation has been developed in order to transfer microalgal metabolism to lipid production. Chisti (2007) and Abugrara et al. (2019), have previously investigated the cellular storage lipids of microalgae used in biodiesel processing. *Nannochloropsis* is a candidate applied for the development of biofuel due to its high level of lipid (Moazami et al., 2012).

For the new cellular lipid storage synthesis sufficient supplies of dissolved inorganic carbon in the environment media are needed for carbon fixation. Sodium bicarbonate was used as a carbon source for examination of the growth and biochemical composition of microalgae (Yeh et al., 2010). Most of the inorganic form of the carbon dissolved in seawaters falls in the form of bicarbonate (HCO_3^-), and the conversion speed from HCO_3^- to CO_2 is low (Skirrow, 1975). Intensive commercial microalgae production could be supported with the addition of bicarbonate salts as sources of carbon (Chi et al., 2011). The use of SB from the external media can differ from one species to another (Dason et al., 2004). Species of *Nanochloropsis* take bicarbonate ions from external media to cytosol via the plasma membrane and extract CO_2 from HCO_3^- through the action of carbonic anhydrase (Li et al., 2018). Some species of green algae and cyanobacteria are found to be a good source of biodiesel (Skjånes et al., 2013; El-Sheekh et al., 2020). This CO_2 captured through microalgae cultivation could be used for potential carbon sources to produce lipids for biofuel production without reducing crop and food supply (Mondal et al., 2017). Gardner et al. (2012) demonstrated that the addition of sodium bicarbonate enhanced triacylglycerol accumulation in the *Scenedesmus* sp. and the diatom *Phaeodactylum tricornutum*, that converted into biodiesel. Algae can use sodium bicarbonate to promote growth and lipid content as an alternative source of carbon, where several microalgae have been examined to detect the effect of sodium bicarbonate addition and findings indicated that fatty acids such as triacylglycerols and n3 fatty acids accumulate rapidly (Guihéneuf & Stengel, 2013). It was observed in a previous study for marine alga *Dunaliella salina* that addition of sodium bicarbonate significantly enhanced lipid and fatty acid content with increasing the activities of carbonic anhydrase enzymes and reduced the oxidative stress caused by ROS (Srinivasan et al., 2018).

On the industrial production scale of marine hatcheries, optimizing an effective media for cultivating microalgae species for nutritional cultivation is very necessary. The microalgae nutrient media should prepare quickly, economically, hit high growth, and fulfill the quality and quantity of all microalgae. Although the medium of F/2 Guillard is regarded as the most popular medium of *Nanochloropsis* cultivation in marine hatcheries, F/2 medium has some drawbacks, such as difficulties in preparation and preparation of outdoor and costly mass culture.

This study was designated to assess the effects of the addition of different levels of $NaHCO_3$ on the biochemical composition of marine alga *N. oculata* and the rate of lipid and amino acid production. Therefore, different media were prepared by using different levels of SB (25, 50, 75, and 100%) for culturing *N. oculata* to replace F/2 medium for reducing the production cost. However, the question is does *N. oculata* cultured on the different levels of SD concentrations achieved the biochemical composition (protein, carbohydrate, and lipids), fatty acids, and amino acids like those cultured on F/2 Guillard medium?

Materials and Methods

Cultivation and growth conditions

Nannochloropsis oculata strain was obtained from an algal unit of the marine

hatchery presented in the National Institute of Oceanography and Fisheries, NIOF Aquaculture Division, Alexandria, Egypt. *N. oculata* was maintained under controlled conditions of illumination (55 µmol photons m^{-2} s^{-1}), salinity (35±2ppt), and temperature (25±2°C) using F/2 medium (Guillard & Rhyter, 1962), with continuous aeration and 16:8 h light to dark cycle in three replicates. Cultures were incubated for homogenous mixing on a shaker at 80rpm. The cellular dry weight (CDW) and biochemical composition of algal cells were monitored in the late exponential growth phase (after 10 days culturing). The cellular dry weight (CDW) was determined, according to Abomohra et al. (2013).

