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Studies on Growth of Marine Microalgae in Batch Cultures: III. *Nannochloropsis oculata* (Eustigmatophyta)

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Abstract: In this study, growth of *Nannochloropsis oculata* in batch culture was investigated in relation to some physical and chemical factors. For this purpose, batch culture of *N. oculata* was prepared in sea water and the cultures were kept under four combinations of light regime and carbondioxide supply. Three salinity levels, 25, 30 and 35‰ S were employed. Growth of the alga in culture varied with respect to number of individuals and size of maxima under different culture conditions. *N. oculata* showed better growth in batch culture that was kept under 24 h illumination and 24 h of carbondioxide supply.

Key words: *Nannochlorapsis oculata,* Eustigmatophyta, batch culture

INTRODUCTION

Nannochloropsis species are known to be abundant in waters with high-nutrient loading such as coastal waters and estuaries[1]. Several species of *Nannochloropsis* were identified since the genus was first presented[2]. The eustigmatophyte *Nannochloropsis* is widely used in aquaculture hatcheries to establish the initial step of an artificial food chain. The alga has unique fatty acid composition that is carried to fish larvae through rotifers which consume this alga.

There are many studies related to the culture of *Nannochloropsis*. Optical properties of *Nannochloropsis* was given by Owens *et al.*[3]. In recent years, Lubzens *et al.*[4] reported the potential advantages of frozen *Nannochloropsis* sp. for rotifer (*Branchionus plicatilis*) culture and Yamasaki and Hirata[5] suggested that some kind of carbon source should be supplied to obtain high density of *Nannochloropsis* sp. Richmond and Cheng-Wu[6] carried out a research on mass production of the alga outdoors and Gitelson *et al.*[7] investigated inherent and apparent optical properties of *Nannochloropsis* in culture.

The main goal of this study was to investigate the influence of salinity, light duration and CO_2 on growth of *N. oculata* in batch culture.

MATERIALS AND METHODS

Materials and methods has been given by Sen *et al.*[8] in the studies on growth of marine microalgae in batch cultures I. *Chlorella vulgaris.*

RESULTS

As it is seen from the Fig. 1-4 the alga displayed similar growth patterns in all cultures kept under different conditions. Cell numbers of the alga remained almost unchanged during 1-8 days (lag phase). The alga then started to increase in cell numbers and continued to increase until a maximum was reached. This active multiplication phase usually started on 7th or 8th day and exclusive of one experiment lasted for 24-26 days. Cell numbers always decreased rapidly after all maxima.

N. oculata reached to a maximum of $7200x10^6$ cells mL^{-1} on 30th day in culture under continuous illumination and CO_2 supply at 25‰ S. A maximum of $4800x10^6$ cells mL^{-1} at 30‰ S and a maximum of $5000x10^6$ cells mL^{-1} at 35‰ S occurred on 33rd an 23rd days, respectively (Fig. 1).

In culture with 24 h illumination and 12 h CO_2 supply maximum cell densities for 25, 30 and 35‰ S were $3500x10^6$, $3800x10^6$ and $2800x10^6$ cells mL^{-1}, respectively (Fig. 2). Maxima were reached between 29th-33rd days.

Cultures kept under 12 h illumination and 24 h CO_2 supply, maximum cell numbers of $6850x10^6$, $3550x10^6$ and $6800x10^6$ cells mL^{-1} were recorded at 25, 30 and 35‰ S, respectively (Fig. 3). Maxima were reached between 28th-32th days as in the previous experiment.

In cultures with 24 h illumination and without CO_2 maxima at 25, 30 and 35‰ S occurred in the order of $720x10^6$, $780x10^6$ and $275x10^6$ cells mL^{-1}, respectively (Fig. 4). This experiment differed from the previous experiments in that alga reached its maxima in a shorter time.

