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Response of growth, protein and fatty acid content of *Desmodesmus cuneatus* to the repletion and depletion of nitrogen

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Abstract

Introduction: Biomass and biochemical composition content (particular fatty acid profile, protein, Pigment) play an important role in commercial scale production of microorganisms. Nitrogen concentration is the most effective factor in biochemical composition and growth rate changes.

Materials and methods: The unicellular fresh water microalgae, *Desmodesmus cuneatus* (Scenedesmaceae, Chlorophyta), was grown on BM medium under various concentration of nitrogen at 25°C and pH 7.0in a period of 12 days. Daily cell counting was done using Neubauer haemocytometer to determine the growth rate, cell density and exponential time. The grown cells were harvested in the stationary phase to determine fatty acid and protein content.

Results: The maximum growth rate ($0.35 \pm 0.09 day^{-1}$) and cell density ($36.00 \pm 1.01 \times 10^5$ $cell.ml^{-1}$) was achieved in 2.5 mM of nitrogen concentration**.** The maximum level of PUFA, was 25.31% of the total fatty acids under N- sufficient conditions (5 mM nitrogen) compared to 17.01% under N- free. The maximum precentage of protein content (36.81%) was found at 5 mM nitrogen content and minimum (11.14%) was seen at nitrogen free medium.

Discussion and conclusion: The results showed that control of nutrients plays an important role in microalgae culture. Any increase or decrease, depending on the type of nutrient, causes metabolic changes as well as changes in physiology and the nutritional value of microalgae. The nitrogen concentration of 2.5 mM may be more beneficial than other concentrations, as cell number is sustained in exponential phase longer.

Key words: *Desmodesmus cuneatus*, Fatty acid, Growth, Nitrogen, Protein

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Introduction

Microalgae are classified under a varied group of photoautotrophic organisms which play important role as primary producers for feeding of zooplanktons and other animals in the food chain of aquatic systems. The ability of microalgae in production of bioactive compounds such as pigments, vitamins, proteins and fatty acids make them a good source to use them as bio energy and pharmaceutical resources in aquaculture systems (1, 2, 3 and 4). The growth and biochemical composition of microalgae is affected by culture conditions (5, 6 and 7). Microalgal cells have shown different mechanisms (such as enzyme reaction, cell permeability, and cell composition) to survive and optimize their growth in response to strong variations in physicochemical conditions (7 and 8). As a nutrient, nitrogen is required in high concentration and is counted as one of the most important chemical nutrients that require microalgae growth. After carbon, nitrogen plays the second important role in production of biochemical compounds such as amino acids, nucleic acids and other nitrogen- enriched molecules in the grown microalga biomass. In recent years, the importance of nitrogen in microalgae biomass has been studied by many researchers (9, 10 and 11). In normal condition, the chlorophyll accumulation and cell division is. In contrast, depletion of nitrogen resources result in the changes in some biosynthesis pathway of microalgae such as a decline in nitrogenous photosynthetic pigments, reduction in photosynthetic efficiency, cell division prevention, lower growth rate, and a reduction in cell size (12 and 13). Further, nitrogen limitation leads to accumulation of carbon compounds such as polysaccharides, fats and reduction of carbohydrate production. Moreover, differences in fatty acid profile occurring response to various nitrogen concentrations (14 and 15). The nitrogen limitation also resulted in the higher saturation in lipid profiles (increase in C18:1, and decrease in C18:2 and C18:3) and fatty acid reduction (13 and 16). Numerous studies have shown different concentrations of nutrients that have a wide effect on the biochemical composition, growth and morphology of fresh water algae (12 and 17). Nitrogen starvation and limitation, on the other hand, cause very different nitrogen stresses which may lead to various physiological responses.

The green algae *D. cuneatus* is a chlorococcal coenobial (Scenedesmaceae, chlorococcales, chlorophyta) which is widely distributed in freshwaters with moderate temperature. The green algae are highly polymorphic (18). Accordingly in this study, the influence of different nitrogen concentrations on fatty acid profile, total protein, growth rate and chlorophyll content of *Desmodesmus cuneatus* was investigated.

