Global Journal of Science Frontier Research: B
Chemistry
Volume 21 Issue 1 Version 1.0 Year 2021
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Measuring the Effect of using two Carbon Sources (Sodium Bicarbonate with Rice Washing Water) on the Chemical Composition, Fatty Acids and Amino Acids of Marine Microalgae *Nannochlorpsis Oceanica*

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GJSFR-B Classification: *FOR Code: 039999*

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Abstract- Microalgae breeding media must be cost-effective, enable high growth, meet exact requirements and be readily available. The effect of different levels of sodium bicarbonate and rice wash water [25, 50, and 75%] in the growth medium on the biochemical constituents (protein, carbohydrates, lipids, fatty acids, and amino acids) of the *Nannochloropsis oceanica* was assessed compared to the F/2 Guillard standard medium. The obtained results revealed that the chemical constituents of *Nannochloropsis oceanica* affected by the level of urea used and the rice washing water. The highest protein, carbohydrate contents, and highest EAA (51.39%) obtained using ME1. medium (25% RWW and 75% SB) as compared to the control (100% F/2). The highest total lipid content was achieved by using the ME3 medium (75% RWW & 25% SB) producing (42.71 %), were the obtained the of the highest biomass productivity and lipid productivity in ME3 medium. In accordance, the highest total saturated fatty acids percentage (TSFA) of *Nannochlorpsis oceanica* recorded by ME3 medium. However, the highest total unsaturated fatty acids percentage (USFA) obtained by the ME3 Medium. The present study recommended taming results for aquaculture feeding by using the proposed ME1 and ME3 medium as a lipid promoter and as a protein promoter.

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I. Introduction

Micro-organisms with extremely high growth rates in various cultural conditions, such as microalgae, have major chemical diversity applications in many fields, including biotechnology, food science, and aquaculture this is due to their nature (Templeton & Leuins, 2015). As microalgae placed as a staple food for humans in the future. Microalgae are a source of many essential elements not only in biomedicine and balanced nutrients but also in technology.

In addition to natural use in aquaculture, microalgae are used directly in feed for larvae and juveniles (Sarker *et al*., 2016), provide a supply of n-3 LC-PUFA for fish farmed in marine hatcheries, and *Nannochloropsis* is considered the species. The main component of algae is of great importance in aquaculture (Bundioli *et al*., 2012). More aspects are requirement in to increase aquaculture production to create new high-quality microalgae species and to use microalgae species as feed sources (Hemaiswarya *et al*., 2011).

The amino acid requirements of prawns were analyzed to determine protein quality in dinoflagellates (Lim *et al*., 2018). Microalgae help improve the traditional nutritional value of foods and promote the growth and development of targeted and main products (Tokuşoglu & Ünal, 2003). The chemical profile of microalgae varies with cultural conditions and age (Carvalho *et al*., 2009). Diverse cultures influence a large number of microalgae species that have been studied for understanding organ function and generating a group culture (Grobbelaar, 2010). El-Muhsnawi *et al*. (2020) show the distinct diets of *Chlorella Vulgaris* have improved the productivity of omega-3 and omega 9 fatty acids, resulting in higher food quality for humans and aquaculture . Suain *et al*. (1987) to Alonso *et al*. (2000) suggested that nitrogen reduction was to transfer microalgal metabolism to lipid production. Chisti (2007) and (Abugrara *et al*. 2019). For a novel cellular lipid storage synthesis, an adequate supply of inorganic carbon dissolved in the medium of the environment is require for carbon fixation. Sodium bicarbonate was used as a carbon source to examine the growth and biochemical composition of microalgae (Yeh *et al*., 2010). Most of the inorganic form of dissolved carbon in seawater is find in the form of bicarbonate (HCO_3^-), and the conversion speed from HCO_3^- to CO_2 is low (Skirrow, 1975). The intensive production of commercial microalgae can be supported by addition bicarbonate salt as sources of carbon (Chi *et al*., 2011). The use of SB from external media can vary from one species to another (Dason *et al*., 2004). Species of *Nanochloropsis* take bicarbonate ions from extras through media to the