Experimental design

The F/2 medium contained (mg. L^{-1}) $NaNO_3$, 75; $NaH_2PO_4.H_20$, 5; Na_2 EDTA. H_20, 4.16; $FeC1_3.6H_20$, 3.15; $CuSO_4.5H_20$, 0.01; $ZnSO_4.7H_20$, 0.022; $COC1_2.6H_20$, 0.01; $MnC1_2.4H_20$, 0.18; $Na_2MoO_4.2H_20$, 0.006; Vitamin B12, 0.0005; Vitamin B1, 0.1; and Bi-tin, 0.0005 (Guillard & Rhyter, 1962).

Carboys loaded with 5 liters of filtered disinfected saline water (35±2ppt), then enriched by sodium bicarbonate (SB) as a percentage against F/2 medium (control) as shown in Table 1. The seawater used in the study was obtained from Alexandria Beach (Egypt). SB stock solution was prepared by dissolving 10 g of $NaHCO_3$ (SB) in 100 ml of distilled water. The cultures were inoculated by the experimented alga for the last harvesting after 10 days.

Estimation of the biochemical constituents of N. oculata

Total protein and carbohydrate content

The extraction of protein content was carried out by the procedure described by Lowry et al. (1951) using Bovine Serum Albumin (BSA) as standard. Dubois et al. (1956) were followed for extraction and estimation of total carbohydrates "phenol-sulfuric acid" by using D-glucose µg/ml as standard.

Total lipid content and fatty acids profile

Total lipid and fatty acids were extracted as described by Folch et al. (1957) and Bligh & Dyer (1959). Preparation of fatty acids methyl ester from total lipids was performed according to the procedure of (Radwan, 1978). All analyses for identification of fatty acids fractions were performed on GS-MS, model HP (Hewlett Packard) 7890GC equipped with a flame ionization detector. GC Conditions: Device Model: HP (Hewlett Packard) 6890GC, Column: HP-INNOWax (Polyethylene glycol), 60m, 0.25mm ID, 0.2µm film thickness. Detector: FID (Flame Ionization Detector). Detector temperature: 250°C. Injector temperature: 220°C, injection volume 3µl, split ratio 50:1.

Amino acids determination

Amino acids of *N. oculata* were analyzed by hydrolysis in 6N HCL for 22hrs at 110°C; after hydrolysis, the acid was evaporated in a vacuum oven. The residue of the algal sample was dissolved in 1 ml of sample dilution (diluting buffer) (0.2M, pH 2.2) to complete the sample dissolving. Automatic amino acid analyzer was used for amino acid determination (Dionex ICS-3000**)** (Block, 1948).

Generated biodiesel theoretical properties

The Biodiesel Analyzer program analyzes the generated biodiesel properties depending on the fatty acid profiles obtained through GC analysis, version 2.2 (2016) http://www.brteam.ir/biodieselanalyzer or http://www.brteam.ir/analysis/ or (Damiani et al., 2010; Talebi et al., 2013, 2014).

Statistical analysis

The statistics have been conducted using the General Linear Univariate Model (ANOVA) analysis. The differences between means were assigned as significant at $P< 0.05$ with the use of the least significant difference LSD for multiple ranges of post hoc comparisons to resolve the differences between the replication means by using SPSS (2007).

TABLE 1. The experimental design used in the cultivation of *Nannochloropsis oculata.*

Medium	Percentage (%)				
	CO	**B1**	**B2**	**B3**	**B4**
F/2	100	75	50	25	---
Sodium bicarbonate (SB)	---	25	50	75	100

Results

Nannochloropsis oculata was cultured under different concentrations of sodium bicarbonate (25%, 50%, 75%, and 100%) in the early stationary phase, where samples were harvested for analysis of biochemical composition after late stationary phase (10 days). The cellular dry weight and biochemical compositions of the isolated species were examined. Moreover, the characteristics of biodiesel were examined by international standards and compared to the previous studies. The presented results indicated that there is no significant difference in the cellular dry weight (CDW) between the media contained different levels of SB and the control. The obtained data Table 2 showed significant variations in the biochemical composition of *N. oculata* between different treatments. The highest total protein and carbohydrate percentages of dry weight (15.58%± 0.02 and 49.17%± 0.02, respectively) were achieved by B1 medium (75% F/2 and 25% SB) in comparison with control and other treatments. The highest total lipid content (20.09%± 0.01) was exhibited by B4 medium (100% SB) relative to the control and other treatments.

