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THE EFFECTS OF CERTAIN ECOLOGICAL FACTORS ON THE GROWTH OF TETRASELMIS SP. IN LABORATORY

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ABSTRACT The green alga, Tetraselmis sp., contain relatively large amounts of valuable lipids and are commonly used as food source for commercial application in aquaculture. In this study we evaluated the effect of certain ecological factors such as light intensity, temperature, salinity, initial density, phosphate and nitrogen concentration on the growth of Tetraselmis sp. which continuously grew under laboratory-controlled conditions, aimed to apply these results for mass-culture of Tetraselmis sp. in aquaculture hatcheries.

Tetraselmis sp. tolerates to a broad range of salinity and prefers high salinity at 35-45 ppt. The best suitable initial density for algal growth was about 15-20 x 10^4 cells/ml. The best temperature for the Tetraselmis sp. growth was 28^{o}C. The suitable fluorescent light intensities were from 150-200μmol $s^{-1}m^{-2}$. In $F/_2$ (Guillard 1975) medium, the critical range of N concentration for growth of the algae was from 7.36 – 22.36 mg/l, the optimum phosphate concentration for growth were from 0.77 –3.27 mg/l.

ANH HÖÔNG CUA MOT SO YEU TO SINH THAI LEN TANG TRÖÔNG TAO TETRASELMIS SP. TRONG PHONG THÍ NGHIEM

Ha Le Thò Loc
Vien Hai Döông Hoc

TOM TAT Tetraselmis sp. la loai tao chöa nhieu thanh phan lipid co gia trò do ño la nguon thöc an ñöôc sö dung pho bien trong nuoi trong thuy san. Nghien cöu nay nham ñanh gia sö anh höông cua mot so yeu to sinh thai nhö: Anh sang, nhiet ño, ño man, mat ño ban ñau, ham löông muoi ni-tô va phot-pho len sö tang tröông quan the Tetraselmis sp. trong ñieu kien phong thí nghiem vôi muc ñích öng dung nhöng ket qua nay vao nuoi sinh khoi tao trong cac trai san xuat giong hai san.

Tetraselmis sp. co bien ño dao ñong ño man rong va öa thích ño man cao tö 35-45ppt. Mat ño ban ñau phu hôp cho tang tröông vao khoang 15-20 van te bao/ml. Nhiet ño tot nhat cho sö phat trien Tetraselmis sp. la 28°C. Cöông ño anh sang phu hôp tö 150 – 200 μmol $s^{-1}m^{-2}$. Trong moi tröông $F/_2$ (Guillard 1975), ham löông ni-tô dao ñong tot nhat cho tang tröông tao la 7,36 - 22,36mg/l. Ham löông phot-pho cöc thuan trong khoang 0,77 – 3,27mg/l.

INTRODUCTION

Microalgae play an important role in aquaculture. They are the first link of the food chain in nature. Microalgae are widely used as food in mariculture and they were considered as excellent food for zooplankton and larvae of many marine species. The success of seed production depends on microalgae.

Microalgae contain a high proportion of unsaturated fatty acids that are vital for larvae of crustacean, bivalve and early stage of many marine species (Volkman et al 1993). High unsaturated fatty acids ($HUFA_s$) are very

important for larvae. The survival rate, pigmentation process and viability depend on $HUFA_s$, especially EPA (eicoxapentaenoic acid) and DHA (docoxahectaenoic acid) (Reitan 1994). The importance of those fatty acids has been demonstrated in the growth of oyster larvae (Chu and Webb 1984; Enricht 1986; cited by Hong 1999). DHA and EPA were also vital for Baramundi Lates calcarifer fingerlings (Kongkeo 1991). However, the important roles of EPA or DHA are different for certain marine species. DHA was the most important HUFA for the development of mud crab Eurypanopeus depressus Increasing EPA content in diets resulted in increasing survival rate of seabass larvae Dicentrachus labrax, while the growth of this species was associated with increasing DHA content (Sorgeloos et al 1988; cited by Hong 1999).

De novo biosynthesis of n-3 fatty acids can only be taken in plant cells and marine algae containing high concentration of n-3 PUFA (Polh 1982; Olsen 1989, cited by Reitan 1994). Therefore, marine microalgae are a source of PUFA for marine cultured animals.

