COMPARING METHODS IN PICOPLANKTON ABUNDANCE ESTIMATION

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Abstract

In order to test and compare different methods for picoplankton abundance estimation, a 20-day growth experiment of marine *Picochlorum* sp. was conducted. Cells were harvested daily and its abundance was estimated using three methods (i) counting cells with Birken-Türk haemocytometer, (ii) flow cytometry and (iii) estimation of biomass through Chlorophyll *a* concentrations. Chl *a* concentration showed more similar trend as haemocytometer count suggesting the need for optimisation of each method when higher densities are considered.

Keywords: Analytical methods, Biomass, Chlorophyll-A, Phytoplankton, South Adriatic Sea

Introduction

Picochlorum sp. is a unicellular halotolerant picoalga (Trebouxiophyceae) that has been used multiple times for investigations of its biotechnological properties and potential usage in industry [1]. Importance of marine picoalgae in general have been recognized since their discovery in late 1970's as the "missing link" in the controversial carbon supply since they can contribute greatly to global carbon cycling, biomass and productivity in the sea [2]. Since their importance and challenges in its detection and biomass estimation due its size, we performed a study using *Picochlorum* sp. as model organism to distinguish the best-fit method for accurate estimation of its abundance/biomass during long term cultivation.

Material and Methods

Picochlorum sp. was isolated from south-eastern Adriatic Sea, Croatia and taxonomically identified using nuclear 18S rDNA and chloroplast 16S rDNA phylogeny. Xenic strain PMFPPE4 was used for laboratory growth rate experiment during 20 days. Growth was maintained in Guillard's F2 Marine Water Enrichment Solution (Sigma-Aldrich, United Kingdom) under constant conditions: temperature – 22°C to 22.5°C; light – 30 µmol photons m^{-2} s^{-1} with photoperiod of 16 h of light: 8 h of dark; continuously shaking on Orbital Shaker OR100 (Cole Parmer, UK) at the shaking frequency 80 rpm for 12 h during the day. Starting inoculate of Picochlorum sp. (cca. 10^6 cells mL^{-1}) was established in 200 mL Erlenmeyer flasks in triplicates. Cells were harvested daily and analysed with three different methods. For abundance estimation, cells were examined under inverted light microscope (Olympus BX51TF (Olympuse corporation, Japan) equipped with camera ARTCAM-300MI (Artray Co. Ltd, Japan) and counted using Birken-Türk haemocytometer chamber. Additionally, 1 mL of each triplicate from fresh culture was preserved with 0.1% glutaraldehyde (final conc.), deep frozen in liquid nitrogen, stored at -80°C and analysed with FACSCalibur flow cytometer (Becton Dickinson, San Jose, California). The samples were diluted to approx. same densities with F2 to avoid coincidence, and count was kept below 800 events/s. Number of cells mL^{-1} was then calculated and standard deviation (SD) was included in generating the growth rate graph. For HPLC analysis 1 mL of culture filtered through 0.7-µm-pore-size GF/F filters with syringe and a filter holder (Whatman, United Kingdom) and flesh frozen in liquid nitrogen. Chl *a* concentration was determined by reversed phase HPLC following the protocol of Barlow et al. [3]. Extracts were mixed 1:1 (v/v) with 1 M ammonium acetate and injected into an HPLC system equipped with 3 mm Thermo-Hypersil column MOS2 (C-8, 120 A pore size, 150 × 4.6 mm) (Thermo-Hypersil-Keystone).

Results and Discussion

Growth curve of *Picochlorum* sp. during 20-day experiment is shown in Fig 1. *Picochlorum* sp. showed acclimatization and steady growth during first 7 days of culturing after which entered exponential phase of growth that lasted until day 14. Afterwise stationary phase occurred with day 15, when cells started to aggregate on the bottom of Erlenmeyer flasks. Average daily growth was $1 \times 10^6 \pm 3 \times 10^5$ cells mL^{-1} (haemocytometer counts) and $2 \times 10^6 \pm 4 \times 10^5$ (flow cytometer counts). Average daily abundances in acclimatization ($9 \times 10^6 \pm 6 \times 10^5$), exponential ($3 \times 10^7 \pm 2 \times 10^6$) and stationary ($4 \times 10^7 \pm 2 \times 10^6$) phase according to haemocytometer counts were higher than those counted by flow cytometer: $2 \times 10^6 \pm 5 \times 10^5$ (stationary), $2 \times 10^7 \pm 3 \times 10^6$ (exponential) and $3 \times 10^7 \pm 5 \times 10^6$ (stationary). According to Chl *a* concentrations, daily concentrations in batch cultures during acclimatization phase were 5.84×10^4 ng L^{-1} after which concentrations increased during exponential phase (2.15×10^5 ng L^{-1}), and stabilized (5.14×10^5 ng L^{-1}) in stationary phase. Likewise, large peaks in Chl *a* concentrations after day 12 (as observed in haemocytometer counts) can be explained different behaviour of cells observed in older cultures (i.e. cultures that are in stationary/dying phase). The standard deviation of data obtained from triplicate by flow cytometer counts increases after 12 days, when cell densities are higher. This suggests that in spite of the sample dilution prior to analysis, the abundance counts are more accurate in lower cell densities in this instrument. To conclude, all tested methods give more accurate counts during exponential phase. So, that's not just the method, but the culture growth phase that needs to be considered.

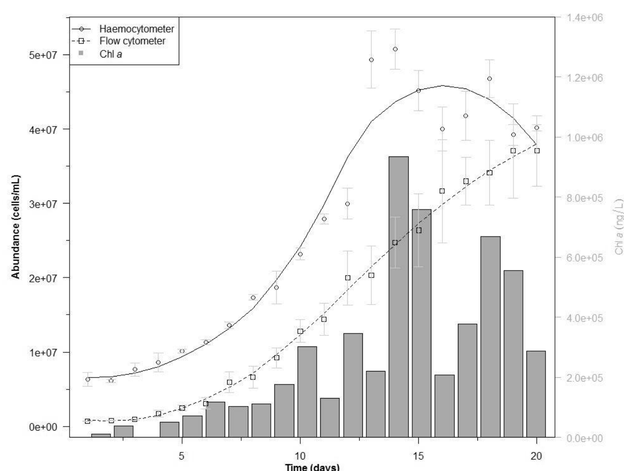

Fig. 1. The growth curve of *Picochlorum* sp. presented through three different methods (haemocytometer, flow cytometer and Chl *a* values) for three replicate cultures.

Acknowledgments

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References

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