The fatty acids analysis

Not every fatty acid is appropriate as a source of biodiesel; fatty acids analysis considered essential requirement criteria for good biodiesel. The fatty acids profile of *N. oculata* was presented in Table 3. The data revealed that there is no change in the fatty acids profile between the different treatments. In contrast, there is a noticeable change in the content of each individual fatty acid between the different treatments. The most abundant saturated fatty acid was the palmitic acid (C16:0), which recorded its highest value (27.68%) with B4 medium (100% SB) than the other media. Following the palmitic acid, is the stearic acid (C18:0), which has nearly the same percentage values in all used media. In addition, Oleic acid (C18:1) was remarkably the most prevalent monounsaturated fatty acid in all treatments, however its value reduced to reach its minimum amount (20.51%) with B4 medium, which means that the Oleic acid content decreased with the addition of sodium bicarbonate to the culture medium. Also, palmitoleic acid (C16:1) showed an increase (5.87%) with B1 (25%SB) medium, while it recorded the lowest percentage value with B4 medium (5%). Moreover, linoleic acid (C18:2) was the most common polyunsaturated fatty acid with all treatments, where the data revealed that the highest value of this fatty acid (15.55%) was recorded with B2, B3, and B4 media relative to the control medium (13.69%). Ecosapentaenoic acid (EPA) was the second polyunsaturated fatty acid, where its maximum percentage value (10.17%) was recorded with B1 medium. Similarly, dcosahexaenoic acid (DHA) was the third polyunsaturated fatty acid which recorded its highest value (5.12%) with the same medium. Low percentage values of linolenic acid (C18:3) were detected by B2 and B4 media. However the highest value of linolenic acid was achieved by B1 medium (1.67%). It is worth pointing out that nearly all such fatty acids have been identified as the most popular biodiesel fatty acids. The results revealed that the highest percentage of total saturated fatty acids TSFA (41.21%) was achieved by B4 medium (100% SB), which was higher than TSFA percentage (35.97%) recorded by the control medium (CO) (100% F/2). The present study explained that the highest rate of the total unsaturated fatty acids USFA (65.75%) was detected by B1 medium (25% SB), where this percentage is mainly consisting of 33.60% MUFA and 32.15% PUFA. On the other hand, the highest ratio (0.70) between SFA/USFA was achieved by B4 medium (100%SB). In addition, the highest ratios between n-3/n-6 and DHA/EPA were 1.01% and 0.50 % respectively, which exhibited by B1 medium (25% SB) (Table 3).

TABLE 2. The Average biochemical composition (in % dry basis) mg/g DW of *N. oculata* at different levels of sodium bicarbonate and F/2 medium harvested after 10 days incubation period.

Medium	CDW (g L^{-1})	Protein (%CDW)	Carbohydrate (%CDW)	Lipid (%CDW)
CO (F/2)	1.04±0.03^{a}	14.29± 0.01^{b}	46.19±0.02^{d}	12.33± 0.01^{d}
B1	1.04±0.03^{a}	15.58±0. 02^{a}	49.17±0.02^{a}	12.15± 0.02^{e}
B2	1.03±0.02^{a}	12.64± 0.02^{d}	48.53±0.01^{b}	13.23± 0.01^{c}
B3	1.02±0.01^{a}	13.19± 0.02^{c}	46.53±0.02^{c}	13.98±0.01^{b}
B4	1.03±0.02^{a}	11.19± 0.01^{e}	41.58±0.01^{e}	20.09±0.01^{a}

Data are statistically analyzed using ONE-WAY ANOVA. Significant result is obtained at P= 0.05.

TABLE 3. Total fatty acids profiles and their individual (%) of *N. oculata* at different levels of sodium bicarbonate and F/2 medium harvested after 10 days incubation period.