Tetraselmis sp. are considered of high nutritional value. The gross chemical composition of Tetraselmis sp. (strain NT18 and TEQL01) consists of protein: 29.9% and 26.4% (dry weight); lipid: 12,6% and 13.8%; carbohydrate: 8% and 9.4%; ash: 13.6 and 13.9%, respectively (Renaud et al. 1999); very high eicoxapentaenoic acid (EPA) (29.7% total of fatty acid) (Zhukova and Aizdaicher 1995). So, it was commonly cultured all over the world. Tetraselmis sp. are widely used as food for rotifers, shrimp larvae culture (in Japan) (Okauchi 1991), bivalves (in Singapore) (Lim 1991), fish larvae (in Norway, Thailand, Hawaii) (Tamaru et al. 1993) (Reitan et al 1997) ….

In Vietnam, Tetraselmis sp. strain was imported from Thailand in 1999. The knowledge about the tolerances to Vietnam environmental conditions of this species was not well defined. So, the effect of certain main ecological factors on the growth of Tetraselmis sp. needs to be studied, that is the baseline for outdoor mass-culture to feed rotifers and fish larvae in aquaculture hatcheries.

MATERIAL AND METHODS

Tetraselmis sp. was obtained from the Asian Institute of Technology Bangkok-Thailand. The experiments were carried out at the Laboratory of Techno-Biology of Aquaculture Faculty, University of Fisheries. Tetraselmis sp. was purified based on microbiology method on agar and liquid media. Then the inoculation was scaled up from small volume to higher volume: 10ml, 20ml test tubes; 125ml, 250ml, 500ml, 1000ml flasks.

Seawater was treated with chlorine at 25 ppm and aeration for three days, exposed to the sun and filtered through sandy filters. After that, water was sterilized by autoclaving in 121°C/15'. Flasks, tubes, petri dishes were heated at 140°C / 30' before using. Aeration used during the experiments was filtered through sterile cotton wool filter to prevent ciliate and bacterial contaminations. The $F/_2$ (Guillard, 1975) was used.

Culture flasks (500ml) were placed 15-20 cm away from 5 fluorescent lights to avoid overheating and to ensure optimum light intensities for the experiments. Room temperature was maintained about 28°C by air- conditioning and the temperature experiments were adjusted by Visi - therm in water bathes. Light intensity was measured using LI-1400 data LOGGER with a quantum sensor. Daily light regimes of 12:12 h light / dark were maintained using an automatic electric time clock. Cell number was determinated by a Burker counting chamber and spectrophotometric method. Spectrophotometre procedure: Transferred culture to a 1ml cuvette and measured optical density at 750 nm. Absorbencies were converted to cell densities using a standard line of regression equation. Triplicates were made for each level of experiments.

Experimental design

_**Experiment 1**: Effect of different salinities on the growth of Tetraselmis sp. Salinities: 10 ppt; 15 ppt, 20 ppt; 25 ppt; 30 ppt; 35ppt; 40 ppt; 45 ppt. Light intensity: 150 µm photon.s^{-1}.m^{-2}. Initial density: 10.10^4 cells/ml. Temperature: 28°C.

_**Experiment 2**: Effect of temperature on the growth of Tetraselmis sp. Temperatures: 22 ± 0.5°C; 25 ± 0.5°C; 28 ± 0.5°C; 31 ± 0.5°C; 34 ± 0.5°C. Salinity: 35 ppt. Light intensity: 150 µm photon.s^{-1}.m^{-2}. Initial density: 25.10^4 cells/ml.

_**Experiment 3:** Effect of initial density on the growth of Tetraselmis sp. Initial densities: 5.10^4; 10.10^4; 15.10^4; 20.10^4; 25.10^4 cells/ml. Salinity: 35 ppt. Light intensity: 150µm photon.s^{-1}.m^{-2}. Temperature: 28°C.

_**Experiment 4**: Effect of light intensity on the growth of Tetraselmis sp. Light intensities: 30µm; 75µm; 125µm; 150µm; 175µm; 200 µm photon.s^{-1}.m^{-2} (fluorescent light) and 91.28µm photon.s^{-1}.m^{-2} (natural light). Salinity: 35 ppt. Initial density: 18.10^4 cells/ml. Temperature: 28°C.

