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Effects of Different Salinities and Luminance on Growth Rate of the Green Microalgae *Tetraselmis chuii*

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Abstract: In this study, the effects of (20, 30, 40 and 50 ppt) salinities and (500, 2500, 4500 and 6500 lux) luminance on the changes of the amount of biomass and the growth rate of the microalgae *Tetraselmis chuii.* have been studied in the controlled conditions. The growth rate, has been expressed as the number of cells mL^{-1}. The results have indicated that the growth rate of this microalgae has varied and influenced by the salinity and light intensities. In this research, growth rate has been decreased by the salinity and light intensity and the highest biomass was observed in 40 ppt salinity × 4500 lux and the lowest was in 20 ppt salinity × 500 lux. We conclude that as like as higher plants high and low (in halophytes) salinities and light intensities cause some changes in different phases of growth rate and other biochemical aspects in unicellular green microalgea. So to maintain such these researches help us to find out the best condition that suits for the growth and culture of microalgea specially those that more important economically and are used by other organisms in food chain.

Key words: *Tetraselmis chuii*, salinity, luminance, growth phases, growth rate

INTRODUCTION

Green algae and land plants (Viridiplantae) are two sister groups which are phylogenetically subdivided into the Chlorophyta and the Streptophyta (Bhattacharya and Medlin, 1998). The common ancestor of this lineage is believed to be a member of the Prasinophyceae (Bhattacharya *et al*., 1998). From many years ago, man was interested in to study aquatic plants, he has made experimental lakes (220 years ago) to research about algae. Microalgae are rich sources of proteins, carbohydrates and especially essential fatty acids. *Tetraselmis* sp. (prasinophyte) is a green, motile and about 10×14 μm microalgea. This organism as a phytoplankton is used greatly by the artemia, mussels, oysters, clams, scallops and corals (Eirik, 1998). It is also one of the preferred foods for rotifer cultures (Makridis, 2006). *Tetraselmis chuii* is very important due to its higher proteins, lipids, essential fatty acids and sterols. *Tetraselmis* maintain natural amino acids of marine organisms (Chung *et al*., 2003). Two documented natural antibiotics are present in *Tetraselmis* (when are used as a food supplement, it inhibited laboratory-induced infection in Atlantic salmon (Shahin, 2001). It has suggested that there may be some bioactive compounds in the algal cells exudates and there appears to be a significant role for *Tetraselmis* in the control of fish diseases (Hemtanon, 2005). This very diversity makes microalgae, as a group, a potentially rich source of a vast array of chemical products with applications in the food, nutritional, cosmetic, pharmaceutical and even fuel industries (Brown *et al*., 2006). Light and salinity as environmental factors have very significant effects on the growth and biochemical composition of marine algae as like as all other plants. The effects of varying light intensity range from the seasonal slowing acceleration of growth rates in marine ecosystems, or marine microalgae sinking through the water column and out of the photic zone due to light attenuation (Barnes and Mann, 1999). Varying light intensity may also be used to control growth rates of algal cells experimentally. For example, the growth rate and time to harvest for large-scale bag cultures of microalgae can be controlled to meet varying biomass production demands in the hatchery (Bolch, 2004a, b). The algal cells compositions also alter by varying light intensities, for example, pigments (e.g., chlorophyll a), unsaturated fatty acids, carbohydrates and protein contents all change in response to increased or decreased light intensity (Thompson *et al*., 1990). So because of the importance of *Tetraselmis* sp. as a member of food chain, in this research

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the effects of varying irradiance and salinity on the growth rate of this organism. and to determine maximum growth rates under typical hatchery growth condition have been studied.