Fatty acid	Media				
		CO	B1	B2	B3	B4
Carbon atom(n) µg/100gDW µg/100gDW µg/100gDW (µg/100gDW)	**Fatty acid**					
	Saturated fatty acids (SFAs)					
C14:0	Myristic acid	3.94	4.36	4.78	4.63	4.65
C15:0	Pentadecanoic acid	0.68	0.68	0.71	0.73	0.75
C16:0	Palmitic acid	23.13	22.00	23.51	24.96	27.68
C17:0	Heptadecanoic acid	0.24	0.30	0.61	0.75	0.81
C18:0	Stearic acid	4.39	4.21	4.30	4.35	4.36
C21:0	Heneicosanoic acid	1.92	1.12	1.09	1.18	1.20
C24:0	Lignoceric acid	1.67	1.59	1.73	1.75	1.76
∑ Saturated (SFA)		**35.97**	**34.26**	**36.73**	**38.35**	**41.21**
	Monounsaturated (MUFAs)					
C14:1	Myristoleic acid	0.13	0.14	0.15	0.13	0.15
C15:1	cis-10-Pentadecenoic acid acid	0.07	0.08	0.07	0.08	0.07
C16:1	Palmitoleic acid	5.78	5.87	5.65	5.62	5.00
C17:1	cis-10-Heptadecenoic aacid	0.51	0.53	0.5	0.47	0.44
C18:1n9	Oleic acid	25.87	24.63	23.70	22.20	20.51
C20:1	Eicosenoic acid	2.92	1.69	1.78	1.71	1.68
C22:1	Erucic acid methyl	0.72	0.66	0.59	0.68	0.62
∑ Monounsaturated (MUFA)		**36.00**	**33.60**	**32.44**	**30.89**	**28.47**
	Polyunsaturated (PUFA)					
C18:2n6	Linoleic acid	13.69	14.63	15.55	15.55	15.55
C18:3n3	Linolenic acid	1.43	1.67	1.37	1.48	1.37
C20:2n6	Eicosadienoic acid	0.91	0.56	0.63	0.79	0.55
C20:5n-3	Ecosapentaenoic acid acid	8.17	10.17	9.47	9.39	9.29
C22:6n-3	Docosahexaenoicacid	3.81	5.12	3.81	3.77	3.69
∑ polyunsaturated (PUFA)		**28.01**	**32.15**	**30.83**	**30.98**	**30.45**
∑ Unsaturated (USFA)		**64.01**	**65.75**	**63.27**	**61.87**	**58.92**
SFA/MSFA		1.00	1.02	1.13	1.24	1. 45
SFA/PSFA.		1.28	1.06	1.19	1.24	1.35
SFA/USFA		0.56	0.52	0.58	0.62	0.70
∑ n3		13.15	15.71	14.42	14.38	14.11
∑ n6		14.86	15.44	16.41	16.38	16.21
∑ n3/ n6		0.88	1.01	0.88	0.88	0.88
DHA/EPA		0.47	0.50	0.40	0.40	0.40

Biodiesel properties

Table 4 results reveal that theoretical CN ranged between 50.24 of control and 49.87 of B4 medium, where all within the appropriate range (more than 47). IV data also ranged between 106.17 of the control and 105.91 of B4 medium, where the EN 14214 range was approved (maximum 120). The obtained data was provided with respect to CP

and PP under ASTM D-6751, −3.12, and −15:10 respectively. The EN 14214 and ASTM D-6751, respectively, accepted viscosity (1.9: 6) and oxidation stability (OS) (minimum 3).

Amino acids analysis

Amino acid profiles of different culture media of *N. oculata* diets were presented in Table 5. The present study revealed that there is no change in the amino acid profile between the different media. In contrast, there is a clear variation in the content of each individual amino acid between the different treatments. The results showed that *N. oculata* recorded the highest percentage of essential amino acids EAA (59.68%) by B1 medium (25% SB), while the lowest value was achieved by B4 medium (100% SB). The results presented that the highest five EAA in the B1 medium were arginine (16.69%), leucine (8.84%), phenylalanine (6.39%), histidine (5.63%) and methionine (5.59%) (Table 5). The vice versa for non-essential amino acids (NEAA), where the highest percentage of non-essential amino acids NEAA (61.25%) was detected by B4 medium (100% SB), while the lowest value of NEAA was achieved by B1 medium. The most abundant five NEAA in the B4 medium were glutamine (17.54%), serine (13.61%), aspartate (8.69%), proline (7.26%) and alanine (4.12%).

TABLE 4. Assessment of theoretical biodiesel properties of determined fatty acids under F/2 Guillard medium (control) and B4 medium (100%SB) using Biodiesel Analyzed software, version 2.2 (2016) for *N. oculata*.

Properties	F/2 Guillard medium control	B4 medium (100%SB)	Accepted range	Ref.
CN_{min}	50.24	49.87	47	ASTM D6751-02
IV_{max}	106.17	105.91	120	EN 14214
CP_{min}	7.17	9.57	-3.12	ASTM D-6751
PP	0.97	3.57	-15:10	ASTM D-6751
$V_{mm2/s}$	3.54	3.53	1.9:6	EN 14214
OS_{min}	11.04	10.06	3	ASTM D-6751

TABLE 5. Amino acids profile (%) in *N. oculata* at different levels of sodium bicarbonate and F/2 medium harvested after 10 days incubation period.