Materials and methods

Desmodesmus cuneatus was obtained from algae bank Artemia and aquatic research institute, Urmia, Iran. Experiments were performed on culture of 450 ml, grown in 500 ml glass flasks. All the glassware and media were sterilized prior to inoculation. Culture was grown in BM medium (Table 1) (19). Stock culture of *D. cuneatus* was performed at 25°C under continuous fluorescent illumination (100 µmol photons m^{-2} s^{-1}) and pH 7.5.

Table 1- The content of BM medium

Constituents	($mg.l^{-1}$)
KNO_3	100
$MgSO_4.7H_2O$	40
$Cl_2Co.6H_2O$	0.110
$MnCl_2.4H_2O$	0.108
$Na_2MoO_4.2H_2O$	0.0075
$ZnSO_4.7H_2O$	0.066
$FeCl_3.6H_2O$	5.88
Ca (NO_3) $_24H_2O$	150
β- Na_2 glycerophosphate	50
EDTA- Na2	2.27
Biotin	0.1
Thiamine- HCL	0.01
Trisaminomethane	0.5

Stress conditions

Initial density for *D.cuneatus* was set on 3.2×10^5 algal cells. Cultures of cells were washed in double- distilled water (DDW) and resuspended in the medium with different concentrations of nitrogen (KNO_3) (5 mM, 2.5 mM, 1.25 mM, 0.62 mM and nitrogen free medium).

In order to assess the cell densities during culture period, cells were daily counted using a haemocytometer. Growth rates and doubling time (DT) were calculated on cell basis which were calculated based on following equation Guillard and Omori.

μg (day^{-1}) = (Ln (n_2/n_1) $/t_2$-t_1); (20) DT = $log_e{}^2/\mu$ (21)

Where μ is the specific growth rate, N_2 and N_1 are biomass concentrations at the start (t_1) of exponential phase ($cell.ml^{-1}$) and at the end of experiment (t_2) respectively.

In order to determine chlorophyll a and b, 5ml acetone was added to each tube containing 5ml of culture and then filtered (using GF/C filters). Each sample was maintained in the dark at 4°C for 24 h, then cells were broken using an ultrasound (on ice) up to 90s and subsequently centrifuged at 12, 000×g for 5 min. Chlorophyll a and b was determined using the equation based on the method of Ritich (22).

Total protein was extracted according to Meijer and Wijffels' method (23). First 500 ml of the algal sample was centrifuged at 4°C at 500 g for 10 min, washed and centrifuged again and then freeze–dried. For protein analysis, 20 mg aliquots of the freeze–dried biomass were suspended for 20 min in 10 ml of lysis buffer in a Falcon tube and then resuspended in 10 ml phosphate buffer with 1% (w/v) SDS (sodium dodecyl sulfate) to facilitate the extraction of proteins. Total protein was determined by means of the method of Bradford, using bovine serum albumin standards (24).Quantitive measurement of fatty acids was performed using GAS chromatography (Agilent 6890 Gas Chromatography device) according to the method of Cohen*et al.* (25). Accordingly, Biomass was transmethylated with 2% H_2SO_4 in the dry methanol/toluene mixture (90:10, v/v) at 80°C for 1.5 h and heptadecanoic acid was added as an internal standard. FA methyl esters were determined by co- chromatography with authentic standards (Sigma) and by comparison of their equivalent chain length (26).

The SDS- PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) method was used for total protein analyses of Algae samples (27).

Two hundred milligrams of each sample were prepared for SDS- PAGE in liquid nitrogen and pulverized in 1.5 ml microtube by appropriate tips. Fifty milligrams of the resulting powder were transferred into 500 µl of protein extraction buffer (buffer K). The extraction buffer for SDS- PAGE contained: 5 mM $MgCl_2$, 5 mM nitrogen H_2PO4, 40mM Hepes, 70mM

Potassium gluconate, and 150mM Sorbitol, and pH was set at 6.5 containing a protease inhibitor cocktail (Invitogene™ Mini from Roche Diagnostics GmbH) (28). Protein concentration was determined by the Bio photometer apparatus (Eppendorf). Samples were heated for 5 min at 95° C and subsequently cooled to room temperature in tap water. After low speed centrifugation (1600 g, 5 min) to remove insoluble fragments, supernatants were electrophoresed. Proteins were separated by 12.5% SDS- PAGE (27). Each lane was loaded with 15 μg total proteins run at 200 V for 45 min. Protein bands were stained with 0.125% Coomassie Brilliant Blue R-250 in 40% methanol, 10% acetic acid for 1 h at a 50°C water bath with gentle agitation. Destaining was performed in 5% methanol, 7.5% acetic acid for 2 h at 50°C with gentle agitation.