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cytosol via the plasma membrane and extract CO_2 from HCO_3^- through the action of carbonic anhydrase (Li *et al*., 2018). Several microalgae were examined for the effect of adding sodium bicarbonate and the results indicated that fatty acids such as triglycerides and n3 fatty acids accumulate rapidly (Guihéneuf & Stingle, 2013). It was observed in a previous study of seaweed *Dunaliella salina* that the addition of sodium bicarbonate significantly enhanced the lipid and fatty acid content while increasing the activities of carbonic anhydrase enzymes and reducing the oxidative stress induced by ROS (Srinivasan *et al*., 2018). Despite this, the animal lacks the enzymes required to synthesize PUFA, it must be obtained from food and, therefore, it is often known to be biotic (Milledge, 2011), so deficiency in PUFAs appears to be the main reason behind low larval survival rates (Patel). *et al*., 2005). As a result, microalgae have been used as a food source for aquatic organisms, with fatty acid contents being the central factor in selecting the microalgae species (Huerlimann *et al*., 2010). The full use of algal biomass may involve a combination of different techniques (Wiley *et al*. 2011).

is increasing and can be saturated by lipid biosynthesis using appropriate nutrients as well as by optimizing harvesting strategies that lead to cell / biomass recovery. Various physico-chemical conditions such as production cost. However, the question is, did N. oceanicacultured at different levels of SD concentrations achieve biochemical composition (protein, carbohydrates and fats) and fatty acids and amino acids such as those temperature, stress, light intensity, culture grown on F / 2 Guillard medium? time, organic carbon and inorganic nutrients including iron (Fe), phosphorous (P), nitrogen

II. Materials and Methods

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. Among the different nutritional factors in particular, nitrogen is one of the most important nutrients for growth, as it is a component of all structural and functional proteins such as peptides, enzymes, chlorophyll, energy transport molecules and genetic material in algal cells (Cai *et al*., 2013). The nitrogen concentration in the middle of the culture affects the rate of cell growth and the biochemical compositions of the microalgae (Wang *et al*. 2013), and several studies have shown that when nitrogen is limited in the middle of the culture, the microalgae slow down the rate of cell growth and increase their fat or carbohydrate content, Which reduces protein synthesis (Ho *et al*., 2014).The demand for algae-based fats (N), manganese (Mn), zinc (Zn), sulfur. (S), cobalt (Co), and others, affect and regulate the growth and lipid accumulation of many types .of microalgae (Bajpai *et al*., 2014) *Nannochloropsis sp*. has been widely accepted as a productive strain of microalgae due to its high growth rate, high lipid content, and strong resistance to biological contamination (Biondi *et.al*., 2013). Therefore, commercial agricultural fertilizers (CAGF) should be used more commonly in place of F / 2 cultivation medium (Lopez-Elias *et al*., 2005). As aquatic organisms, microalgae need water, salts, and carbon dioxide to thrive. The main primary nutrients are nitrogen (N), phosphorous (P) and silicon (Si, for diatoms only). Certain vitamins and micronutrients are also required for algae growth (such as magnesium, sulfur, iron, etc.). Of all the nutrients, nitrogen and phosphorous are the main nutrients that limit growth, lipid content and microalgae productivity (Bajpai *et al*., 2014). At the level of industrial production of marine hatcheries, the improvement of the effective culture medium for microalgae species for food culture is absolutely necessary. Nutrient media should quickly prepare microalgae, hit high growth and meet the quality and quantity of all microalgae.

This study was designed to assess the effects of adding different levels of $NaHCO_3$ and (RWW) on the biochemical composition of *N. oceanica* and the rate of lipid and amino acid production. Therefore, different media were prepared using different levels of SB and (RWW) (25, 50 and 75%) to cultivate *N. oceanica* to replace F / 2 medium to reduce Cultivation and growth conditions *Nannochloropsis oceanica* 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. oceanica* 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).

III. 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. 2H20, 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 NaHCO3 (SB) in 100 ml of distilled water.

Use liquid plastic bottles of 1.5 liters and 1 liter of sterile saline water (35 ± 2ppt) and 1kilo of rice was washed (RWW) with 1.5 liters of water in a first wash. 50 ml of water was taken and filtered using filter paper, and water was used as a medium, rice washing water was used without fermentation (Table 1).

The cultures were inoculated by the experimented alga for the last harvesting after 10 days

a) Estimation of the biochemical constituents of N. oceanica

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.

$$\text{Biomass productivity (mg } L^{-1} \text{ day}^{-1}) = (CDWL - CDWE) \text{ x } (tL - tE)^{-1}$$

With CDW_E representing the CDW (mg L^{-1}) at the days of early exponential phase (t_E) and CDW_L at the days of late exponential phase (t_L). (Abomohra , *et al.,* 2016).

b) Total lipid content and fatty acid 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, 0.2*µ*m film thickness.