	Medium				
Amino acid (AA) %	**CO**	**B1**	**B2**	**B3**	**B4**
Essential amino acids (EAA)					
Arginine	4.1	16.69	4.14	4.18	4.56
Histidine (HIS)	1.7	5.63	1.83	5.75	2.21
Isoleucine (ILE)	5.1	3.85	4.16	4.12	4.35
Leucine (LEU)	9.2	8.84	4.07	8.13	4.26
Lysine (LYS)	3.1	3.43	4.92	4.23	4.67
Methionine (MET)	1.5	5.59	5.96	2.8	3.36
Phenylalanine (PHE)	4.9	6.39	3.82	5.7	4.17
Threonine (THR)	4.6	1.66	5.82	5.77	4.15
Tryptophan (TRP)	14.5	4.57	12.93	6.84	3.33
Valine (VAL)	6.1	3.03	3.16	6.29	3.69
Total EAA	54.8	59.68	50.81	53.81	38.75
Non-essential amino acids (NEAA)					
Alanine (ALA)	6.6	3.42	3.63	3.61	4.12
Aspartate (ASP)	9.7	8.17	8.39	8.33	8.69
Cystine (C-C)	1	2.69	3.18	3.03	3.48
Glutamine (GLU)	11.3	12.65	9.22	9.26	17.54
Glycine (GLY)	5.2	2.58	4.82	2.79	3.28
Proline (PRO)	4.9	4.53	12.71	7.13	7.26
Serine (SER)	4.2	3.62	4.17	6.02	13.61
Tyrosine (TYR)	2.3	2.66	3.07	6.02	3.27
Total NEAA	**45.2**	**40.32**	**49.19**	**46.19**	**61.25**

Discussions

The improvement of culture conditions is essential to raise efficiency and economic value for microalgae productivity in the future. New methods of extraction, production, and cultivation can be efficiently established to improve productivity and reduce costs. For more than 50 years, Guillard F/2 medium has been popular for marine aquaculture in the cultivation of microalgae, currently, because of the different use of microalgae in various biotechnological domains; the F/2 Guillard medium has many drawbacks. Our results investigated that some sodium bicarbonate levels achieved significant biochemical constituents higher than F/2 medium (control).

The present study showed that low addition of SB to B1 medium (25% SB) could improve protein, carbohydrate, PUFA and EAA contents of *N. oculata*, which may be exhibited by increasing inorganic dissolved carbon concentration as additional sources of energy. Similar findings have been found with *Chlorella pyrenoidosa* and *Scenedesmus obliquus* exposed to increased CO_2 (Yang & Gao, 2003; Srinivasan et al., 2018). Pancha et al. (2015) recorded that bicarbonate addition raises the protein content of freshwater alga *Scenedesmus* sp. Jegan et al. (2013) recorded that the protein and carbohydrate contents of *Desmococcus* sp., *Chlorococcum* sp., and *Chlorella* sp. strains elevated when they were cultivated in media provided with bicarbonate. Microalgae protein content can be explained by intake of nitrogen internally, possibly due to the high level of nitrate intake. The decrease in the nitrogen level in the B1 medium (25%SB) than that in F/2 medium (control) caused an increase in the carbohydrate content in B1 medium due to nitrogen limitation. This result is following Millán-Oropeza et al. (2015), who revealed that nitrogen starvation caused the accumulation of carbohydrate in *Chlorella* sp. In this research, the replacement of all nutrient salts from the culture by 100% sodium bicarbonate (B4 medium) resulted in a significant decrease in protein content of *N. oculata*. Similarly, Pancha et al. (2015) showed a decrease in protein content under nutrient-starved conditions. The present study also revealed that B4 medium (100 % SB) significantly decreases the carbohydrate content; this finding in contrast to Pancha et al. (2015).

The present work demonstrated an increase in polyunsaturated fatty acids (PUFAs) yield as eicosapentaenoic fatty acid (EPA) and docosahexaenoic fatty acid (DHA) with the addition of different sodium bicarbonate levels in relative to the control. This is in agreement with Ma et al. (2016), who detected that EPA is the dominant PUFA in *Nannochloropsis*, which makes it a possible partial replacement of fish oil for fish foods (Sørensen et al., 2017).