The obtained growth rate data were analyzed by one- way ANOVA. Tukey's test was performed to identify differences among the means, and differences were considered statistically significant at *Pvalue*<0.05. SPSS version 18 (IBM Company) was used for statistical analyses.

Results

The effect of five level of nitrogen concentrations on the growth of *D. cuneatus* was investigated for 12 days. The effect of nitrogen concentration was monitored by counting the cells number of *D. cuneatus* (Fig. 1). Based on Fig. 1, during stress, lowest growth rate (0.23 ± 0.06 μgday^{-1}) was observed in the nitrogen free treatment group. On the other hand, maximum specific growth rates (0.35 ± 0.09 μgday^{-1}) and (0.33 ± 0.09 day^{-1}), were observed in groups treated with 2.5 mM nitrogen and 5 mM nitrogen, respectively (Table 2). In 1.25 mM nitroge, the maximum growth rate (0.29 ± 0.05 μgday^{-1}) was observed with density of 22.72× 10^5 cells ml^{-1} (Fig. 1). In 0.62 mM nitrogen, the maximum growth rate was 0.27 ± 0.07 μgday^{-1} (Table 2).

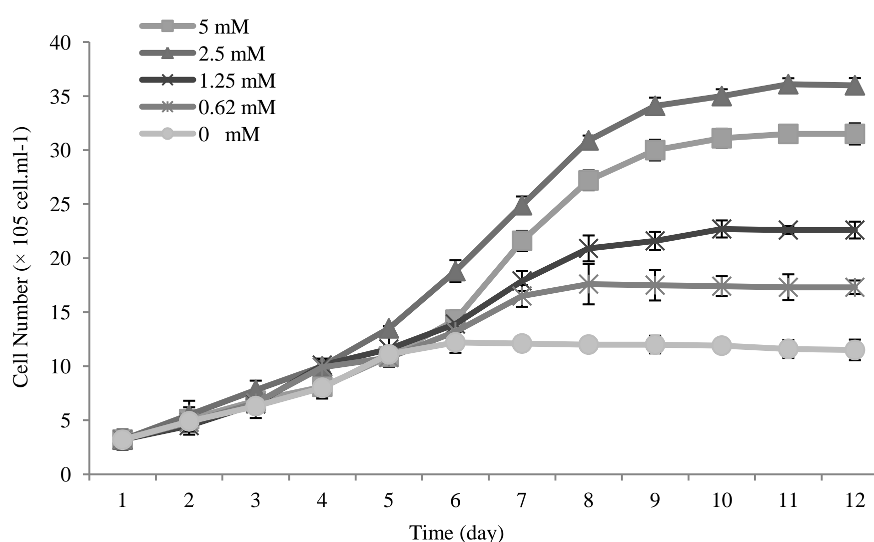

Fig. 1- Cell number of *D. cuneatus*cultured under different nitrogen concentrations

Table 2- Cell number of *D. cuneatus*cultured under different nitrogen concentrations

N concentration (mM nitrogen)	Maximum density ($*10^5$ cells.ml^{-1})	Growth rate, μgday^{-1} (d^{-1})	Doubling time (d^{-1})
5	31.51 ±0.68 ^{a}	0.33 ± 0.09^a	2.14
2.5	36.00 ± 1.01^b	0.35 ± 0.09^a	1.98
1.25	22.72± 0.87^c	0.29 ± 0.05^b	2.39
0.62	17.50 ± 0.86^d	0.27 ± 0.07^b	2.56
0	12.11 ± 0.47 ^{e}	0.23 ± 0.06 ^{c}	3.01

Values sharing the same letter in each row are not significantly different, ANOVA; *P value*>0.05

Table 3- Chlorophyll α, b and protein contents of *D. cuneatus* cultured under different nitrogen concentrations