_**Experiment 5**: Effect of nitrogen concentration on the growth of Tetraselmis sp. Nitrogen concentrations: 2.36; 7.36; 12.36; 17.36; 22.36; 27.36; 32.36 mg/l. Light intensity: 150 µm photon $m^{-2}s^{-1}$. Salinity: 35ppt. Initial density: 18.10^4 cells/ml. Temperature: 28°C.

_**Experiment 6:** Effect of phosphate concentration on the growth of Tetraselmis sp. Phosphate concentrations: 0; 0.27; 0.77; 1.27; 1.77; 2.27; 3.27 mg/l. Light intensity: 150µm photon.s^{-1}.m^{-2}. Salinity: 35ppt. Initial density: 18.10^4 cells/ml. Temperature: 28°C.

Statistic method: Used Excel 97 for treatment of the data. Algal growth between the batches was compared by t-test: paired two samples for means.

RESULTS

1. Taxonomy (Throndsen, 1997)

Phylum Chlorophyta

Class Prasinophyceae Moestrup & Throndsen 1988.

Order Chloredendrales Fritsch 1917

Family Chlorodendraceae Oltmanns 1904.

Genus Tetraselmis Stein 1878.

Species Tetraselmis sp.

Characteristics of Tetraselmis sp.: cell length: 14-18µm; width: 8-12µm; thickness: 5-7µm. Shape: bilaterally compressed, with a depression where the flagella originate. Organic scales cover cell body and flagella, which may assemble to form a theca. Flagella: 4. Color: slightly olive-green. Eyespot: in the chloroplast. Storage product: starch in shield around pyrenoid.

2. Effects of salinity on the growth of Tetraselmis sp.

Salinity is one of important factors effecting the distribution and growth of algae. Some species stenohaline and the others euryhaline.

Cell densities from 8 batches of salinity were analysed and compared in Figure 3. The result of experiments showed Tetraselmis sp. tolerated to broad range of salinity (10 – 45ppt) and preferred high salinity (35-45ppt).

The optimum salinity for the growth of Tetraselmis sp. was 35ppt with the highest cell density (266.33 x 10 cells/ml) in the 16^{th} day. pH increased to the highest in these flasks (from 7.89 to 9.69) (Table 1). At 40ppt level, cell density was lower (247 x 10 cells/ml). The time reach to the maximum cell density was longer than previous cultures (35ppt), after 19 days of culture.

The lower the salinity, the worse the algal growth. At salinity of 10ppt, algal growth is the lowest. Maximum cell density only obtained 24.85 x 10^4 cells/ml. So, Tetraselmis sp. showed highest growth at 35-45 ppt. There was significant difference of algal growth between the batches of different salinities (t-test>t-critical): between 10-15ppt; 15-20ppt; 20-25ppt; 25-30ppt; 30-35ppt; 35-40ppt and 40-45ppt.

