Evaluate the effect of using source of nitrogen as urea and a rice wash water (RWW) on the chemical composition, fatty acids and amino acids for marine microalgae *Nannochlorpsis oceanica*

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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 urea 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 *N. oceanica* was assessed compared to the F/2 Guillard standard medium. The obtained results revealed that the chemical constituents of *N. oceanica* were influenced by the used level of urea and rice wash water. The highest total protein, carbohydrate contents, and the maximum percentage of essential amino acids (EAA) (55.16%) were obtained by using the MF3 medium (75% RWW) as compared to the control (100% F/2). The highest total lipid content was achieved by using the MF3 medium (75% RWW) producing (41.72 %), were the obtained of highest biomass productivity and lipid productivity in MF3 medium . In accordance, the highest total saturated fatty acids percentage (TSFA) of *N. oceanica* was recorded by MF3 medium. However, the highest total unsaturated fatty acids percentage (USFA) was exhibited by the MF3 medium.
The present study recommended taming results for aquaculture feeding by using proposed MF3 and MF2 medium as a lipid promoter and as a protein promoter.

Keywords: Amino acids, Fatty acids, *Nannochloropsis oceanica*, Proximate composition

INTRODUCTION

Microalgae are a large group of photoautotrophic eukaryotic organisms that play important roles in marine, freshwater and even terrestrial ecosystems on earth (Zhu y. Donford NT.,2013; Piggott *et al*., 2015). For example, marine microalgae are among the most significant contributors of biological fixation and cycling of atmospheric CO_2 (Riebesell *et al*., 2009).Microalgae 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). 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*.,

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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). In spite of that, animal lack the required enzymes to synthesize PUFA, it must be obtained from food and, therefore, is often known to be vital (Milledge, 2011).Therefore, deficiency in (PUFAs) seems to be the main cause of the low survival rates of larvae (Patil *et al*., 2005). As a result, microalgae have been used as a dietary source for aquatic organisms, with fatty acid contents being the centric agent in the selection of microalgal species (Huerlimann *et al*., 2010). The complete utilization of algal biomass may involve the combination of different technologies (Wiley *et al*. 2011).

Particularly among various nutritional factors, nitrogen is considered one of the most critical nutrient for growth, since it is a constituent in all structural and functional proteins such as peptides, enzymes, chlorophylls, energy transfer molecules, and genetic materials in algal cells (Cai *et al*. 2013). The concentration of nitrogen in culture medium considerably affects both cell growth rate and biochemical compositions of microalgae (Wang*et al*. 2013), and numerous studies have shown that when the nitrogen is limited in culture medium, microalgae slow down cell growth rate and increase their lipid or carbohydrate content, reducing protein synthesis (Ho *et al*., 2014). Most microalgae are able to utilize various forms of nitrogen, including nitrate, nitrite, ammonium and organic nitrogen sources such as urea (Becker 1994); each nitrogen source is first reduced to the ammonium form and assimilated into amino acids through a variety of pathways (Cai *et al*. 2013).

Demand for algae-based lipids is increasing and can be satisfied by an efficient lipid biosynthesis using proper nutrients as well as by optimizing harvesting strategies that lead to high cell/biomass recovery. Different physico-chemical conditions such as temperature, stress, light intensity, culture time, organic carbon and inorganic nutrients including iron (Fe), phosphorous (P), nitrogen (N), manganese (Mn), zinc (Zn), sulfur (S), cobalt (Co) and others, affect and regulate growth and lipid accumulation of several microalgae species (Bajpai *et al.*,2014).