Concentration mM ntrogen	chl α mg.l^{-1}	chl b mg.l^{-1}	Protein (%)
5	8.01 ± 1.04 ^{a}	6.23 ± 0.43^a	36.81± 1.53 ^{a}
2.5	7.11 ± 1.44 ^{ab}	5.11 ± 0.42 ^{b}	22.13 ± 2.11 ^{b}
1.25	6.41 ± 0.78 ^{ab}	4.51 ± 0.86 ^{b}	18.08± 3.14 ^{c}
0.62	4.62 ± 0.55 ^{c}	2.1± 0.57 ^{c}	19.23± 1.71 ^{c}
0	2.1 ± 0.89 ^{d}	1.3± 0.92 ^{c}	11.14± 2.53 ^{d}

Values sharing the same letter in each row are not significantly different, ANOVA; *P value*>0.05

High pigment values probably attributed to the high cell density. Content of chlorophyll α and b significantly varied (*Pvalue*<0.05) under various nitrogen concentrations. The maximum (8.01 mg.ml^{-1}) and the minimum (2.1 mg.ml^{-1}) chlorophyll a content were measured at 5 mM nitrogen and nitrogen free respectively (Table 3). The chlorophyll b content of the cells also was affected by different nitrogen concentrations. The maximum chlorophyll b (6.23 mg.l^{-1}) was measured at 5 mM nitrogen, while the minimum (1.3 mg.l^{-1}) was observed at nitrogen free treatment (Table 3). Different nitrogen concentrations positively affected protein content (Table 3). The maximum protein concentration was 36.81% at 5 mM nitrogen, and the minimum was 11.14% at nitrogen free.

D. cuneatus showed significant differences in total protein content at various level of nitrogen concentration (Fig. 2). A major protein of approximately 68 kDa was observed in stress conditions. In addition, several proteins with molecular weight of approximately 36, 48 and 60 kDa were observed in 5 and 2.5 mM of nitrogen concentration treatments (Fig. 2).

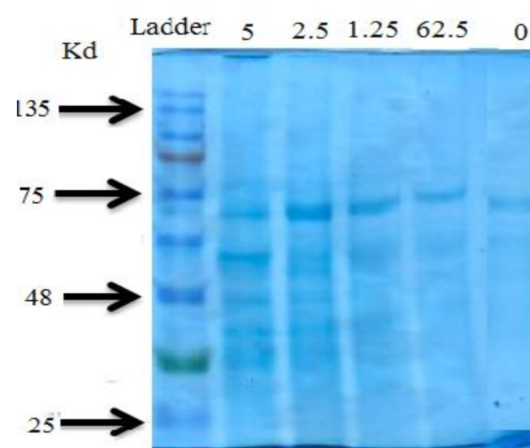

Fig. 2- SDS- PAGE gel electrophoresis of *D.cuneatus* cultured under different nitrogen concentrations

The fatty acid composition of *D.cuneatus* was measured in stationary phase of the cultured cells at different nitrogen concentration levels (Table 4). The most abundant saturated fatty acid was 16:0 (palmitic acid), which constituted 26.01% of the total fatty acids at the nitrogen free treatment. Among the PUFAs, 18:3 (n–3) was the major fatty acid (by 13.51%) of the total fatty acids. The total percentage of PUFA (Polyunsuturated fatty acids) decreased at low nitrogen concentration, so the minimum percentage of PUFA (17.01%) was observed at the nitrogen free treatment group. In contrast, the highest percentage of the PUFA (25.31%) was recorded at the maximum nitrogen concentration (5 mM nitrogen). As for SFA (Saturated fatty acids) and MUFA (Monounsaturated fatty acids), highernitrogen concentration was associated with low percentage of these groups of fatty acids. The maximum percentages of MUFA (22.10 and 23.00%) and SFA (29.89 and 31.54%) were observed at 0.62 mM nitrogen and nitrogen free respectively.