In the present study, the highest percentage of essential amino acids EAA was detected by B1 medium (25% SB), which recorded the highest percentage of protein content. In contrast, the highest percentage of non-essential amino acids NEAA revealed by B4 medium (100% SB), which recorded the lowest percentage of protein content. The data are supported by those published by Barkia et al. (2019), who mentioned that amino acids differ with growth conditions as do other bioactive compounds synthesized by the microalgae. The most abundant EAA in the profile of *N. oculata* cultured on B1 medium is Arginine. The percentage of Arginine (16.69%), is higher than that of *Tetraselmis* spp. (Brown, 1991), *Chlorella* sp. (Brown & Jeffrey, 1992), and dinoflagellates as represented by Lim et al. (2018). Leucine as the second largest EAA (8.48%) is higher than that of *Heterocapsa rotundata* and *Ansanella granifera* (7.5 and 8.3%, respectively) as recorded by Lim et al. (2018). Therefore, *N. oculata* cultured on B1 medium can be used as arginine and leucine-rich mixed algal diets in aquaculture.

Our results showed an increase in the lipid content of *N. oculata* especially at B4 culture medium (100%SB). Pancha et al. (2015) recorded an increase in the cellular storage lipids (namely triacylglycerides or TAGs) of microalgae with the addition of bicarbonate to the nutrient deficiency medium. The present data revealed that the highest lipid content and percentage of total saturated fatty acids TSFA were obtained by culturing *N. oculata* on B4 medium (100%SB). Therefore, these results in accordance with other authors who showed that increasing concentration of bicarbonate in algal cultures will increase the number of fatty acids (Xia & Gao 2005; Chiu et al., 2009). El-Sheekh et al. (2013), indicated that sodium bicarbonate addition in *Scenedesmus obliquus* culturing medium has a negative effect on the production of the fatty acid. Similarly,

White et al. (2013) showed that there is no effect of sodium bicarbonate addition on the composition of fatty acids in *Tetraselmis suecica* cultures, while a marked effect was observed on *N. salina* cultures. Our data revealed that Palmitic acid (C16:0) was the main saturated fatty acid and this result was in agreement with Abugrara et al. (2019), who declared that palmitic acid is the common component in the crude lipids of the *N. oceanica*. The data explained that SFA was predominantly in *N. oculata* cultured on B4 medium (100% SB) than MUFA and PUFA recorded at the same medium. This finding was recommended by Guihéneuf & Stengel (2013).

As C16–C18 (palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid) are the most widely obtained fatty acid esters in our biodiesel and are considered to be the most prevalent biodiesel components (Li et al., 2010), estimation of the properties must be advantageous to the usage of the *N. oculata* as a source of biodiesel. Usage of *N. oculata* demonstrated clearly that sodium bicarbonate supply has a positive impact on FAME content and therefore on the parameters of biodiesel efficiency (Islam et al., 2013). The limiting factors for biodiesel quality are considered cetane number (CN), oxidation stability (OS), cloud point (CP), iodine value (IV), and viscosity (V), and by matching data collected in this study against those obtained in the United States by standardization (ASTMD-6751) and European standardization (EN 14214), such range is acceptable (Damiani et al., 2010). In addition, the biodiesel procured is very similar to traditional diesel fuel, so it needs no improvement to the engine (Knothe, 2006, 2008; Damiani et al., 2010). Viscosity, Density, Cetane Number, Cloud, Pour Points, and Flash point, which is agreed by ASTM D-6751 or EN 14214, represent the most important features of biodiesel produced (Knothe, 2006, 2008). Linoleate (C18:2) and linolenate (C18: 3) can serve as indirect estimates of oxidative biodiesel stability (Park et al., 2008). CN number and IV have been determined using fatty acid methyl esters (FAME) composition (Ramos et al., 2009). Good biodiesel should agree with standard cetane number (CN), which specifies the good quality of inflammation, low pollutant level, proper density, and viscosity (Gopinath et al., 2009). According to the ASTM D6751-02 standard for biodiesel, the minimum CN should be 47.0, whereas the IV is set to a maximum of 120g I2/100g fat. Cetane number (CN), combustion heat, and viscosity usually increase with an increasing length of the chain, indicating a better fatty acid (C16–18) (Francisco et al., 2010). The CN improves the ignition quality in fuels that contain high levels of saturated fatty acids. The proportion of long and short-chain constituent fatty acids and the presence of a single or more two bonds (saturated or unsaturated) affect the properties of obtained biodiesel (Damiani et al., 2010).

