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

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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 71.375×10^6 cells/mL and 46.787×10^6 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 CO_2 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 30×10^6 cells/mL using culture medium without nitrogen for 10 days. The experiment evaluates sodium nitrate ($NaNO_3$) 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 (NaH_2PO_4). 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-Tϋrk 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).

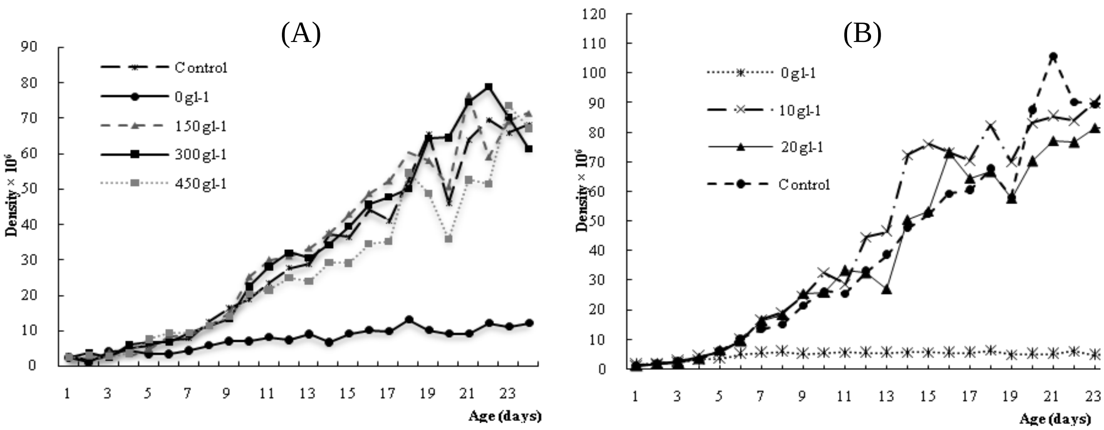

Fig.1. (A) Cell density growth rate of *Nannochloropsis gaditan* grown in different concentrations of $NaNO_3$; (B) Cell density growth rate of *Nannochloropsis gaditan* grown in four concentrations of NaH_2PO_4

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 NH_4^+ and NO_3 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 NH_4^+. 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*, requires1-6 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 150g/L of nitrogen ($NaNO_3$), 10g/L of phosphorus (NaH_2PO_4) instead of 300 g/L and 30 g/L, respectively.

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