Nannochloropsis oceanica has been widely accepted as a production microalgal strain because of its high growth rate, high lipid content and strong resistance to biotic contamination (Biondi *et.al*.,2013). Therefore, commercial agricultural fertilizers (CAGF) should be commonly used instead of F/2 culture medium (Lopez-Elias *et al*.,2005). As aquatic organisms, microalgae need water, salts and CO_2 to grow. The major essential macronutrients are nitrogen (N), phosphorus (P) and silicon (Si, for diatoms only). Some vitamins and micronutrients are also required for algal growth (such as magnesium, sulfur, iron, etc). Among all nutrient elements, nitrogen and phosphorus are the main nutrient limiting the growth, lipid percentage and productivity of microalgae (Bajpai *etal*.,2014).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 CH_4N_2O and rice wash water on the biochemical composition of marine alga *N. oceanica* and the rate of lipid and amino acid production. Therefore, different media were prepared by using different levels of for culturing *N. oceanica* to replace F/2 medium for reducing the production cost. However, the question is does *N. oceanica* 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

Microalgal strains

*Nannochloropsis oceanica*strain was from an algae unit of the marine hatchery at the kilo 21 Alexandria - Egypt.

N. oceanica were kept Institute of Oceanography and Fisheries (NIOF), Egypt and cultured under controlled conditions of temperature (22± 2C˚), salinity (35 ± 2 ppt).

Using F/2 medium (Guillard and Rhyter,1962), with continuously 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; $NaH_2PO_4.H_2O$, 5; Na_2 EDTA. H_2O, 4.16; $FeC1_3.6H_20$, 3.15; $CuSO_4.5H_20$, 0.01; $ZnSO_4.7H_2O$, 0.022; $COC1_2.6H_20$, 0.01; $MnC1_2.4H_2O$, 0.18; $Na_2MoO_4.2H_2O$, 0.006; Vitamin B12, 0.0005; Vitamin B1, 0.1; and Bi-tin, 0.0005 (Guillard & Rhyter, 1962).

Culture conditions

Use liquid plastic bottles of 1.5 liters and 1 liter of sterile saline water (35 ± 2) 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.

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.

Biomass productivity (mg L^{-1} day^{-1}) = $(CDW_L - CDW_E) \times (t_L - t_E)^{-1}$

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).

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) 7890 GC 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.

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

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).

Statistical analysis

Statistical analysis was performed using analysis of the one way (ANOVA) was used to test the effects of urea on chemical composition of microalgae. Duncan.

One- way ANOVA was used to match the mean differences by the Statistical Package for the Social Sciences SPSS(2007). As such, the differences were small to be significant at $p<0.05$.

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

	Control (CO)	MF1	MF2	MF3
F/2	100	---	---	--
Urea	----	0.75	0.50	0.25
Rice wash water (RWW)	------	0.25	0.50	0.75

RESULTS

Nannochloropsis oceanica was cultured under different concentrations as shown in table (1) 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. The cellular dry weight and biochemical compositions of the isolated species were examined.The presented results indicated that there is no significant difference in the cellular dry weight (CDW) between the media contained different levels of medium mixture 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 (22.35%± 0.02 and 21.81%± 0.02, respectively) were achieved by MF3 medium (75% RWW and 25% Urea) in comparison with control and other treatments. The highest total lipid content (41.72%± 0.03) was exhibited by MF3 medium relative to the control and other treatments.

Biomass productivity and lipid productivity.