Table 4- Fatty acids (mean ± SD %) (Expressed on the *percentage* of total fatty acids) of *D. cuneatus*cultured under different nitrogen concentrations

Fatty acid	5 mM nitrogen	2.5 mM nitrogen	1.25 mM nitrogen	0.62 mM nitrogen	free nitrogen
C14:00	0.64 ± 0.08^{b}	0.32 ± 0.09^{b}	1.43 ± 0.06^{a}	0.48 ± 0.02^{b}	0.26 ± 0.01^{b}
C16:0	21.99 ± 0.20^{c}	22.81 ± 0.33^{c}	24.64 ± 0.90^{b}	25.72 ± 0.22^{ab}	26.01 ± 7.42^{a}
C18:0	0.61 ± 0.41^{c}	1.16 ± 0.16^{a}	0.51 ± 0.06^{d}	1.01 ± 0.13^{b}	0.98 ± 0.09^{b}
C20:0	0.56 ± 1.84^{c}	0.92 ± 0.07^{b}	$0.24 \pm .04^{d}$	0.91 ± 0.01^{b}	1.15 ± 0.37^{a}
C22:0	1.51 ± 2.15^{a}	1.64 ± 0.13^{a}	1.8 ± 0.02^{a}	1.53 ± 0.06^{a}	2.1 ± 0.19^{a}
C24:0	0.64 ± 1.14^{b}	0.17 ± 0.01^{c}	0.22 ± 0.22^{c}	0.24 ± 0.24^{c}	1.04 ± 0.04^{a}
SAF	25.95 ± 0.76^{c}	27.02 ± 1.06^{c}	28.84 ± 0.97^{b}	29.89 ± 1.22^{b}	31.54 ± 0.66^{a}
C14:1n5	2.10 ± 0.71^{ab}	2.71 ± 0.06^{a}	2.83 ± 0.01^{a}	1.07 ± 0.05^{c}	1.40 ± 0.10^{bc}
C16:1n7	0.37 ± 0.33^{b}	0.51 ± 0.01^{b}	1.15 ± 0.03^{a}	0.36 ± 0.03^{b}	0.12 ± 0.01^{b}
C18:1n9	6.76 ± 0.58^{c}	7.90 ± 2.42^{b}	8.34 ± 0.40^{b}	10.35 ± 1.02^{a}	10.64 ± 0.02^{a}
C18:1n7	10.12 ± 0.79^{a}	9.75 ± 1.10^{a}	7.45 ± 0.11^{b}	9.35 ± 0.11^{a}	10.39 ± 0.79^{a}
C20:1n9	$0.15 \pm .09^{d}$	0.43 ± 0.03^{c}	0.79 ± 0.04^{a}	0.64 ± 0.01^{b}	0.15 ± 0.15^{d}
C22:1n9	-	-	-	-	-
C24:1n	0.24 ± 0.09^{c}	0.1 ± 0.03^{d}	0.51 ± 0.02^{a}	$0.33 \pm .02^{b}$	0.3 ± 0.04^{bc}
MUFA	19.74 ± 0.66^{c}	21.4 ± 0.46^{b}	21.07 ± 0.33^{b}	22.10 ± 0.82^{ab}	23.00 ± 1.1^{a}
C18:2n6	10.7 ± 1.13^{a}	7.81 ± 0.26^{b}	7.50 ± 0.82^{b}	6.61 ± 0.23^{b}	4.79 ± 0.26^{c}
C18:3n3	10.45 ± 0.74^{b}	13.51 ± 0.02^{a}	12.92 ± 0.23^{a}	10.52 ± 0.03^{b}	8.52 ± 0.46^{c}
C20:2n6	-	-	-	-	-
C20:4n6	-	-	-	-	-
C20:3n3	3.01 ± 2.10^{a}	1.29 ± 0.04^{bc}	0.28 ± 0.02^{c}	1.29 ± 0.14^{bc}	$2.32 \pm .10^{ab}$
C20:5n3	0.83 ± 0.08^{ab}	0.04 ± 0.02^{b}	0.21 ± 0.07^{b}	0.34 ± 0.03^{b}	1.22 ± 0.19^{a}
C22:6n3	0.32 ± 0.32^{a}	0.07 ± 0.07^{a}	0.09 ± 0.02^{a}	0.34 ± 0.01^{a}	0.16 ± 0.16^{a}
PUFA	25.31 ± 0.29^{a}	22.72 ± 0.96^{b}	21.00 ± 0.53^{c}	19.1 ± 0.76^{c}	17.01 ± 1.12^{e}

Values sharing the same letter in each row are not significantly different, ANOVA; *P value*>0.05