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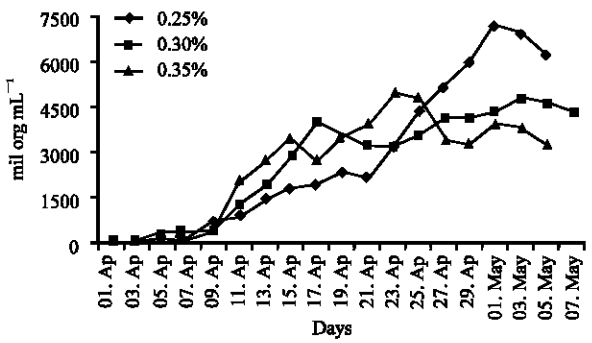

Fig. 1: Growth of *N. oculata* in culture with 24 h illumination and 24 h CO_2 supply at 25, 30 and 35‰ S

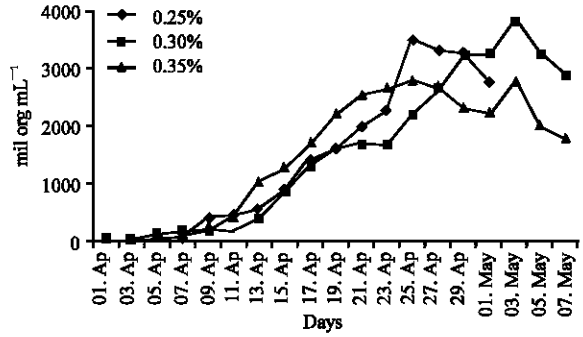

Fig. 2: Growth of *N. oculata* in culture with 24 h illumination and 12 h CO_2 supply at 25, 30 and 35‰ S

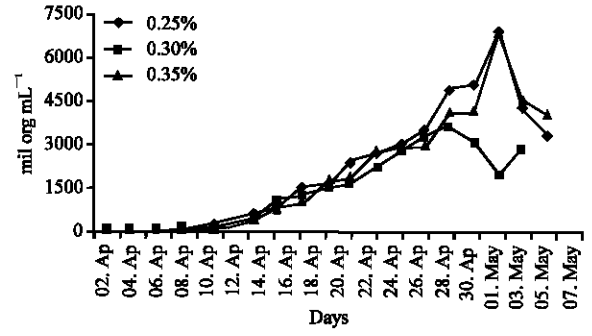

Fig. 3: Growth of *N. oculata* in culture with 12 h illumination and 24 h CO_2 supply at 25, 30 and 35‰ S

DISCUSSION

Of all, best growth of *N. oculata* occurred in the culture subjected to 24 h illumination and 24 h CO_2 supply at 25‰ S since the size of the maximum in this experiment was by far greater than those recorded under other culture conditions. This may indicate that continuous illumination and CO_2 supply clearly supported the growth of this alga. This finding was in harmony with that of Rocha *et al.*[9] who reported that *Nannochloropsis* species are able to metabolise the inorganic carbon CO_2 and there is an equilibrium trend for the pH to increase which can be controlled by the addition of CO_2 in culture. Yamasaki and Hirata[5] are also reported some kind of carbon source should be supplied to obtain high density of *Nannochloropsis* sp.

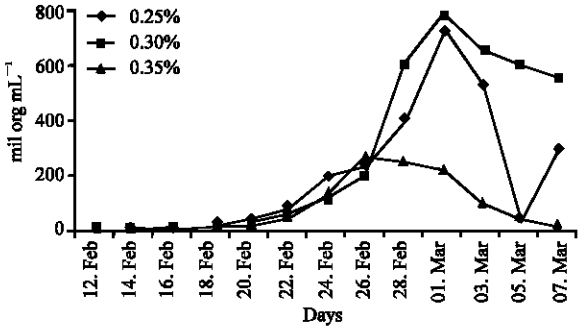

Fig. 4: Growth of *N. oculata* in culture with 24 h illumination with no CO_2 supply at 25, 30 and 35‰ S

The cell density of the alga was observed to be lowest in the culture supplied no CO_2 despite continuous illumination. Microalgae do not make a distinction between natural and artificial light, but they are much more sensitive to light intensity and light/dark cycles. The results of the present study supported this finding since best growth of *N. oculata* was found to be related to light duration. The present finding supported the study of Yamasaki and Hirata[5] who also reported some kind of carbon source should be supplied to obtain high density of *Nannochloropsis* sp. under strong light intensity.

The salinity showed slight effect on the growth of *N. oculata* and 25 and 30‰ S levels appeared to be more suitable for the growth of the alga than 35‰ S. The same result was obtained for *Isochrysis galbana* that was kept under the same culture conditions[8].

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