MATERIALS AND METHODS

The green unicellular *Tetraselmis chuii* (Prasinophyte) was supplied by the university of Tarbiat Moddares of Noor. Microalgae was grown photoautotrophycally in filtered and sterilised natural seawater medium (20 min at 121°C) enriched with Walne medium (Laing, 1991). Cells were grown in 500 mL Erlenmeyer flasks containing 450 mL of medium at 20°C with different irradiance 500, 2500, 4500 and 6500 lux and different salinity of seawater 20, 30, 40 and 50 ppt for 9 days and the initial pH of the culture was 8. Hemacytometer is used for countingthe number of cells in ml of medium culture. Estimating the cells densities of all cultures were made daily using a Coulter Counter and their growth rates were calculated from the formula (Alix *et al*., 2004; Shannon *et al*., 2005).

$$\text{No. of cells mL}^{-1} = (n_1 + n_2)/(2\times20)\times250\times10^3\times d = (n_1 + n_2)/160\times10^6\times d$$

Where:

n_1 = No. of cells counted in upper rafter.
n_2 = No. of cells counted in lower rafter.
d = Dilution factor.

RESULTS

The effects of different salinities and luminance on growth rates of *Tetraselmis chuii* cells over 9 days is shown in Fig. 1. The growth rate was the highest at 40 ppt and tended to decrease at lower 20 ppt and higher salinity 50 ppt. Significant decrease in growth rates was observed at 20ppt salinity × 500 lux luminance (Fig. 1a). Reduced growth at 20 ppt salinity has also been reported in all 500, 2500, 4500 and 6500 lux luminances (Fig. 1a-d). Increasing salinity from 40 to 50 ppt has reduced. The specific growth rate in all cases. At the 40 and 30 ppt salinity and continuous illumination of 4500 and 6500 lux the growth rate and cell division of *Tetraselmis* was the most rapid.

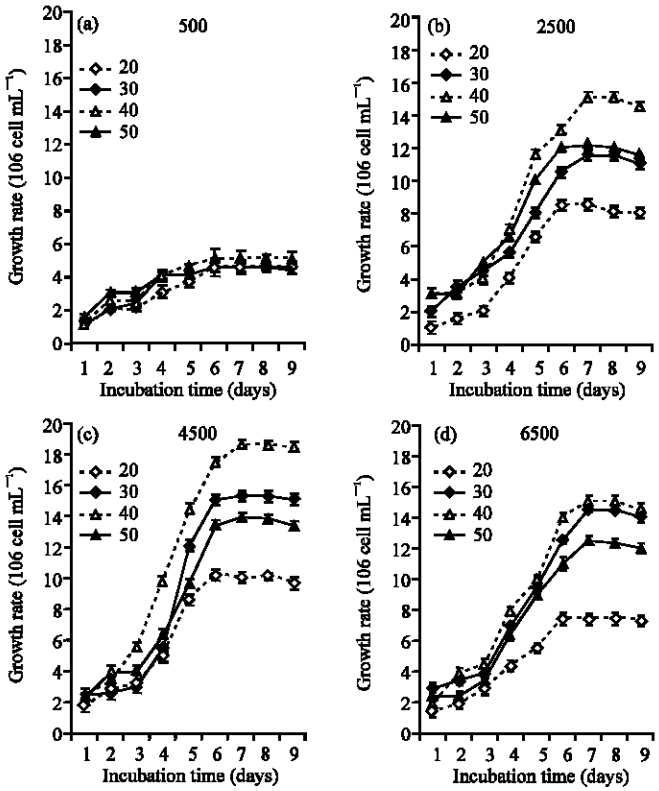

Fig. 1: Effects of different salinity (20, 30, 40 and 50 ppt) and different luminance(500, 2500, 4500 and 6500 lux) on different fases of growth rate in *Tetraselmis chuii*. Data represents the mean of three replicates±SE

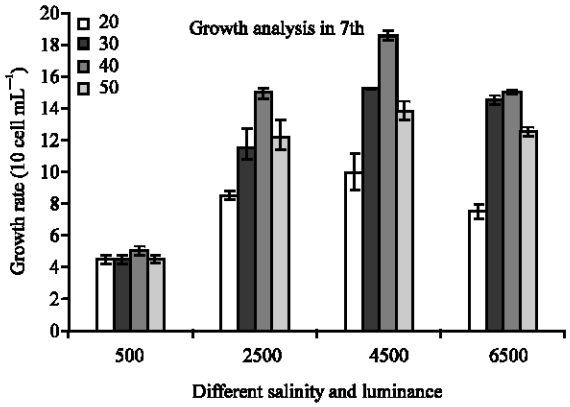

Fig. 2: Effects of different salinity (20, 30, 40 and 50 ppt) and different luminance(500, 2500, 4500 and 6500 lux) on Growth rate in *Tetraselmis chuii* at 7th Data represents the mean of three replicates±SE