Discussion and conclusion

Total biomass, quantity and quality of biochemical composition particular fatty acid profile, protein and pigment content, play an important role in commercial scale production of microalgae. Changes in nitrogen concentration have been led to significant differences in pigment and growth rate (29 and 30). The present study has also proved significant differences in the growth rate of *D. cuneatus* cultured under various levels of nitrogen concentrations. The biomass concentration was increased as nitrate source increased in the medium (5 and 2.5 mM nitrogen), in (Fig. 2). This result is the same as the results of Banerjee who found the growth rate in *D. cuneatus* declined under limited nitrogen sources (31). Similarly, some previous studies showed decreasing trend in growth rate for many microalgae culture under low concentration of nitrogen (15 and 32). Yeesang and Cheirsilp reported loss of biomass when green alga *Botrycoccus sp.* was exposed to deficient nitrogen (33). Li *et al.* noticed that the growth rate in *Pavlova viridis* decreased in the nitrogen- free medium while it increased in nitrate- rich media (34). Decrease in algal biomass concentration in low nitrate concentration was also observed by Hu and Gao in *Nannochloropsis* sp. (35). Studies also indicated that in nitrogen free medium, cells start to metabolize pigments to release nitrogen (34).

We found that the chlorophyll α and b content was increased at high nitrogen concentration (5 and 2.5 mM nitrogen). On the contrary, the chlorophyll α and b value was decreased in algae cultivated at low nitrogen concentration (0.62 mM nitrogen and free N). This result is similar to the findings of Ördög *et al.* who noted that high nitrogen concentration (70 and 700 mg. L^{-1}N) led to increase in the chlorophyll α and chlorophyll b contents (36). In contrast, carotenoid concentrations gradually declined. Similarly in *Neochloris oleoabundus,* there was a decrease in chlorophyll α within 2 days and day 4 in low- nitrogen and intermediate nitrogen treatments, respectively. However there was no decrease in higher nitrogen treatment (14). The changes in pigments content are considered to be an adaptation mechanism to various nitrogen concentrations, so in the higher nitrogen concentration, chlorophyll is utilized for faster growth rates and in depleted nitrogen concentration, chlorophyll was degraded to support cell growth and biomass production (14 and 36). The total protein showed a strong positive relationship with nitrogen concentration. The total protein production increased within high nitrogen concentration and minimum percentage of protein content was observed at lower nitrogen concentration (Table 3).

li *et al.* noted that increase in nitrogen concentration caused increase in protein biosynthesis and low nitrogen concentration led to protein decline in *Pavolva viridis* (34).Various species of microalgae shows different responses to the nitrogen stress. For example, *Chlorella vulgaris* and *C. minutissima* have shown large decrease in protein content in response to nitrogen stress (36 and 37). Whereas, Dean *et al.* concluded that the protein content in *Chlamydomonas reinhardtii* and *Scenedesmus subspicatus* showed a minimal change in response to

nitrogen limitation (38). Also under nitrogen limitation, carbon reallocation resulted in a decrease in cellular protein such as Rubisco and an increase in lipids (16, 39 and 40). Several groups of proteins with different molecular weigth were accumulated under nitrogen stress condition. The most abundant protein in our study was a 68 kDa protein; while in *Haematococcus pluvialis* growing under low and high nitrogen concentration, proteins with molecular weigth of 38kDa-63kDa were accumulatedin the cultured cells. Conversely, in nitrogen starvation condition, proteins with molecular weigth of approximately 28kDa were accumulated in *Chlamydomonas reinhardtii* (41).

