

Short Communication

Enhance growth and biochemical composition of *Nannochloropsis oceanica*, cultured under nutrient limitation, using commercial agricultural fertilizers

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Abstract:

Microalgae culture media should be economic, allow for high growth, satisfy the needs of microalgal cells and easy to prepare. In this study, we evaluate the effect of different media formula prepared from commercial agricultural fertilizers (CAGF), comparing to F/2 Guillard standard medium as a control medium, on growth (cell density, CD; dry weight, DW and specific growth rate, ?) and biochemical composition (lipid, protein, and carbohydrate) of *Nannochloropsis oceanica*. Comparing to N/P ratio (9.6) and actually quantity (12.36 g/l and 1.29 g/l, respectively) of F/2 standard medium, six N/P ratios (19.2, 9.6, 9.6, 4.8, 3.2 and 1.6) were prepared from Nitric Acid (N-Nt) or Ammonium Sulphate (N-Am), as a nitrogen source, with phosphoric acid (P), as a phosphorus source, for culturing media of *N. oceanica*. The results investigated that some CAGF media achieved significant ($P < 0.05$) growth and biochemical composition higher than F/2. Comparing to lipid percentage (30.70 %) of F/2, the lipid percentage of *N. oceanica* cultured on different CAGF media were ranging from 18.40% to 46.12%, depending on nutrient limitation, nitrogen source, N/P ratios and actually atom concentrations. Finally, the use of CAGF constitutes a viable alternative of F/2 medium to reduce the production costs *N. oceanica*, the commonly used in marine hatcheries and also other biotechnological applications.

Keywords

N. oceanica; Aquaculture; Lipid; Biodiesel; Agricultural fertilizers; Nitric acid

Introduction

Nannochloropsis is considered the main algal species cultured in marine hatcheries and play an important role in aquaculture development. In the industrial production scale in marine hatcheries, it is very important to optimize a suitable nutrient culture media for culturing this species. The microalgae nutrient media should be prepared easy, economical, achieve high growth and satisfy all the microalgae quality and quantity. Although F/2 Guillard medium is considered the most commonly medium used in culturing of *Nannochloropsis* in marine hatcheries, F/2 Guillard medium have some disadvantages, like

difficult to prepare and set up for outdoor mass culture and expensive. Microalgae production cost as a live food produced in marine shellfish and shrimp hatcheries are about 30% of the total seeds production cost. The high cost of microalgae production remains an obstacle for marine hatcheries [2]. Therefore, commercial agricultural fertilizers (CAGF) should be commonly used instead of F/2 culture medium [3]. As aquatic organisms, microalgae need water, salts and CO₂ 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 [4]. Many authors noticed that the biochemical composition of microalgae modify as a function of nutrient limitation. However, nutrient limitation may increase the lipid content capacities of some microalgae, by 30-60% of the dry cell weight. Among these factors, nitrogen and phosphorus have strong effects on lipids metabolism in various microalgae [5]. The present study aims to find the economical nutrient medium prepared from CAGF and has the positive effect on growth and lipid content of *Nannochloropsis oceanica*, the main species in marine hatcheries, in regarding to reduce the production cost of marine larvae.

Material and Methods

Microalgal strains *Nannochloropsis oceanica* strain was isolated from East-south of the Mediterranean Sea (31° 13' 48" N, 29° 53' 12" E), Eastern Harbor of Alexandria, Egypt, and cultured in Microalgae Lab., Invertebrate Aquaculture Lab., Aquaculture Division, Alexandria branch, National Institute of Oceanography and Fisheries (NIOF), Egypt. *N. oceanica* were kept and cultured under controlled conditions of temperature (22 ± 2°C), salinity (35 ± 2 ppt), and illumination (750-3000 Lux /24 h.) using F/2 Guillard and Ryther [6], with continuously aeration. Experimental design Depending on the atomic mass weight (g/mol) and total quantity (g/l) of N and P that used in F/2 Guillard medium stock solution, different atomic mass weight (g/mol) and quantity (g/l) of N and P were prepared from Nitric Acid (N-Nt) or Ammonium sulphate (N-Am), as nitrogen source, in combination with phosphoric acid (P), as phosphorus source. The compositions and preparing cost of F/2 standard Guillard medium and other CAGF media were presented in Table 1. In addition to nitrogen and phosphorus sources, vitamin B1 and B12 (in form of local human pharmacy ampoules, commercially named Tri-B, produced by The Nile Co. for Pharmaceuticals and Chemical Industries, Cairo, Egypt) were added in the concentration of 100 µg/l and 1000 µg/l, respectively. All treatments were conducted without aeration in conical flasks 250 ml filled with 100 ml of culture medium (three replicates for each treatment medium) under controlled conditions of temperature (21 ± 1°C), salinity (35ppt), and continuous illumination (1000 Lux/24 h). The samples for growth parameters and biochemical analysis were taken at late exponential phase. All experiments were ended when the growth rate had reached to the death phase.