Conclusion

In summary, this research has the potential to promote the development of industrial algal biodiesel using sodium bicarbonate as a useful inorganic source of carbon to enhance the growth of biomass and lipid production. Our findings suggested that sodium bicarbonate addition in low percentage (25%SB) to the culture medium had significant effects on the production of cellular compounds including protein, carbohydrates, polyunsaturated fatty acids and essential amino acids (especially arginine and leucine), where these valuable substances are used for feeding in aquaculture. The present study nominates *N. oculata* as a promising candidate for biodiesel production by cultivation on a high level of sodium bicarbonate (100%SB) in the culture medium.

Conflict of interests: The authors declare that they have no conflict of interest.

Authors contribution: This work was carried out in collaboration between all authors. Ali M. Abugrara, Hanan M. Khairy, Heba S. El-Sayed, Hoda H. Senousy. Ali M. Abugrara and Hoda H. Senousy wrote the protocol and the design of the work. Ali M. Abugrara, Heba S. El-Sayed and Hoda H. Senousy supervised the experimental work, the data collection and drafted the manuscript. Hanan M. Khairy and Hoda H. Senousy carried out data analysis and interpretation of the results, critical revision of the article and final approval of the version to be published.

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تأثير الإمداد بمستويات مختلفة من البيكربونات على إنتاج وقود الديزل الحيوى وعلى المكونات الكيميائية الحيوية القيمة للطحلب البحرى *Nannochloropsis oculata*

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بيئات الزراعة الميكروبية يجب أن تكون فعالة من حيث التكلفة وتعطى نمو عالى وتفى بالمتطلبات الدقيقة وتكون متاحة بسهولة. تم تقييم تأثير المستويات المختلفة من بيكربونات الصوديوم (25% ، 50% ، 75% 100%) فى بيئة النمو على المكونات البيوكيميائية (البروتين، الكربوهيدرات، الدهون، والأحماض الدهنية، والأحماض الأمينية) لطحلب الـ *Nannochloropsis oculata* بالمقارنة بالبيئة الكنترول (F/2 100% Guillard). ولقد أوضحت النتائج التى تم الحصول عليها أن المكونات الكيميائية للطحلب قد تأثرت بالنسب المختلفة المستخدمة من الصوديوم بيكربونات. ولقد تم الحصول على أعلى نسبة لمحتوي البروتين والكربوهيدرات الكلى وكذلك أعلى نسبة من الأحماض الأمينية الأساسية الكلية وهى (59.68%) بإستخدام بيئة الزراعة (B1) (والتى تحتوى على 25% صوديوم بيكربونات) بالمقارنة بالبيئة الكنترول (F/2 100%). ولقد تم تحقيق أعلى محتوى للدهون الكلية بإستخدام بيئة الزراعة (B4) (والتى تحتوى على 100% صوديوم بيكربونات) حيث انتجت دهون بنسبة (20.09%) نسبة إلى الوزن الجاف. وفقا لذلك، تم تسجيل أعلى نسبة للأحماض الدهنية المشبعة الاجمالية من الطحلب بإستخدام بيئة الزراعة (B4). بينما تم الحصول على أعلى نسبة للأحماض الدهنية الغير المشبعة الاجمالية من الطحلب بواسطة بيئة الزراعة (B1). ولقد أظهرت التحليلات القياسية لـ [EN14214] و [ASTMD-6751] لإنتاج وقود الديزل الحيوى أن وقود الديزل الحيوى المنتج بواسطة بيئة الزراعة (B4) وبيئة الكنترول (F/2 100% Guillard) ذات جودة عالية. لذلك تعد إضافة بيكربونات الصوديوم سياسة ممتازة لزيادة التركيب الكيميائى وتراكم الدهون. لذلك أوصت الدراسة الحالية بتوظيف النتائج إما لإنتاج وقود الديزل الحيوى أو تغذية الأحياء المائية وذلك بإستخدام بيئة الزراعة (B4) المقترحة كمحفز للدهون أو بيئة الزراعة (B1) كبيئة محفزة لانتاج البروتين.