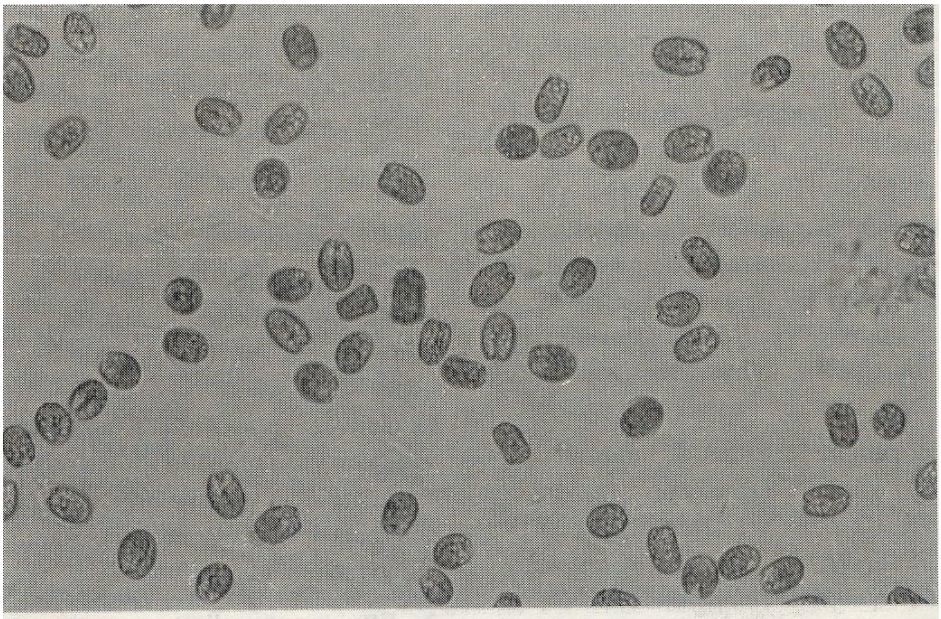

Figure 1: Form of *Tetraselmis* sp. (10x10)

Figure 2: Design of experiments

3. Effects of temperature

The result from Figure 4 showed that high temperature was not suitable for the growth of Tetraselmis sp. At 34°C, the algae collapsed completely on the first days of cultures. At 31°C, the algae grew well and reached the maximum cell density of 176 x 10^4 cells/ml on the 18th day and after that the algae collapsed rapidly because of the increase of pH value (9.71). pH level that is too high or too low will slow algal growth by disrupting cellular processes. pH very high increase can cause complete culture collapse (Ukeles 1971; Fulks and Main 1991).

At 22°C and 25°C, Tetraselmis sp. grew more slowly and reached the maximum cell

densities of 138 x 10^4 and 160 x 10^4 cells/ml, respectively, on 20th day. However, the stationary phase remained more stable.

The optimum temperature for the growth of Tetraselmis sp. was 28^{o}C, at which cell division was rapidly on the first days and reached the maximum cell density of 196 x10^4 cells/ml on 18th day (Table 2). There was significant difference of algal growth between the batches of different temperatures (t-test > t-critical): between 22-25 ^{o}C; 25-28 ^{o}C; 28-31 ^{o}C and 31-34 ^{o}C.

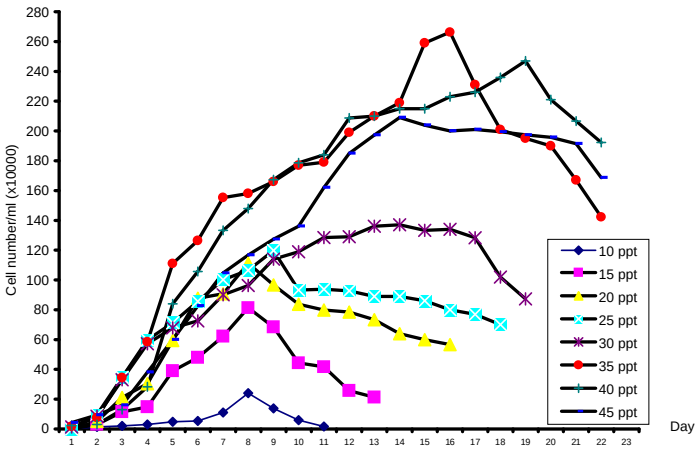

Figure 3: Effect of different salinities on the growth of Tetraselmis sp.

The above result had an important significance in determining a suitable period for effective algal culture and limiting disadvantages in outdoor culture of Tetraselmis sp.

4. Effects of initial densities

The result showed that the suitable initial densities of Tetraselmis sp. in indoor cultures were 10 – 20 x 10^4 cells/ml (Figure 5 and Table 3).

At the initial density of 5x10^4cells/ml, the growth of the algae was slow; the maximum cell density reached 125x10^4cells/ml on 9th day and after that collapsed. At the initial density of 25 x 10^4 cells/ml, although the algae reached the maximum density of 199x10^4 cells/ml on 11th day, the dead phase occurred rapidly because of the increase of pH value (9.72) in cultures.

For the initial densities of 10x10^4, 15x10^4 and 20 x 10^4 cells/ml, the algae grew rapidly with high biomass and the long stable stationary phase. Among of which, the initial density of 20 x 10^4 cells/ml was the best for the growth of Tetraselmis sp. with the maximum density of 158.5 x 10^4 cells/ml on the 11th day, stable biomass and the longest stationary phase. There was significant difference of algal growth between the batches of different initial densities (t-test > t-critical): between 5-10x10^4; 10-15x10^4; 15-20x10^4; 20-25x10^4 cells/ml.