The obtained data Table (2) showed significant variations in the biomass productivity of *N. oceanica* between different treatments. The highest percent of dry weight (104.15±0.02 and 102.06±0.02 (mgL^{-1} $day^{-1)}$, respectively) were achieved by MF3 medium (75% Rice wash water and 25% Urea) and MF2 medium (50% urea and 50 % RWW)in comparison with control and other treatments. The highest lipid productivity content (51.15±0.02 and 49.75± 0.03 (mg L^{-1} day^{-1}) was exhibited by MF3 and MF2medium 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. 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 (27.62%) with MF3 medium than the other media. Following the palmitic acid, is the Myristic acid) (C14:0).
In addition, Oleic acid (C18:1) was remarkably the most prevalent monounsaturated fatty acid in all treatments, It scored the highest values where it was (24.30%) with MF3 medium,Which means that the Oleic acid content increased with the increase in medium of rice washing water . Also, palmitoleic acid (C16:1) showed an increase (5.87%) with MF3 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 (16.25%) was recorded with MF2 and MF3 media relative to the control medium (15.81%). Ecosapentaenoic acid (EPA) was the second polyunsaturated fatty acid, where its maximum percentage value (7.69%) was recorded with MF3 medium. Similarly, dcosahexaenoic acid (DHA) was the third polyunsaturated fatty acid which recorded its highest value (12.35%) with the MF1 medium. Low percentage values of linolenic acid (C18:3) were detected by F/2 and MF1 media. However the highest value of linolenic acid was achieved by MF2 medium (1.71%). The results revealed that the highest percentage of total saturated fatty acids TSFA (42.39%) was achieved by MF3 medium , which was higher than TSFA percentage (30.28%) recorded by the control medium (CO) (100% F/2). The present study explained that the highest rate of the total unsaturated fatty acids USFA (72.73%) was detected by MF3 medium , where this percentage is mainly consisting of 34.51% MUFA and 38.22% PUFA. On the other hand, the highest ratio (0.58) between SFA/USFA was achieved by MF1 and MF3 medium . In addition, the highest ratios between n-3/n-6 and DHA/EPA were 1.26% and 1.74 % respectively, which exhibited by MF1 medium (Table 3).

Amino acids analysis

Amino acid profiles of different culture media of *N. oceanica* diets were presented in Table 4. 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 acids EAA (55.16%) by MF3 medium , while the lowest value was achieved by CO medium (100% F/2). The results presented that the highest five EAA in the MF3 medium were arginine (6.59%),lysine (7.21%) phenylalanine (5.59%), histidine (4.46%) and isoleucine (6.10%) (Table 4). The vice versa for non-essential amino acids (NEAA), where the highest percentage of nonessential amino acids NEAA (50.27%) was detected by CO medium (100% F/2), while the lowest value of NEAA was achieved by MF3 medium. The most abundant five NEAA in the F/2 medium were aspartate (11.02%), glutamine (10.28%), alanine (6.66%), serine (6.21%) and proline (5.33%).

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

Medium	CDW ($g\ L^{-1}$)	Protein (%CDW)	Carbohydrate (%CDW)	Lipid (%CDW)	Biomass productivit ($mg\ L^{-1}\ day^{-1}$)	Lipid productivity ($mg\ L^{-1}\ day^{-1}$)
CO	0.74 ± 0.02^{d}	18.37 ± 0.02^{d}	17.31 ± 0.05^{d}	36.57 ± 0.03^{d}	69.78 ± 0.02^{d}	27.25 ± 0.02^{d}
MF1	0.86 ± 0.01^{c}	20.61 ± 0.03^{c}	21.81 ± 0.03^{a}	38.43 ± 0.02^{c}	91.65 ± 0.02^{c}	29.01 ± 0.02^{c}
MF2	0.88 ± 0.01^{b}	21.41 ± 0.03^{b}	20.45 ± 0.03^{c}	40.32 ± 0.03^{b}	102.06 ± 0.02^{b}	30.93 ± 0.03^{b}
MF3	0.96 ± 0.01^{a}	22.35 ± 0.02^{a}	21.22 ± 0.03^{b}	41.72 ± 0.03^{a}	104.15 ± 0.02^{a}	32.30 ± 0.02^{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 rice wash water and urea medium harvested after 10 days incubation period.

