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# Growth and fatty acid profile of the marine microalga *Picochlorum* Sp. grown under nutrient stress conditions

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**Abstract** Growth, proteins, carbohydrates and chlorophyll a as well as fatty acid compositions of a native marine microalga *Picochlorum* Sp. were studied in batch culture at light intensity 100 μ mol photons $m^{-2}$ $s^{-1}$, temperature 25 ± 1 °C and 16:8 h light and dark diurnal cycles using Walen medium. Under nutrient stresses, the cell counts and biomass productivity of the tested alga decreased as compared by control culture after 12 days. Carbohydrate content increased by nearly 21%, and 44% in the cultures grown in media supplemented with (−50% & −100% $NaNO_3$), respectively. The proteins showed a remarkable decrease by 54% and 69.7% under the same conditions, respectively. chl *a* contents of *Pichochlorum* Sp. culture grown under N-starvation (−100%) decreased and also, a yellowish colour was recorded in the culture. The lipids increased by about 0.55, 1.6 folds in the cultures growing on the medium containing (−50% & −100% $NaNO_3$), respectively and a decrease by 0.6% in those growing on the media containing nitrogen (+100% $NaNO_3$), respectively. Considering phosphorus stress, the carbohydrate content increased by nearly 30.27%, 62.38% for the culture growing on −50% and −100% $NaH_2PO_4{\cdot}2H_2O$ with respect to the control culture. The proteins showed a remarkable decrease by 24.5%, 37.3% in the culture medium containing the same concentrations, respectively. On the other hand, phosphorus deficiency (−50%) caused an increase in the chl *a* level of the cultures. Similarly, the lipids increased by about 2.2% and 2.5 folds in the cultures growing on the phosphorus deficient media (−50% & −100%), respectively. Fatty Acid Methyl Ester (FAME) was also found to be improved in algal cultures grown under nitrogen & phosphorus stress (−50% & −100%) which are mainly saturated fatty acids. The unsaturation of the FAME profile is crucial for the overall performance of the final produced biofuel. With further augmentations of lipid & carbohydrate content and improved fatty

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acids, the native microalga strain could be a potent candidate for aqua-culture feeding and or biofuel production.



## Introduction

Microalgae biomass contains products with high commercial importance like proteins, lipids, carbohydrates (Torzillo and Vonshak, 2004).

Nutrient availability has a significant impact on growth and propagation of microalgae and broad effects on their lipid and fatty acid composition. Environmental stress condition when nutrients are limited, invariably causes a steadily declining cell division rate. Surprisingly, active biosynthesis of fatty acids is maintained in some algae species under such conditions, provided there is enough light and $CO_2$ available for photosynthesis (Thompson, 1996).

Nitrogen and phosphate are two important macronutrients for growth and metabolism of algal cells. Nitrogen is a fundamental element for the formation of proteins and nucleic acids. Being an integral part of essential molecules such as ATP, the energy carrier in cells, phosphate is another very important nutrient. Phosphate is also a part of the backbone of DNA and RNA, which are essential macromolecules for all living cells. Phosphorus is also a key component of phospholipids. It is not unusual for algae to become nutrient-limited (*i.e.*, nitrogen- and phosphorus-limited) in the natural environment (Harris, 1986).

One who has grown microalgae under laboratory or outdoor condition is well aware of the fact that to obtain high lipid content, external stress or lipid induction techniques need to be applied. Many microalgae produce saturated and unsaturated fatty acids naturally under ideal growth conditions, which have high nutritional value, but are less ideal for biofuels. However, the synthesis of neutral lipids in the form of Triacylglycerides (TAGs) can be induced in many species under stress conditions. (Miao and Wu, 2006; Hu et al., 2008). There has been a wide range of studies carried out on lipid induction techniques in microalgae such as the use of nutrients stress, including nitrogen and/or phosphorus starvation (Juneja et al., 2013). Due to the high growth rates and ease of cultivation Chlorophyta is considered to be a promising phylum for biofuel production.

The green alga Picochlorum Sp. was considered previously as promising feed stocks for large scale production of biofuels (De la Vega et al., 2011). The unsaturation of the FAME profile is crucial for the overall performance of the final produced biofuel. For instance, biodiesel is mainly constituted of SFA and MUFA, since PUFA decrease the final stability of biodiesel. Furthermore, the fatty acid methyl ester profile of Picochlorum Sp. seems ideal for biodiesel production due to a low degree of polyunsaturated fatty acid methyl esters and high amount of palmitic and oleic acids (Demirbas, 2009; Pereira et al., 2013).

Therefore, the main objectives of this study were: (i) monitoring the growth of Picochlorum Sp. under normal conditions and nutrient stress ones i.e. nitrogen and Phosphorus, (ii) estimation of cellular contents of proteins, carbohydrates, chlorophyll and lipid as well as fatty acid profile in the algal species under nitrogen limitation and phosphorus limitations.

## Materials and methods

### *The alga strain and growth conditions*

The alga strain *Picochlorum* Sp. was obtained from Southern Company for Fish Farming, Djerba, Tunisia. It was cultivated axenically as batch cultures in 1 L Erlenmeyer flasks with Walens medium (Walne, 1970) at an initial counts of $10 \times 10^6$ colony $ml^{-1}$. For the production of biomass, exponentially growing algae culture was transferred into fresh sterile medium [10% (v/v) of inoculums]. Cultures were illuminated by tubular fluorescent lamps (PHILIPS Master TL-D 85 W/840). The light intensity at the surface of the culturing vessels was 100 μ mol photons $m^{-2}$ $s^{-1}$ with a photoperiod of 16:8 h light: dark at 25 ± 1 °C.

The effect of different nutrients namely $NaNO_3$ [(control (20.0 g $L^{-1}$), −100% (0 g $L^{-1}$), −50% (10 g $L^{-1}$) and +100% (40 $L^{-1}$)], $NaH_2PO4{\cdot}2H_2O$ [(control (100 g $L^{-1}$), −100% (0 g $L^{-1}$), −50% (50 g $L^{-1}$) and + 100% (200 g $L^{-1}$), on growth and biochemical composition of *Picochlorum* Sp. were studied.

### *Monitoring of algal growth*

The growth of alga was monitored by determining the cell density using a haemocytometer slide, where cell numbers were estimated at 48 h intervals in addition to the determination of algal cellular dry weight (CDW) and biomass productivity that was calculated as according to Abomohra et al. (2012).

Biomass productivity g CDW $L^{-1}$ $d^{-1}$ = ($CDW_L - CDW_E$)·($t_L$ _ $t_E$) with $CDW_E$ represents the CDW (g $L^{-1}$) at days of early exponential phase ($t_E$) and $CDW_L$ at days of late exponential phase ($t_L$). Biomass was determined as cellular dry weight (CDW) and measured gravimetrically. A known volume of culture was filtered through pre-weighed and pre-combusted GF/C filter paper. The filtered cell mass was oven dried at 80 °C for 6 h until constant weight, cooled down to room temperature in desiccators and measured the dry weight of the sample using an analytical balance with a precision of 0.1 mg. Biomass was expressed in grams per litre (g $L^{-1}$).

### *Biochemical composition of Picochlorum Sp.*

Total protein was extracted from the algal cells, according to the method of Rauch, 1981. Protein content, both total and water-soluble, was determined according to Hatree (1972). Chlorophyll a (chl *a*) content of an algae was determined spectrophotometrically after extraction with acetone (Parsons and Strickland, 1963). Carbohydrate content was estimated according to the methods of Dubois et al. (1956). The total