5. Effects of light intensity

Among seven treatments of light effect on Tetraselmis sp. growth were set up, six indoor treatments were provided by fluorescent light, which was considered the best light for micro-algal growth because of its suitable wave-length and less heat emitting (Guillard, 1975). One outdoor treatment was provided by natural light. However, the algal cultures were placed next to the window of the laboratory to avoid direct sunlight and too high temperature caused by small culture volume. The average of light intensity was 91.28µm photon.s^{-1}.m^{-2}. The temperature was recorded

daily with an interval of every 1-hour. The temperature ranged from 23.64°C at 8 a.m. to 27.5°C at 2 p.m. and 25°C at 5 p.m. (Figure 6). The average of temperature was 25.75°C.

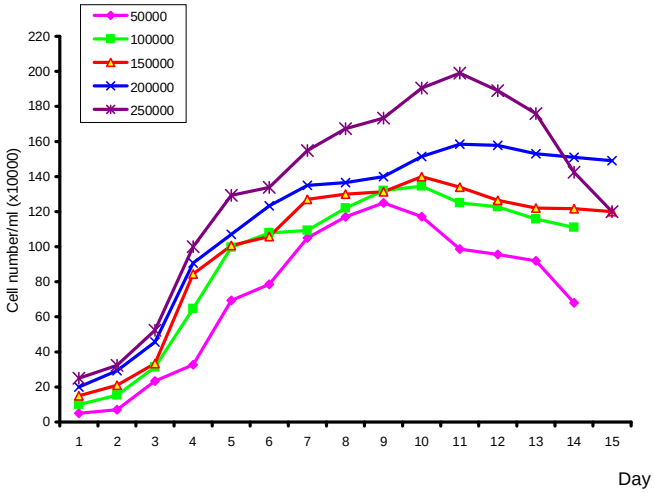

Figure 4: Effect of temperature on the growth of Tetraselmis sp.

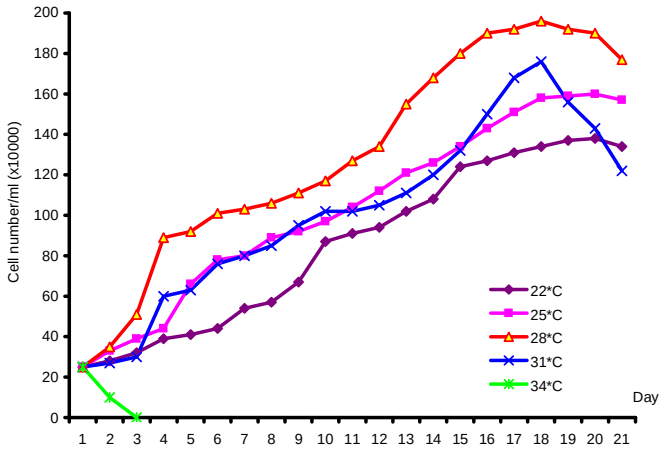

Figure 5: Effect of initial density on the growth of Tetraselmis sp.

For the in door treatments, the algal growth increased along with increase of light intensities. At light intensity of 30µm photon.s^{-1}.m^{-2}, the growth of Tetraselmis sp. occurred slowly on the first days of culture, the maximum cell density was only 96x10^4 cells/ml. However, the algal biomass was rather stable and the stationary phase could remain one month. Similarly, as increasing of light intensities of 75, 125, 150, and 17µm photon.s^{-1}.m^{-2}, the algal biomass also increased and reached the maximum biomass on days 9, and 11 of culture, respectively. At 200µm photon.s^{-1}.m^{-2}, the maximum cell density on day 10 was 152 x 10^4cells/ml, after that algae collapsed rapidly because of the high pH variety of cultures.

