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Studies on Growth of Marine Microalgae in Batch Cultures: I. *Chlorella vulgaris* (Chlorophyta)

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Abstract: In this study, growth of *C. vulgaris* in batch culture was investigated in relation to some physical and chemical factors. For this purpose, culture medium for *C. vulgaris* was prepared in sea water and the culture was 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. *C. vulgaris* showed better growth in culture that was kept in 25‰ salinity under 24 h illumination and 24 h of carbondioxide supply.

Key words: *Chlorella vulgaris*, Chlorophyta, batch culture

INTRODUCTION

Use of microalgae as food sources in both freshwater and marine fish culture has now become very common. The commercial utilization of microalgae has especially gained attention with the discovery that some algae are they best natural source of β-carotene[1]. Further interest in the microalgae as food organisms has been particularly generated in recent years since microalgae has now extensively used as live food for commercially important molluscs, fish and crustaceans during at least part of their life cycles. In fact, aquaculture is now one of the most rapidly growing areas in the field of food production all over the world.

Microalgal culture is one of the modern biotechnologies and *Chlorella* has been one of most commonly used algae in algal biotechnology. *Chlorella vulgaris* (Chlorophyta) is also one of the most significant algae as a food organism for fish larvae.

The first unialgal cultures were achieved by Beijerink 1980 with *Chlorella vulgaris* and *Chlorella* was also one of the first algae to be isolated as a pure culture[2]. In addition commercial large-scale culture of microalgae commenced in the early 1960s in Japan with the culture of *Chlorella*[3]. By 1980 there were 46 large-scale factories in Asia producing more than 1000 kg of microalgae (mainly *Chlorella*) per month and 1996 about 2000 t of *Chlorella* were traded in Japan alone[4]. Later *Chlorella* was also cultured as health foods for human consumption[5] or as by-products in waste water treatment[6].

Cultural characteristics of *Chlorella* were investigated by several researchers since the late 1950s[7-11]. The present study was aimed to investigate the influence of light duration and CO_2 on growth of *C. vulgaris* together with salinity.

MATERIALS AND METHODS

C. vulgaris was obtained from the a culture center and culture medium of *C. vulgaris* was prepared in sea water filtered through 55-60 μm diatomite filter. Sea water was then subjected to UV and autoclaved at 170°C 4 h. The culture medium was prepared as in Table 1. Experiments were carried out in erlenmayer flasks (500 mL) with 250 mL of medium and maintained in a controlled culture room. All glassware was washed in HCl 10% and autoclaved at 170°C before use. 250,000 cells mL^{-1} was inoculated into each erlenmayer and closed with aluminium foil. Burker counting chamber was used for cell counts.

Four combinations of light regimes and CO_2 supply were employed.

- 24 h light phase and 24 h CO_2 supply
- 12 h light phase and 24 h CO_2 supply
- 24 h light phase and 12 h CO_2 supply
- 24 h light phase and no CO_2 supply

Light intensity was adjusted at 2000-3000 luxes from flourescent lamps. CO_2 was supplied to the cultures by connecting an industrial CO_2 tube to the air blower of the culture room. All cultures were kept at a constant temperature of 24°C and pH of the culture medium was maintained at 7.5 since the rate of the growth was inhibited at higher pH levels[12]. Three salinity levels (25, 30, 35‰ S) were used and salinity of sea water was reduced with distilled freshwater.

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Table 1: Preparation of enriched sea water medium

Salt solution (Stock solution)		Trace elements solution	
$NaNO_3$	300 g	Solution A	
KH_2PO_4	30 g	$ZnSO_4.H_2O$	30 g
NH_4Cl	20 g	$CuSO_4.5H_2O$	25 g
Distilled water	1 L	$CoSO_4.7H_2O$	30 g
		$MnSO_4.H_2O$	20 g
Vitamin solution		Distilled water	1 L
Biotin	100 mg	Solution B	
B_{12}	100 mg	$FeCl_3.6H_2O$	50 g
Thiamin	10 mg	Distilled water	1 L
Each vitamin is		Solution C	
dissolved in disstilled water separately		$Na_2MoO_4.2H_2O$	25 g
Stock vitamin solution		Distilled water	1 L
Biotin	10 mL	Solution D	
B_{12}	10 mL	$Na_2EDTA.2H_2O$	50 g
Thiamin	10 mL	Distilled water	1 L
Distilled water	970 mL	Trace elements stock solution	
		Solution A	10 mL
		Solution B	10 mL
		Solution C	100 mL
		Solution D	10 mL
		Distilled water	870 mL

All solutions exclusive of vitamin solution were autoclaved at 120°C for 30 min. 1 mL from each solution is added to 1 L culture medium.

RESULTS

As 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 1-3 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 5th or 6th day and lasted for 17-33 days according to the culture conditions. Cell numbers always decreased rapidly after all maxima.

