Influence of nitrogen and phosphorus concentrations for the intensive cultivation of marine microalga, *Nannochloropsis gaditana*, in the local conditions of M’diq, Morocco.

Imane Haoujar¹,³*, Jamal Abrini¹, Housni Chadli², Kamal Chebbaki³, Francesco Cacciola⁴*, Nadia S. Senhaji¹

1. Laboratory of Microbiology and Applied Biotechnology, Department of Biology, Faculty of Sciences of Tetouan, Abdelmalek Essaadi University, Morocco
2. AQUA M’DIQ, Port of M’diq, Morocco
3. Specialized Center in Zootechnics and Marine Aquaculture Engineering, National Institute of fisheries Research, Morocco
4. Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali, via Consolare Valeria, Italy

*Corresponding authors: cacciola@unime.it; imane.haoujar2015@gmail.com

**ARTICLE INFO**

**Article History:**
Received: April 6, 2020
Accepted: April 28, 2020
Online: April 30, 2020

**Keywords:**
*Nannochloropsis gaditana*; Sodium nitrate; Sodium dihydrogen phosphate; biomass growth.

**ABSTRACT**

The influence of nitrogen and phosphorus of different concentrations on biomass production of the green microalga *Nannochloropsis gaditana* was investigated. The result revealed that there was a significant difference among nitrogen concentrations and among phosphorus concentrations in promotion of algal biomass growth. Sodium nitrate (150 g/L) and sodium dihydrogen phosphate (10 g/L) were found to be preferred concentrations of nitrogen and phosphorus with a cells growth 71.375×10⁶ cells/mL and 46.787×10⁶ cells/mL, respectively.

**INTRODUCTION**

Currently, biodiesel production from vegetable oils has been improved and getting considerable attention as a friendly alternative to fossil fuels. As a result, the use of algae as an alternative source for biofuels helps to maintain the stability of land-based crop production (Chisti, 2008; Griffiths and Harrison, 2009; Weyer et al., 2010; John et al., 2011). Interestingly to mention that the lipid stock in microalgae can arrive at 50-60% of their dry cell weight and that can increase by the influence of environmental and nutritional conditions (Illman et al., 2000; Hu et al., 2008; Breuer et al., 2012; Lin et al., 2012). However, lipid accumulation induced by environmental stress often corresponds to reduced productivity of biomass (Rodolfi et al., 2009). The potential
application of *Nannochloropsis* species to biofuel production has been reported by various studies (*Carrero et al.*, 2011; *Jazzar et al.*, 2015; *Peña et al.*, 2015). This microalga characterized by their high biomass accumulation rate, high lipid content, successful cultivation at large scale using natural sunlight by companies such as Solix Biofuels, Aurora Algae, Seambiotic, Hairong Electric Company/Seambiotic and Proviron (*Radakovits et al.*, 2012). The culture medium influenced directly on the accumulation and lipid production by the microalga. Nitrogen was quantitatively the most important nutrient that affects the biomass growth and lipid production from the microalgae (*Griffiths and Harrison*, 2009; *Xin et al.*, 2010). However, few studies are available on the effect of nitrogen and phosphorus sources on the growth and lipids production of the green alga *Nannochloropsis*. Therefore, the interaction of the different concentrations of the two elements on the cell growth of *Nannochloropsis gaditana* was evaluated.

### MATERIALS AND METHODS

To evaluate the parameters that control the species of microalgae studied; the culture is inoculated by 250 mL of *Nannochloropsis gaditana* sample in exponentially growing. An enriched air stream containing 5% of CO₂ was passing through a water bottle, and then a flow rate of 0.5 vvm was filtered using 0.47 μm filter, before bubbling from the bottom into the culture bottle. The culture medium Gillard F/2 was added in sterilized seawater with a concentration of 1 mL in Erlenmeyer flasks of 1L. The temperature inside the culture chamber was controlled and was set at 21 °C (*Tahiri et al.*, 2000).

**Nitrogen**

The experiment was initiated by 250 mL of *Nannochloropsis gaditana* species of 30 × 10⁶ cells/mL using culture medium without nitrogen for 10 days. The experiment evaluates sodium nitrate (NaNO₃) as a source of nitrogen in order to determine the algal growth during 24 days. Five concentrations of sodium nitrate were prepared in this experiment; 0, 150, 300, 400 and 450 g/L.

**Phosphorus**

The second parameter used in this study as a source of phosphorus was sodium dihydrogen phosphate (NaH₂PO₄). The experiment was initiated in the same laboratory conditions, using four concentrations; 0, 10, 20, and 30 g/L.

**Monitoring of the microalgal culture**

During the experiment, a daily count of algal cells density was performed by a Brker-Türk using an optical microscope.

**Statistical treatment**

The results were analyzed by ANOVA one-factor with a significance level (p<0.05). The individual averages were compared using Tukey's method.
RESULTS

Culture of *Nannochloropsis gaditana* species in different concentration of sodium nitrate

The densities of cells that cultivated in 150g/L, 300g/L and that cultivated as control medium were developed from 2.563×10^6, 2.44×10^6 and, 2.63×10^6 cells/mL to 71.375×10^6, 61.31×10^6, and 68.06×10^6 cells/mL, respectively (Fig.1A).

Culture of *Nannochloropsis gaditana* species in different concentration of phosphorus

In this experiment, the culture medium with 10 g/L of sodium dihydrogen phosphate, registered the highest cell density when compared with that used as control (30g/L); with 46.787×10^6 and 41.923×10^6 cells/mL, respectively (Fig.1B).

![Fig.1](image)

**DISCUSSION**

Sodium nitrate element is important in the growth of microalgae though biological tests on unicellular algal biomass in clear water, and result by a significant difference Robert *et al.* (1982). The presence of NH₄⁺ and NO₃ in the same culture medium induce an inhibition of the nitrate uptake by the ammonium Maestrini *et al.* (1982). Therefore, the absorption of nitrate occurred after the consumption of NH₄⁺. This inhibition is due to the exhaustion of ammonium nitrate reductase (Hattori, 1962; Morris and Syrett, 1963). The total conversion of nitrate to intracellular nitrogen affects the limitation of cells growth; for that, using nitrogen concentrations at the norms supports regular cell growth of microalgae species Fábregas *et al.* (1998). Furthermore, a faster cells growth of *Nannochloropsis gaditana* species required a low concentration (0.5 mg N/A) of nitrates, nitrites or ammonia Lubián (1982). Phosphorus plays an essential role in metabolic pathways by regulating cell division. The cellular concentration of phosphorus as an indispensable element of the cell has a direct influence on the production of biomass.
Also, Chen et al. (2011) study showed that the phosphorus element occurs during many metabolic processes. Therefore, the use of a culture medium containing a lower phosphorus concentration will be possible without compromising the cell growth of Nannochloropsis gaditana. In addition, Sancho et al. (1997) have shown that the affinity of microalgae for the substrate (phosphate) varied from one genus to another. The genus Scenedesmus, requires 1-6 μM, while the genus Chlorella requires 4-5 μM.

**CONCLUSION**

The results obtained during these experiments lead us to the conclusion that the values of the relative growth percentage of Nannochloropsis gaditana obtained under the controlled conditions are 150 g/L of nitrogen (NaNO₃), 10 g/L of phosphorus (NaH₂PO₄) instead of 300 g/L and 30 g/L, respectively.

**REFERENCES**


Influence of nitrogen and phosphorus on *Nannochloropsis gaditana*