Fatty acid	CO	MF1	MF2	MF3
C14:0 (Myristic acid)	3.49±0.03^{d}	5.16±0.03^{c}	5.31±0.03^{b}	5.43±0.03^{a}
C15:0 (Pentadecylic acid)	0.52±0.03^{d}	0.89±0.03^{b}	0.85±0.03^{c}	0.93±0.03^{a}
C16:0 (Palmitic acid)	20.26±0.03^{d}	26.31±0.03^{b}	25.14±0.03^{c}	27.62±0.03^{a}
C17:0 (Margaric acid)	0.29±0.02^{d}	0.55±0.02^{b}	0.65±0.02^{a}	0.51±0.03^{c}
C18:0 (Stearic acid)	3.64±0.02^{d}	4.25±0.03^{c}	4.36±0.02^{b}	4.64±0.02^{a}
C21:0 (Heneicosanoic acid)	0.67±0.02^{d}	1.66±0.03^{c}	1.91±0.03^{a}	1.85±0.02^{b}
C24:0 (Lignoceric acid)	1.41±0.03^{d}	1.89±0.02^{a}	1.81±0.03^{b}	1.69±0.02^{c}
∑Saturated (SFA)	**30.28**	**40.71**	**40.31**	**42.39**
C14:1 (Myristoleic acid)	0.12±0.03^{c}	0.14±0.03^{a}	0.13±0.02^{b}	0.14±0.02^{aa}
C15:1 (cis-10-pentadecenoic acid)	0.06±0.02^{c}	0.09±0.02^{a}	0.07±0.02^{b}	0.07±0.02^{bb}
C16:1 (Palitoleic acid)	4.37±0.02^{d}	5.36±0.02^{c}	5.48±0.03^{b}	5.67±0.02^{a}
C17:1 (cis-10-Heptadecenoic acid)	0.45±0.02^{d}	0.58±0.03^{b}	0.53±0.02^{c}	0.59±0.03^{a}
C20:1 (Paullinic acid)	2.14±0.03^{d}	2.65±0.03^{c}	2.85±0.02^{b}	2.87±0.02^{a}
C18:1n9 (Oleic acid)	14.12±0.03^{d}	23.59±0.03^{c}	24.05±0.03^{b}	24.30±0.03^{a}
C22:1 (Erucic acid methyl)	0.52±0.03^{d}	0.79±0.02^{b}	0.72±0.02^{c}	0.82±0.03^{a}
∑Monosaturated (MUFA)	**21.78**	**33.20**	**33.83**	**34.51**
C18:2n6 (Linoleic acid)	10.34±0.02^{d}	15.31±0.03^{c}	16.25±0.03^{a}	15.81±0.03^{b}
C18:3n6 (y-Linoleic acid)	0.18±0.03^{d}	0.29±0.02^{b}	0.26±0.02^{c}	0.35±0.03^{a}
C18:3n3 (α- Linolenic acid)	1.31±0.02^{d}	1.35±0.02^{c}	1.71±0.03^{a}	1.59±0.02^{b}
C20:2n6 (Eicosadienoic acid)	0.75±0.03^{d}	0.95±0.03^{b}	0.92±0.03^{c}	1.07±0.02^{a}
C20:5n-3 (Ecosapentaenoic acid)	3.66±0.02^{d}	7.12±0.03^{c}	7.35±0.03^{b}	7.69±0.02^{a}
C22:6n-3 (Docosahexaenoic acid)	7.64±0.02^{d}	12.35±0.03^{a}	10.82±0.02^{c}	11.71±0.03^{b}
∑Polyunsaturated (PUFA)	23.88	37.37	37.31	38.22
∑Usaturated	45.66	70.57	71.14	72.73
SFA/MSFA	1.39	1.23	1.91	1.23
SFA/PSFA	1.27	1.09	1.08	1.11
SFA/USFA	0.66	0.58	0.57	0.58
∑n 3	12.61	20.82	19.88	20.99
∑n6	11.27	16.55	17.43	17.23
∑n3/n6	1.12	1.26	1.14	1.22
DHA/EPA	2.09	1.74	1.47	1.52

TABLE 5. Amino acids profile (%) in *N. oceanica* at different levels of rice wash water and urea medium harvested after 10 days incubation period.