C. vulgaris reached to a maximum of $10000x10^6$ cells mL^{-1} on 21st day in culture under continuous illumination and CO_2 supply at 25‰ S. A maximum of $5500x10^6$ cells mL^{-1} at 30‰ S and a maximum of $4700x10^6$ cells mL^{-1} at 35‰ S occurred on 17th an 19th days, respectively (Fig. 1).

In cultures with 24 h illumination and 12 h CO_2 supply maximum cell densities for 25, 30 and 35‰ S were $5100x10^6$, $4400x10^6$ and$3350x10^6$ cells mL^{-1}, respectively (Fig. 2).

Cultures kept under 12 h illumination and 24 h CO_2 supply, maximum cell numbers of $1400x10^6$, $600x10^6$ and $1800x10^6$ cells mL^{-1} were recorded at 25, 30 and 35‰ S, respectively (Fig. 3).

In culture with 24 h illumination and without CO_2 maxima for 25, 30 and 35‰ S occurred in the order of $275x10^6$, $800x10^6$ and $900x10^6$ cells mL^{-1} (Fig. 4).

DISCUSSION

Of all, best growth of *C. vulgaris* occurred in the culture subjected to 24 h illumination and 24 h CO_2 supply at 25‰ S since maximum cell number was reached in a shorter time and the size of the maximum was by far greater than those recorded in other cultures with different conditions. This may indicate that continuous illumination strongly supported the growth of the alga in this study. However, the photosynthetic processes of *Chlorella* was reported to become saturated at relatively low illumination, ranging from 4000-30000 lux depending on the strain[2].

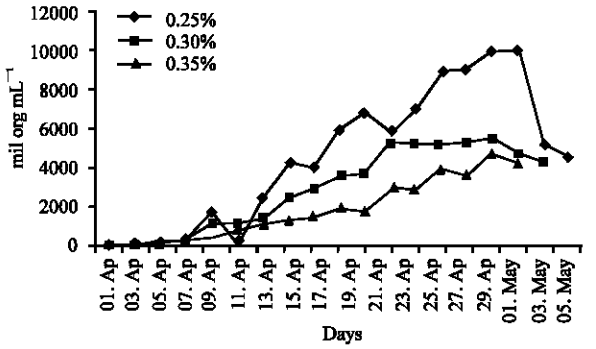

Fig.1: Growth of *C. vulgaris* in culture with 24 h illumination and 24 h CO_2 supply at 25, 30 and 35‰ S

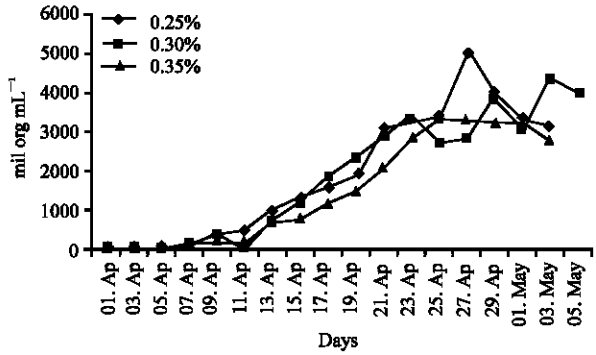

Fig. 2: Growth of *C. vulgaris* in culture with 24 h illumination and 12 h CO_2 supply at 25, 30 and 35‰ S

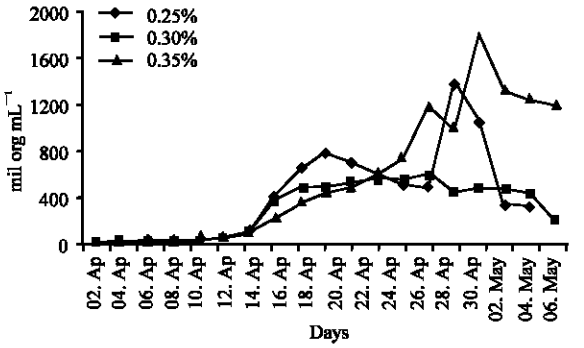

Fig. 3: Growth of *C. vulgaris* in culture with 12 h illumination and 24 h CO_2 supply at 25, 30 and 35‰ S

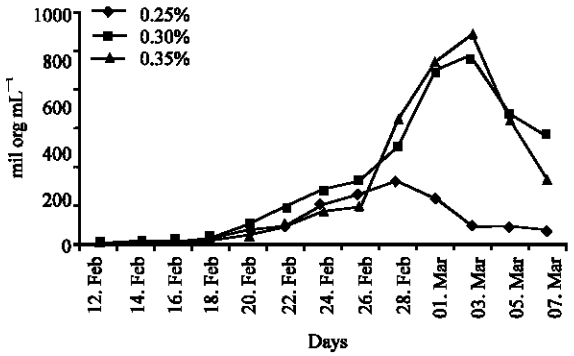

Fig. 3: Growth of *C. vulgaris* in culture with 24 h illumination with no CO_2 supply at 25, 30 and 35‰ S

In addition, 25 and 30‰ S levels appeared to be more suitable for the growth of the alga than 35‰ S when culture was kept under 24 h illumination accompanied with 12-24 h CO_2 supply. This finding is in harmony with the study of Fabregas *et al.*[13] who found salinity and nutrient concentration to be closely related to the growth of marine food algae.