As illustrated in Fig. 1a growth lag phase was longher in 500 luminance. Other phases of growth have also been seen in Fig. 1b-d, optimum growth seen in 7th days and it shown in Fig. 2.

DISCUSSION

During lag or induction phase, little increase in cells density occurs. The lag in growth is attributed to the physiological adaptation of the cells metabolism to growth, such as increase the levels of enzymes and metabolites involved in cells division and carbon fixation (Meyer, 1997; Staal, 2007). As illustrated in Fig. 1a growth lag phase was longher in 500 luminance. A lag phase may occur if the innoculum is transferred from one set of growth conditions to different salinity and luminance condition so in all condition this phase is showed. Exponential phase, the second phase, that the cell density has increased logarithmichally. The specific growth rate is mainly dependent on algal species, light intensity and temperature (Theroux, 2005). The growth rate of a microalgal population is a measure of the increase in biomass over time and it is determined from the exponential phase (Yuehua, 2006) and this result shown in Fig. 2, that maximum biomass produsd in 7th day. Growth rate is one important way to express the relative ecological success of a species or strain in adapting to its natural environment or the experimental environment imposed upon it Yuehua (2006). In growth rate declining phase, Cells division slows down when nutrients, light, pH, carbon dioxide or other physical and chemical factors begin to limit the growth. Stationary phase: In the fourth stage, the limiting factor and the growth rate are balanced, which results in a relatively constant cell density. Death phase, during the final stage, water quality deteriorates and nutrients are depleted to a level incapable of sustaining growth. Cell density decreases rapidly and the culture eventually collapses that all of these phases and changes have been seen in Fig. 1a-d.

Light effects on microalgae growth: Algal cells respond to increased light in three ways: an increase in growth rate, photosynthetic rate and changes in their cellular composition (Bolch, 2004a; Thompson *et al*., 1990). When microalgal cells are exposed to high light intensity, photosynthesis is inhibited and therefore the growth rate of the algal culture has reduced which has shown in 6500 lux (Barnes and Mann, 1999; Toro, 1989). This process is known as photo-inhibition. This effect is caused by photo-oxidation reactions inside the cell due to excess light that can not be absorbed into the photosynthetic apparatus; the increase in ultraviolet light also has detrimental effect on the cell (Barnes and Mann, 1999).

Salinity effects in microalgae growth: Salt stress is one of the many environmental factors that limit growth and productivity of microorganisms. The mechanisms of the hyperosmotic stress-induced and the salt stress-induced inactivation of the photosynthetic machinery, particularly the oxygen evolving machinery of the photosystem II complex, have been shown in *Synechococcus* sp. (Allakhverdiev *et al*., 2000a,b, 2001). *Tetraselmis* sp. was able to grow in all the tested concentrations of salinity (20, 30, 40 and 50 ppt), but the biomass yields has increased with increasing concentration of salinity and maximum biomass was achieved in 40 ppt (Fig. 1c). Hart *et al*. (1991) has showed the reduced growth at higher salinities due to decrease in photosynthetic rate. The decreased yields of biomass as reported by Vazquez-Duhalt and Arredondo-Vega (1991) and Ben-Amotz *et al*. (1985) were probably due to non adaptability of the organism to higher salinity.

CONCLUSION

We know that different algae like all other organisms have different requirements. So for cultivating sucessfully of the algae, several factors must be considered and the meduim culture must be in a temperature range that will support the specific algal species being grown. Nutrients must be controlled so algae will not be starved and so that the nutrients will not be wasted. Light must be not too strong nor too weak.

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