Changes in fatty acid composition in microlagae have been previously reported in response to changes in nitrogen concentration (4, 17, 30, 34 and 42). We detected 17 fatty acids in *D. cuneatus* and observed that cellular content of fatty acids ranged from 0.07 to 26.01% in different treatments. In the present study, as nitrogen concentration increased, the ratio of the total saturated and monounsaturated fatty acids decreased, and in turn the polyunsaturated fatty acids increased. Maximum PUFA contents were found at high nitrate concentration (5 mM nitrogen). This result is similar to the findings of Chu et al. (42). Similar to Li et al. who reported the increasing level of saturated and monounsaturated fatty acids in nitrogen depletion condition of *Pavolva viridis*culture (34). In *Nannochloropsis* sp., the response to nitrogen starvation was a decrease in polyunsaturated fatty acids and an increase in the saturated FA (15). Other studies have found that nitrogen limitation alters the lipid profile towards higher saturation (increase in C18:1, and decrease in C18:2 and C18:3) and further reduction in fatty acids chain (13 and 16). The effect of nitrogen on the cellular fatty acid composition seems to be quite complex and appears to be different according to species. For example, Hu and Gao showed that both low and high nitrate conditions resulted in higher amount of EPA on a dry- mass basis (35), whereas *Parietochloris incisa* revealed increase in arachidonic acid at lower nitrogen level (9). li et al. reported the highest EPA content in total fatty acids at the maximum nitrate (6.2 mM) (34). Thompson noted that under nitrogen deficiency, many microalgal species accumulate lipids mostly TAG (triacylglycerols) which generally contain saturated and monounsaturated fatty acids (43).

The results showed that control of nutrients plays an important role in microalgae culture. Any increase or decrease, depending on the type of nutrient, causes metabolic changes as well as changes in physiology and the nutritional value of microalgae. The nitrogen concentration of 2.5 mM nitrogen may be more beneficial than other concentrations, as cell number is sustained in exponential phase longer.

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واکنش میزان رشد، محتوای پروتئین و اسیدچرب جلبک دسمودسموس کوناتئوس درشرایط کمبود و انباشتگی نیتروژن

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چکیده

مقدمه: میزان بیومس و محتوای بیوشیمیایی (به‌ویژه اسیدهای چرب، پروتئین و رنگدانه‌ها) جلبک‌های تک سلولی نقش مهمی را در تولید تجاری این میکروارگانیسم ایفا می‌کنند. نتایج پژوهش‌ها نشان داده هست که غلظت نیتروژن از عوامل مؤثر بر میزان رشد و ترکیب بیوشیمیایی جلبک‌های تک سلولی است.

مواد و روش‌ها: در این پژوهش، ریز جلبک دسمودسموس کوناتئوس (خانواده سندسماسه، کلروفیتا) در محیط کشت BM، دمای ۲۵ درجه سانتی گراد و اسیدیته ۷ تحت تأثیر غلظت‌های مختلف نیتروژن در یک دوره ۱۲ روزه پرورش داده شد. به منظور تعیین تراکم سلولی و زمان دو برابر شدن جمعیت ریزجلبکی، شمارش جلبک‌ها به شکل روزانه و با استفاده از لام هموسیتومتری انجام گرفت. تعیین میزان اسیدهای چرب و پروتئین ریزجلبک‌ها نیز با برداشت توده جلبک در مرحله سکون انجام شد.

نتایج: بالاترین میزان رشد ویژه (۰/۰۹±۰/۳۵ در روز) و تراکم سلولی (10^5×۱/۰۱±۳۶ سلول در میلی‌لیتر) در غلظت ۲/۵ میلی‌مولار نیتروژن به دست آمد. همچنین، متوسط بیش‌ترین میزان اسیدهای چرب با چند پیوند غیراشباع، ۲۵/۳۱ درصد، در غلظت بالای نیتروژن (۵ میلی‌مولار) و کم‌ترین مقدار، ۱۷/۰۱ درصد، در تیمار فاقد نیتروژن مشاهده شد. بیشینه میزان پروتئین، ۳۵/۸۱ درصد، نیز در غلظت ۵ میلی‌مولار و کمینه آن، ۱۱/۱۴ درصد، تیمار فاقد نیتروژن به دست آمد.

بحث و نتیجه‌گیری: نتایج این مطالعه نشان داد که غلظت مواد مغذی مختلف نقش مهمی در پرورش ریز جلبک‌ها داشته و بسته به نوع مواد مغذی هر گونه کاهش یا افزایش در غلظت آن تغییراتی را در فیزیولوژی و ارزش غذایی ریزجلبک‌ها موجب می‌شود. در این مطالعه، غلظت ۲/۵ میلی‌مولار نسبت به سایر غلظت‌ها در عوامل مؤثر مورد مطالعه همانند میزان رشد نتایج بهتری نشان داد.

واژه‌های کلیدی: اسیدهای چرب، *دسمودسموس کوناتئوس*، پروتئین، رشد، نیتروژن

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