It has been shown that 1 mL of pure CO_2 is needed for the production of 1 mg (dry weight) of *Chlorella* cells. Thus, the most important problem in liquid algal cultures seems to be an adequate carbon supply[2]. The rate of CO_2 transfer into liquid phase was reported to decrease in parallel with the elevation of temperature[14]. The present study was in harmony with the study of Oh-Hama and Miyachi[2] since the growth of the alga was observed to be poorest in the culture with no CO_2 supply despite continuous illumination. In contrast, the best growth was achieved under continuous CO_2 supply. This finding may also show that CO_2 supply can strongly affect the growth of *Chlorella* under the culture conditions employed in this study. Fabregas *et al.*[13] also reported that an increase in the nutrient concentration did not produce an increase in biomass production but CO_2 added to the cultures increased the final biomass production of the alga in the culture of another marine alga *Tetraselmis suecica*. This also holds true for *Chlorella* in this study. In addition, *Chlorella* was reported to show excellent stabilities to high concentrations of CO_2[11] and high growth rates of *Chlorella* species in up to 50% CO_2 were emphasized[9].

REFERENCES

1. De Pauw, N. and G. Persoone, 1988. Micro-algae for Aquaculture. In: Borowitzka, M.A. and L.J. Borowitzka, (Eds.), Micro-algal Biotechnology, Cambridge University Press, Cambridge, pp: 197-221.
2. Oh-Hama and Miyachi, 1988. *Chlorella*. In: Borowitzka, M.A., and L.J. Borowitzka, (Eds.), Micro-Algal Biotechnology. Cambridge University Press, Cambridge, pp: 3-26.
3. Tsukada, O., T. Kawahara and S. Miyachi, 1977. Mass Culture of *Chlorella* in Asian Countries. In: Mitsui, A., S. Miyachi, A. SanPietro and S. Tamura,(Eds), Biological Solar Energy Conversion. Academic Press, New York, pp: 363-365.
4. Kawaguchi, 1980. Microalgae Production Systems in Asia. In: Shelef, G. and C.J. Soeder, C.J., (Eds.), Algae Biomass Production and Use. Elsevier/North Holland Biomedical Press, Amsterdam, pp: 25-33.
5. Durand-Chastel, H., 1980. Production and Use of *Spirulina* in Mexico. In: Shelef, G. and C.J. Soeder, (Eds.), Algae Biomass Production and Use. Elsevier/North Holland Biomedical Press, Amsterdam, pp: 51-64.
6. Sandbank and Hepher, 1980. Microalgae Grown Wastewater as an Ingredient in the Diet of Warm Water Fish. In: Shelef, G. and C.J. Soeder, (Eds.), Algae Biomass Production and Use. Elsevier/North Holland Biomedical Press, Amsterdam, pp: 697-706.
7. Walker, J.B., 1953. Inorganic micronutrient requirement of *Chlorella*. I. Reguirements for calcium (or strontium), copper and molybdenum. Arch. of Biochem. Biophys., 46: 1-11.
8. Eyster, H.C., T.E. Brown and H.A. Tanner, 1958. Mineral Requirements for *Chlorella Pyrenoidosa* under Autotrophic and Heterotrophic Conditions. In: Lamb, C.A., O.G. Bently and J.M. Beattie, (Eds.), Trace Elements. Academic Press, New York, pp: 157-191.
9. Hanagata, N., T. Takeuchi, Y. Fukuju, D.J. Barnes and I. Karube, 1992. Tolerance of microalgae to high CO_2 and high temperature. Phytochemistry, 31: 3345-3348
10. Sung, K.D., J.S. Lee, C.S. Shin, M.S. Kim, S.C. Park and S.W. Kim, 1998. CO_2 fixation by *Chlorella* HA-1 cultured in bubble columns. Korean J. Applied Microbiol. and Biotechnol., 26: 1-6
11. Sung, K.D., J.S. Lee, C.S. Shin, S.C. Park and M.J. Choi, 1999. CO_2 fixation by *Chlorella* sp. KR-1 and its cultural characteristics. Biores. Technol., 68: 269-273.
12. Kang and Fog, 1958. Studies on the growth of marine phytoplankton. II. *Isochrysis galbana*. J. Mar. Biol. Assoc., 37: 781-788 .
13. Fabregas, J., C. Herrero, J. Abalde and B. Cabezas, 1984. Growth of marine micro alga *Tetraselmis suecica* in batch cultures with different salinities and nutrient concentrations. Aquaculture, 42: 207-215.
14. Setlik, I., 1968. Growth and photosynthetic characteristics of algae. Annual Report of the Laboratory of Experimental Algology and Department of Applied Algology for the Year 1967, pp: 71-140.