	Medium			
Amino acid (AA)%	**CO**	**MF1**	**MF2**	MF3
Essential amino acids (*EAA*)				
Arginine	5.52±0.03^{d}	5.63±0.02^{c}	6.10±0.02^{b}	6.59±0.03^{a}
Histidine (HIS)	2.89±0.03^{d}	3.14±0.02^{c}	4.19±0.02^{b}	4.46±0.02^{a}
Isoleucine (ILE)	3.74±0.02^{d}	4.69±0.03^{c}	5.13±0.02^{b}	6.10±0.02^{a}
Leucine (LEU)	9.32±0.03^{a}	5.89±0.03^{d}	6.21±0.02^{c}	6.65±0.02^{b}
Lysine (LYS)	4.32±0.03^{d}	7.36±0.02^{b}	7.59±0.03^{a}	7.21±0.03^{c}
Methionine (MET)	4.27±0.02^{c}	4.24±0.02^{d}	5.29±0.02^{a}	4.50±0.03^{b}
Phenylalanine (PHE)	6.49±0.03^{a}	5.16±0.02^{d}	5.24±0.03^{c}	5.59±0.03^{b}
Threonine (THR)	5.43±0.03^{a}	4.69±0.03^{d}	4.76±0.03^{c}	4.89±0.03^{b}
Tryptophan (TRP)	1.97±0.02^{d}	4.32±0.03^{c}	4.41±0.03^{b}	4.96±0.03^{a}
Valine (VAL)	5.37±0.03^{a}	5.14±0.02^{b}	4.43±0.02^{c}	4.21±0.03^{d}
Total EAA	**49.32**	**50.26**	**53.35**	**55.16**
Non-essential amino acids (*NEAA*)				
Alanine (ALA)	6.66±0.02^{a}	5.25±0.02^{d}	5.29±0.03^{c}	5.41±0.02^{b}
Aspartate (ASP)	11.02±0.01^{a}	8.84±0.02^{b}	8.79±0.03^{c}	7.39±0.03^{d}

Cystine (C-C)	4.34±0.03^{c}	4.65±0.03^{b}	4.72±0.03^{a}	4.16±0.03^{d}
Glutamine (GLU)	10.28±0.02^{a}	9.79±0.02^{b}	8.33±0.03^{c}	7.66±0.03^{d}
Glycine (GLY)	4.32±0.03^{c}	5.49±0.03^{a}	5.34±0.03^{b}	5.49±0.02^{aa}
Proline (PRO)	5.33±0.03^{d}	8.44±0.02^{a}	7.43±0.02^{b}	7.36±0.02^{c}
Serine (SER)	6.21±0.02^{a}	4.52±0.03^{d}	4.61±0.03^{c}	4.82±0.03^{b}
Tyrosine (TYR)	2.11±0.02^{d}	2.25±0.03^{c}	2.31±0.03^{b}	2.63±0.02^{a}
Total NEAA	**50.27**	**49.23**	**46.82**	**44.92**

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 demonstrated that supplementation of Dilute to (RWW) with urea could improve protein, carbohydrate, PUFA, EAA and biomass contents in the alga *N.oceanica*. In protein and it was higher than the results of (Abugrara, *et al*., 2020), where sodium bicarbonate with F / 2 was used in different proportions on *N.oculata* algae and it was higher than the results of (Abugrara, *et al*., 2019), who used starch by 75% with F/2, as well as when using it as a source of nitrogen on *N.oceanica*, and this is due to the presence of urea, and is close to what (Ashour, *et al*., 2019) found, which used (F / 2 100%) on the algae *N.oceanica*, And higher than what reached in (Ashour M. and Abd El-Wahab K., 2017), when he used a source of nitrogen and phosphorus at 50- 100% on the same algae. A lower percentage of (Chun W. *et al*.,2012) results were recorded on the same type with the useof Medium F / 2 .