Detector: FID (Flame Ionization Detector). Detector temperature: 250°C. Injector temperature: 220°C, injection volume 3*µ*l, split ratio 50:1.

$$\text{Lipid productivity (mg } L^{-1} \text{ day}^{-1}) = (LC_L - LC_E) \text{ x } (t_L - t_E)^{-1}$$

with LC_E representing the lipid content (mg L^{-1}) at the days of early exponential phase (t_E) and LC_L at the days of late exponential phase (t_L) . (Abomohra , *et al.,* 2013).

c) Amino acids determination

Amino acids of N. oceanica 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 ICS3000) (Block, 1948).

IV. Statistical Analysis

Table 1: The experimental design used in the cultivation of *Nannochloropsis oceanica*

	CO	ME1	ME2	ME3
F/2	100	---	---	--
Sodium bicarbonate (SB(	----	0.75	0.50	0.25
Rice wash water (RWW)	----- -	0.25	0.50	0.75

V. Results

Samples were harvested for analysis of *Nannochloropsis oceanica* was cultured under biochemical composition after late stationary different concentrations of sodium phase (10 days). The cellular dry weight and bicarbonate and rice washing water (25%, 50% biochemical compositions of the isolated and75%) in the early stationary phase, where species were examined. Moreover, the

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). 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, RWW and the control. The obtained data (Table 2) showed significant variations in the biochemical composition of *N. oceanica* between different treatments. The highest total protein and carbohydrate percentages of dry weight (18.76%± 0.02 and 24.66%± 0.02, respectively) were achieved by ME1 medium (75% SB and 25% RWW) in comparison with control and other treatments. The highest total lipid content (42.71%± 0.02) was exhibited by ME3 medium (25% SB and 75% RWW) relative to the control and other treatments.

a) Biomass productivity and lipid productivity

The obtained data (Table 2) showed significant variations in the biomass productivity of *N. oceanica* between day^{-1}) was exhibited by ME3 and ME2 medium relative to the control and other treatments.

b) The fatty acids analysis

The fatty acids profile of *N. oceanica* 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 (27.14%) with ME3 medium (25% SB and 75%RWW) 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 remarkable the most prevalent monosaturated fatty acid in all treatments, however its value reduced to reach its minimum amount (22.71%) with ME3 medium, which means that the Oleic acid content decreased with the addition of RWW to the culture medium. Also, palitoleic acid (C16:1) showed an highest value (5.91%) with ME3 (25%SB and 75% RWW) medium, while it recorded the lowest value with F/2 medium. 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 (15.85%) was recorded with ME3 media relative to the control medium (13.64%). Ecosa-pentaenoic acid (EPA) was the second polyunsaturated fatty acid, where its maximum percentage value (9.54%) was recorded with ME3 medium. Similarly, dcosa-hexaenoic acid (DHA) was the third polyunsaturated fatty acid which recorded its highest value (3.67%) with the same medium. Low percentage values of linolenic acid (C18:3) were detected by control and ME1 media. However the highest value of linolenic acid was achieved by ME3 medium (1.48%). The results revealed that the highest percentage of total saturated fatty acids TSFA (41.69%) was achieved by ME3 medium (25% SB and75% RWW), which was higher than TSFA percentage (35.75%) recorded by the control medium (CO) (100% F/2). The present study explained that the highest rate of the total unsaturated fatty acids USFA (65.80%) was detected by ME1 medium (25% RWW and 75% SB), where this percentage is mainly consisting of 33.66% MUFA and 32.14% PUFA. On the other hand, the highest ratio (0.66) between SFA/USFA was achieved by control medium (25% SB and 75%%RWWME3), In addition, the highest ratios between n-3/n-6 and DHA/EPA were 1.08% and 0.52 % respectively, which exhibited by ME1 medium (75% SB and 25% RWW), (Table 3).

c) Amino acids analysis

Amino acid profiles of different culture media of *N. oceanica* 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. oceanica* recorded the highest percentage of essential amino acid EAA (51.39%) by ME1 medium (75% SB and 25% RWW), while the lowest value was achieved by ME3 medium (25% SB and 75% RWW). (Table 5). The non-essential amino acids (NEAA), where the highest percentage of nonessential amino acids NEAA (53.61%) was detected by ME3 medium (25% SB and 75% RWW), while the lowest value of NEAA was achieved by ME1 medium.