Results

The effect of different nutrient medium prepared from CAGF, comparing to F/2 medium, on the growth and biochemical composition of *N. oceanica* were shown in Table 2. The results investigated that some CAGF media achieved significant ($P \leq 0.05$) growth and biochemical composition higher than F/2 while other CAGF media achieved significant ($P \leq 0.05$) growth and biochemical composition lower than F/2, depends on nutrient limitations, nitrogen source, nitrogen and phosphorus ratios and atomic concentrations. . These significant differences may be due to N/P ratios, concentrations and sources. However, to optimize the production of *N. oceanica* for aquaculture purposes in marine hatcheries, CAGF should be used with advantages of reduced cost media, high productivity and easy to prepare of culture medium. Our suggestions were in agreement with Guzman-Murillo et al. who suggested that, CAGF media may be used to improve the biochemical composition of microalgae for the purposes of aquaculture, production of bioactive materials and biotechnology. Bae and Hur, (2011) found that the growth of *N. oceanica* cultured on fertilizer medium was similar to that of *N. oceanica* cultured in F/2 medium. On the other hand, our results disagree with Simental and Sanchez-Saavedra (2003) [22] who pointed that, comparing to F/2 medium, the using of liquid CAGF did not achieve any significant differences in cell concentration and growth rate of *Navicula incerta*, *Nitzschia thermalis* and *Nitzschia laevis*. This disagree may be due to the experiment conditions, N/P ratios, concentrations and sources. In F/2 medium, the nitrogen (in form of sodium nitrate) and phosphorus (in form of sodium hydrogen orthophosphate.) concentrations in medium stock solution were 12.36 g/l and 1.29 g/l, respectively, and 0.0124 g/l and 0.0013 g/l in microalgae culture solution, respectively, with ratio N/P (9.6). Hsieh and Wu [23] reported that nitrogen sources were strongly affecting microalgae quality and quantity. Our study investigated that in the case of nitric acid, the treatment medium N-Nt100+P100, which has the same N/P ratio and concentrations of F/2 medium, achieved growth (cell density, dry weight and specific growth rate) higher than F/2 and/or ammonium sulphate nitrogen based media. Until now, there is no recorded data available about using of nitric acid as nitrogen source in medium composition of marine microalga *N. oceanica*. To date, nitrate is a commonly studied as a nitrogen source used to understand nutrient limitation to induce lipid accumulation [24]. In the current study, we compare ammonium sulphate and nitric acid, as CAGF nitrogen sources, with sodium nitrate used in F/2 medium. The results illustrated that nitric acid candidate to be best investigated nitrogen source achieved the highest quantity (cell density, dry weight and growth rate) of *N. oceanica*, while ammonium sulphate candidate to be best investigated nitrogen source achieved high lipid content of *N. oceanica*, depending on (1) N/P ratio, (2) N, P concentrations, and (3) N, P sources. From our results we concluded that *N. oceanica* may be need to a specific N/P ratio, concentration and sources to achieve the highest significant growth and/or biochemical composition. However, the results suggests that in the algal strain *N. oceanica*, the optimal N/P ratio, concentra-

tion and sources that achieved the highest growth may be not the optimal to achieve the highest biochemical composition. Finally, from our results we can conclude that to determine the optimal N/P ratio, concentration and sources for each species, we should examine that according to the final purpose of culture. According to literature, *Nannochloropsis* have a wide range of lipid percentage (28% to 68.5%), as shown in Table 3, and dry weight (0.05 to 2.67 g/l), as shown in Table 4, which make *Nannochloropsis* have respect for biotechnological applications, beside its important role as a live food source in marine hatcheries. The production cost of microalgae culture mediums is another favorable factor should be discussed. Microalgae productivities (lipid and biomass) are the most important key parameters affecting the economic feasibility of microalgae production in marine hatcheries, as well as in the industrial scale [25]. Simental and Sanchez-Saavedra (2003) reported that the production cost of microalgae cultured on CAGF is lower than F/2 medium about eight times. According to our conditions, sodium nitrate used in F/2 Guillard medium is much expensive more than CAGF, however, some CAGF media achieved *N. oceanica* quality and quantity higher than F/2 standard medium, with advantage of reduced production cost [26]. Finally, our results reported that the cost of CAGF media (prepared from nitric acid and/ or ammonium sulphate in the same N/P ratio and concentration of F/2 standard medium) was about 1/37:1/39 times lower than F/2 culture medium.

Conclusion

From the present study we can concluded the following: 1. To reduce production cost and enhance quality and quantity of *N. oceanica*, CAGF may be used rather than F/2 medium 2. To increase lipid cell content of *N. oceanica*, CAGF of ammonium sulphate and phosphoric acid must be considered, depending on N/P concentrations and ratio. 3. Microalgae nutrient medium prepared from CAGF, have many advantages more than F/2 standard medium, like (1) easy to prepare, (2) reduce production cost, as well as depending on N/P concentrations and ratio, (3) increase dry weight, (4) increase cell density, and (5) enhance lipid percentage.