For the outdoor treatment, although the light intensity and temperature were lower than those of the indoor treatments, in addition, the temperature variation was depended on the light intensity, but the

maximum density of algae reached 171 x 10^4 cells/ml on the 13rd-14th day (Figure 7 & Table 4). There was significant difference of Tetraselmis sp. growth between the batches of different light intensities: between 30-75; 75-125; 125-150; 150-175 and 175- 91.28(natural light) µm photon.s^{-1}.m^{-2} (t-test > t-critical) and insignificant difference of growth between 175-200µm photon.s^{-1}.m^{-2} (t-test < t-critical).

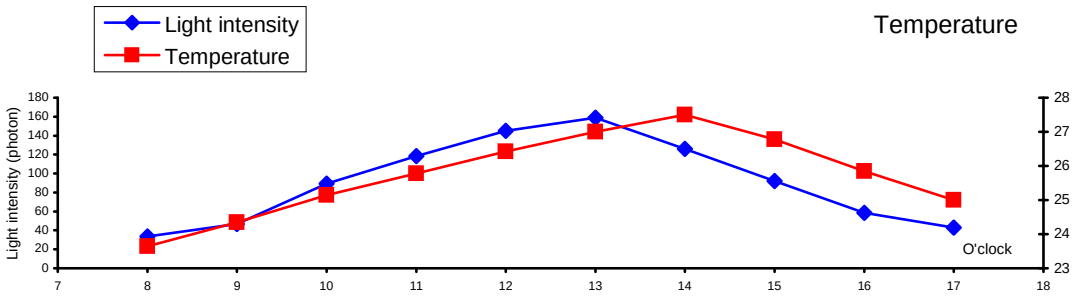

Figure 6: Average variable temperature and light intensity in a day

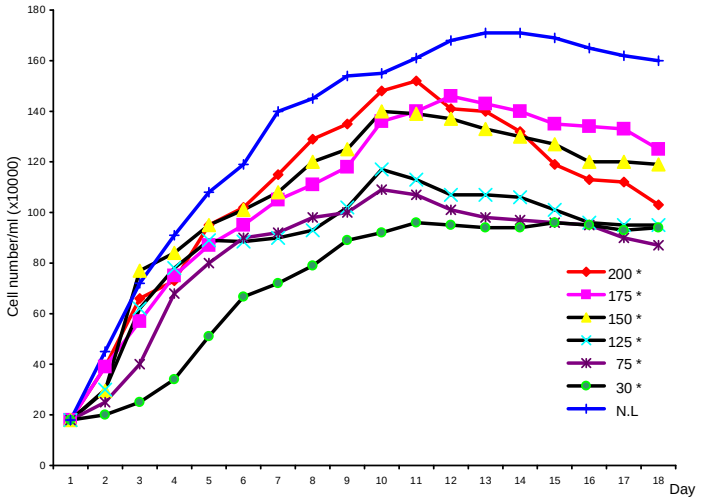

Figure 7: Effect of light intensity on the growth of Tetraselmis sp.
(* : µm photon.s^{-1}.m^{-2}; N.L : natural light intensity)

The result above showed that under natural light condition, Tetraselmis sp. grew better than under fluorescent light condition. This result confirmed that the algal growth was not only affected by light intensity but also by light quality (Guillard, 1975; Fulks & Main 1991).

6. Effects of nitrogen concentration

In the cultures with 2.36 mg N/l, the algae grew slowly, after 10 days of culture, the maximum cell density obtained 64 x 10^4 cells/ml. Whereas, in the cultures with 7.36 mg N/l, the algal biomass reached 121 x 10^4cells/ml on the10th day and continued increasing to reach the maximum density of 169 x 10^4 cells/ml on the 17th day of culture.

In the cultures with 12.36 mg N/l, after 17 days of culture, the maximum cell density was 200 cells/ml, concurrently with pH high increase of these cultures (9.61).

In the cultures with 17.36 and 22.36 mg N/l, after 16 days of culture, the maximum cell densities reached 232 x 10^4 and 270 x 10^4 cells/ml and concurrently with pH increase 9.72 and 9.89, respectively.

The above results showed that in the critical range of N concentrations, the algal biomass increased along with increase of N concentrations in cultures. However, out of the critical range, the very high N concentration could cause negative effects to the growth of the algae. With 27.36 and 32.36 mg N/l in the cultures, the maximum cell densities reached only 102 x 10^4 and 91 x 10^4 cells/ml, respectively.