Table 2: The Average biochemical composition (in % dry basis) mg/g DW of *N. oceanica* at different levels of sodium bicarbonate (SB) and rice wash water (RWW) medium harvested after 10 days incubation period

Medium	CDW (g L^{-1})	Protein (%CDW)	Carbohydrate (%CDW)	Lipid (%CDW)	Biomass productivity (mg L^{-1} day^{-1})	Lipid productivity (mg L^{-1} day^{-1})
CO	0.74 ± 0.02^{d}	21.64 ± 0.03^{a}	17.29 ± 0.02^{d}	36.55 ± 0.03^{d}	69.76 ± 0.03^{d}	27.13 ± 0.02^{d}
ME1	0.84 ± 0.00^{c}	18.74 ± 0.02^{b}	24.66 ± 0.02^{c}	38.43 ± 0.02^{c}	91.64 ± 0.03^{c}	29.01 ± 0.02^{c}
ME2	0.87 ± 0.00^{b}	16.86 ± 0.02^{c}	22.74 ± 0.02^{b}	41.64 ± 0.03^{b}	102.05 ± 0.03^{b}	32.23 ± 0.02^{b}
ME3	0.97 ± 0.00^{a}	14.97 ± 0.01^{d}	20.26 ± 0.02^{a}	42.71 ± 0.02^{a}	104.14 ± 0.03^{a}	33.27 ± 0.03^{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. oceanica* at different levels of sodium bicarbonate (SB) and rice wash water (RWW) medium harvested after 10 days incubation period

Fatty acid	CO	ME1	ME2	ME3
C14:0 (Myristic acid)	3.49 ± 0.03^{d}	4.37 ± 0.02^{c}	4.78 ± 0.03^{b}	4.92 ± 0.03^{a}
C15:0 (Pentadecylic acid)	0.63 ± 0.02^{d}	0.75 ± 0.02^{c}	0.85 ± 0.02^{b}	0.96 ± 0.02^{a}
C16:0 (Palmitic acid)	23.27 ± 0.02^{d}	22.45 ± 0.03^{c}	24.62 ± 0.03^{b}	27.14 ± 0.02^{a}
C17:0 (Margaric acid)	0.29 ± 0.02^{d}	0.37 ± 0.02^{c}	0.62 ± 0.02^{b}	0.67 ± 0.02^{a}
C18:0 (Stearic acid)	4.41 ± 0.02^{d}	4.63 ± 0.02^{c}	4.75 ± 0.02^{b}	4.86 ± 0.02^{a}
C21:0(Heneicosanoic acid)	1.93 ± 0.02^{d}	1.15 ± 0.03^{c}	1.17 ± 0.02^{b}	1.22 ± 0.02^{a}
C24:0 (Lignoceric acid)	1.73 ± 0.02^{b}	1.78 ± 0.02^{d}	1.86 ± 0.03^{c}	1.92 ± 0.02^{a}
∑Saturated (SFA)	53.53	53.33	56.13	96.14