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 starchby 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 El-Wahab K., 2017) in its use of 50-50% nitrogen and phosphorous, and higher than (Zhang, *et al.,* 2016), which used Different nitrogen levelsand 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 El-Wahab 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 PUFA scored higher in MF3 than (Abugrara , *et al*., 2020) found, as it used sodium bicarbonate on the same algae, as well as it was in DHA and it was lower in EPA, and higher than (Ashour, *et al*., 2019) results, who used Medium F / 2 on the same algae, and (Madhusree, *et al*., 2016) results were lower in some proportions. Used and higher in proportions on the same algae as

it used wastewater at different levels with some types of media, and it was higher than (Jean H. B.& Sung Bum H.,2011) when using Medium F / 2 on algae *Nannochloropsis spp*. , *Nannochloropsis sp*.

In the present study, the highest percentage of essential amino acids , EAA was in MF1 level higher than (Abugrara, *et al*., 2020) reached, NEAA was lower in this level, and was higher than (Jean H. B.& Sung Bum Hu., 2011).

Conclusion

In summary, this research has the ability to enhance the growth of biomass and the results indicate that the use of rice washing water because it contains starch, which is a useful carbon source, as well as with the presence of urea as a source of nitrogen and their use in proportions of 75 - 25% as a culture medium for *N.oceanica* ز algae had a significant impact on the production of cellular compounds. Including protein, carbohydrates, polyunsaturated fatty acids and essential amino acids (especially arginine and leucine) as they have value in materials used for feeding in aquaculture.

تقييم تأثير استخدام مصدر النيتروجين مثل اليوريا ومياه غسل الأرز (RWW) على التركيب الكيميائي والأحماض الدهنية والأحماض الأمينية للطحالب البحرية الدقيقة نانوكلوربسس أوشينيكا

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المستخلص: يجب أن تكون وسائط تربية الطحالب المجهرية فعالة من حيث التكلفة ، وتمكن من النمو العالي ، وتفي بالمتطلبات الدقيقة وتكون متاحة بسهولة. تم تقييم تأثير المستويات المختلفة من اليوريا وماء غسيل الأرز [25 و 50 و 75٪] في وسط النمو على المكونات البيوكيميائية (البروتين والكربوهيدرات والدهون والأحماض الدهنية والأحماض الأمينية) في *نانوكلوربسس أوشينيكا* مقارنة مع الوسط القياسي F / 2 Guillard. أوضحت النتائج المتحصل عليها أن المكونات الكيميائية لنوع نانوكلوربسس أوشينيكا قد تأثرت بمستوى اليوريا المستخدم وماء غسيل الأرز. تم الحصول على أعلى نسبة بروتين ، ومحتويات كربوهيدرات ، وأعلى نسبة من الأحماض الأمينية الأساسية (EAA) (55.16٪) باستخدام وسط MF3 (75٪RWW) مقارنة بالكنترول (100٪ F / 2). تم تحقيق أعلى محتوى إجمالي للدهون باستخدام وسط (75٪MF3 RWW) ينتج (41.72٪) ، حيث تم الحصول على أعلى إنتاجية للكتلة الحيوية وإنتاجية الدهون في وسط MF3. وفقًا لذلك ، تم تسجيل أعلى نسبة إجمالي للأحماض الدهنية المشبعة (TSFA) من نانوكلوربسس أوشينيكا بواسطة وسط MF3. ومع ذلك ، تم عرض أعلى نسبة إجمالية للأحماض الدهنية غير المشبعة (USFA) بواسطة وسط MF3.

أوصت الدراسة الحالية بنتائج التربية للإستخدام في الإستزراع المائي باستخدام وسط MF3 و MF2 كمحفز للدهون وكمحفز للبروتين.

الكلمات المفتاحية: الأحماض الأمينية ، الأحماض الدهنية ، *نانوكلوربسس أوشينيكا* ، التركيب الكيميائي .

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