C14:1 (Myristoleic acid)	0.12±0.02^{c}	0.18±0.02^{a}	0.23±0.02^{b}	0.28±0.02^{cc}
C15:1 (cis-10-pentadecenoic acid)	0.06±0.02^{a}	0.08±0.02^{b}	0.06±0.02^{bb}	0.09±0.02^{c}
C16:1 (Palitoleic acid)	5.76±0.02^{d}	5.84±0.02^{c}	5.75±0.02^{b}	5.91±0.02^{a}
C17:1(cis-10-Heptadecenoic acid)	0.50±0.02^{a}	0.40±0.02^{c}	0.33±0.02^{d}	0.42±0.02^{b}
C18:1n9 (Oleic acid)	25.84±0.02^{d}	24.83±0.02^{c}	23.62±0.02^{b}	22.71±0.02^{a}
C20:1 (Paullinic acid)	2.84±0.02^{a}	1.67±0.02^{d}	1.79±0.02^{c}	1.67±0.01^{b}
C22:1 (Erucic acid methyl)	0.63±0.02^{b}	0.66±0.02^{d}	0.59±0.02^{c}	0.67±0.02^{a}
∑Monosaturated (MUFA)	53.53	55.11	53.55	56.53
C18:2n6 (Linoleic acid)	13.64±0.02^{d}	14.72±0.03^{b}	15.75±0.02^{c}	15.85±0.02^{a}
C18:3n6 (y-Linoleic acid)	0.20±0.01^{a}	0.12±0.01^{d}	0.13±0.02^{c}	0.14±0.02^{b}
C18:3n3 (α- Linolenic acid)	1.41±0.02^{d}	1.65±0.02^{c}	1.36±0.02^{b}	1.48±0.02^{a}
C20:2n6 (Eicosadienoic acid)	0.87±0.02^{a}	0.64±0.02^{c}	0.75±0.02^{d}	0.63±0.02^{b}
C20:5n-3 (Ecosapentaenoic acid)	8.15±0.02^{d}	9.87±0.02^{c}	9.65±0.02^{b}	9.54±0.02^{a}
C22:6n-3 (Docosahexaenoic acid)	3.81±0.02^{d}	5.14±0.02^{b}	3.74±0.02^{c}	3.67±0.02^{a}
∑Polyunsaturated (PUFA)	36.36	53.69	56.56	56.56
∑Usaturated	15.65	13.63	15.53	15.31
SFA/MSFA	6.33	6.66	6.35	6.56
SFA/PSFA	6.35	6.66	6.35	6.55
SFA/USFA	3.31	3.39	3.16	3.11
∑n 3	65.51	61.11	69.53	69.14
∑n6	69.56	63.96	61.15	61.13
∑n3/n6	3.46	6.36	3.64	3.66
DHA/EPA	3.95	3.33	3.54	3.54

Table 4: Amino acids profile (%) in *N. oceanica* at different levels of sodium bicarbonate (SB) and rice wash water (RWW) medium harvested after 10 days incubation period

	Medium			
Amino acid (AA)%	CO	ME1	ME2	ME3
Essential amino acids (*EAA)*				
Arginine	4.13±0.02^{d}	6.16±0.02^{c}	6.32±0.02^{a}	6.20±0.02^{b}
Histidine (HIS)	1.68±0.02d	3.62±0.02^{c}	3.75±0.02^{b}	3.85±0.02^{a}
Isoleucine (ILE)	5.09±0.02^{a}	3.25±0.02^{b}	3.16±0.02^{d}	3.22±0.02^{c}
Leucine (LEU)	9.22±0.02^{a}	7.12±0.02^{b}	6.42±0.01^{c}	6.16±0.02^{d}
Lysine (LYS)	3.11±0.02^{d}	7.41±0.02^{a}	5.45±0.02^{c}	5.66±0.02^{b}
Methionine (MET)	1.56±0.02^{b}	4.16±0.02^{d}	4.63±0.02^{a}	4.21±0.02^{c}
Phenylalanine (PHE)	4.89±0.03^{a}	5.47±0.02^{b}	5.14±0.02^{d}	5.31±0.02^{c}
Threonine (THR)	4.58±0.02^{a}	4.62±0.02^{b}	4.11±0.02^{c}	4.07±0.02^{d}
Tryptophan (TRP)	14.48±0.02^{d}	4.24±0.02^{a}	3.61±0.03^{b}	3.12±0.01^{c}
Valine (VAL)	6.09±0.02^{a}	5.34±0.02^{b}	4.69±0.02^{c}	4.59±0.03^{d}
Total EAA	39.6	36.54	95.34	91.54
Non-essential amino acids (*NEAA*)				
Alanine (ALA)	5.59±0.02^{a}	4.55±0.02^{d}	4.76±0.02^{c}	4.92±0.03^{b}
Aspartate (ASP)	9.68±0.01^{a}	9.44±0.02^{b}	9.23±0.02^{c}	9.46±0.02^{d}
Cystine (C-C)	3.31±0.02^{c}	4.34±0.02^{d}	6.47±0.02^{a}	5.86±0.02^{b}
Glutamine (GLU)	11.55±0.02^{a}	11.23±0.02^{b}	9.62±0.03^{d}	10.13±0.02^{c}
Glycine (GLY)	4.39±0.02^{d}	5.65±0.02^{b}	5.76±0.02^{a}	5.20±0.02^{c}
Proline (PRO)	4.34±0.02^{d}	5.83±0.02^{c}	7.77±0.02^{a}	7.34±0.02^{b}
Serine (SER)	4.22±0.02^{d}	5.41±0.03^{c}	6.55±0.02^{b}	8.37±0.02^{a}
Tyrosine (TYR)	2.12±0.01^{d}	2.16±0.02^{c}	2.55±0.02^{a}	2.34±0.02^{b}
Total NEAA	93.3	96.16	33.56	35.16

VI. 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 and rice

washing water levels achieved significant biochemical constituents higher than F/2 medium (control).

The present study showed that low addition of SB and RWW to ME1 medium (25% RWW and 75% SB) could improve protein, carbohydrate, PUFA and EAA contents of *N. oceanica*, 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 ME1 medium (25% RWW and 75% SB) than that in F/2 medium (control) caused an increase in the carbohydrate content in ME1 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 25% SB and 75% RWW (ME3 medium) resulted in a significant decrease in protein content of *N. oceanica*. Similarly, (Pancha *et al*. 2015) showed a decrease in protein content under nutrient-starved conditions. The present study also revealed that ME3 medium (25% RWW and 25%SB) significantly decreases the carbohydrate content; this finding in contrast to(Pancha *et al*., 2015).

As for the lipid, it was higher than (Abugrara, *et al*., 2020) as 100% sodium bicarbonate was used on *N.oculata*, the percentage was higher than (Abugrara, *et al.,* 2019) in using starch by 75% on the same algae, higher than (Ashour, *et al*., 2019's results when using Medium F/2, and higher than (Ashour M. and Abd ElWahab K., 2017) in its use of 50-50% nitrogen and phosphorous, and higher than (Zhang, *et al.,* 2016), which used Different nitrogen levels and the carbohydrates were less than what (Abugrara, *et al.,* 2020) reached when different levels of sodium bicarbonate were used on *N.oculata*, and it was higher than (Ashour, *et al*., 2019) by using medium F / 2 on the same algae, and less than (Ashour M. and Abd ElWahab K., 2017) when using N - P by 50 - 50%, and less than (Chun W. *et al*.,2012) when it used me to medium F/2 on the same algae.

The biomass productivity was higher than (Ashour, *et al*., 2019) using Medium F/2 on the same alga, higher than (Mata, et al., 2010) reported on *N.oculata*.

The lipid productivity was higher than (Ashour *et al.,* 2019) results for its use of Medium F / 2 on the same algae It was similar to (Aarón Millán *et.al*., 2015) results that used nitrate and carbon dioxide on the alga *N.oculata* and higher than (Chun Wan, *et al.,* 2013) results on the same algae with different sources of nitrogen used Below is what (Mata, *et al*., 2010) has found on *N.oculata*.

The present work demonstrated an increase in polyunsaturated fatty acids (PUFAs) yield as eicosapentaenoic fatty acid (EPA) and docosa-hexaenoic 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 ME1 medium (75% SB and 25% RWW), which recorded the highest percentage of protein content. In contrast, the highest percentage of non-essential amino acids NEAA revealed by ME3 medium (25% SB and 75% RWW), 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. oceanica cultured on ME1 medium is Arginine. The percentage of Arginine (6.16%), 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 (7.12%) is higher than that of *Heterocapsa rotundata* (7.5 %) as recorded by Lim et al. (2018). Therefore, *N. oceanica* cultured on ME1 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. oceanica* especially at ME3 culture medium (25%SB and 75% RWW). 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. oceanica* on ME3 medium (25%SB and 75% RWW). 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. oceanica* cultured on ME3 medium (25% SB and 75% RWW) than MUFA and PUFA recorded at the same medium. This finding was recommended by Guihéneuf & Stengel (2013).

VII. Conclusion

In summary, this research has the potential to promote the development of industrial 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 and rice wash water addition in low percentage (25%SB and RWW) 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. oceanica* as a promising candidate for lipid production by cultivation on level of sodium bicarbonate and rice wash water (25%SB and 75% RWW) in the culture medium.

As C16 - C18 (palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid) is the most widely obtained fatty acid ester in biodiesel, and is considered the most widespread component of biodiesel, the use of *N. oceanica* has shown Clearly, the supply of sodium bicarbonate as well as rice wash water has a positive effect on FAME content and therefore it can be used in the production of biofuels.

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