The algae reached the lowest cell density (64 x10^4 cells/ml) in cultures with 2.36 mg N/l and the highest cell density (270 cells/ml) in cultures with 22.36 mg N/l. Out of the N critical range, although N concentration increased, the algae biomass decreased rapidly. There was significant difference of the growth between different treatments (t-test, > t-critical): between 2.36-7.36mg/l; 7.36-12.36mg/l; 12.36-17.36mg/l; 17.36-22.36mg/l; 22.36-27.36mg/l; 27.36-32.36mg/l.

7. Effects of phosphate concentration

In Figure 9 and Table 6, the result showed that in the treatment without enrichment of phosphate concentration (0.0 mg P/l), the algal growth and cell division were slow. The algae reached the maximum density of 60x10^4 cells/ml after 4 days of culture in all replicates and then collapsed rapidly. Although there was no P supplement in this treatment, there was always a very low phosphate concentration with the N:P ratio of 16:1 in natural seawater (Redfiled & al.1960 ; cited by Diep 1999).

Along with the increase of phosphate concentration was enriched such as 0.27, 0.77, 1.27, 1.77 and 2.77 mg P/l, the algal density also increased and reached the maximum cell density of 175 x 10^4 cells/ml at the treatment with highest phosphate concentration (2.27mg P/l).

For the treatment with cultures was provided 3.27 mg P/l, the maximum cell density decreased to 155x10^4 cells/ml. There was significant difference of the growth between different treatments (t-test > t-critical): between 0.0-0.27mg/l; 0.27-0.77mg/l; 0.77-1.27mg/l; 1.27-1.77mg/l; 1.77-2.27mg/l and 2.27-3.27mg/l.

The above results indicated that Tetraselmis sp. grew well in $F/_2$ medium with phosphate concentration from 0.77 to 3.27 mg/l.

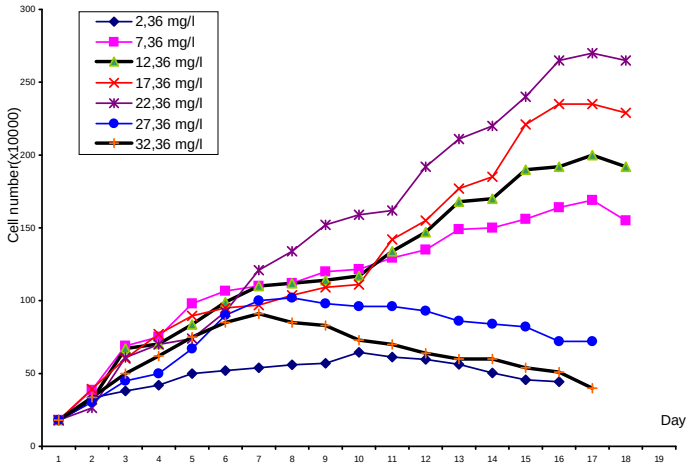

Figure 8: Effect of nitrogen concentration on the growth of Tetraselmis sp.

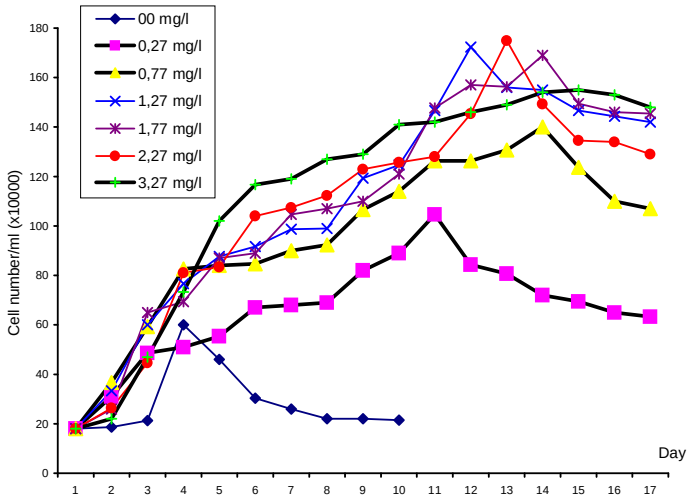

Figure 9: Effect of phosphate concentration on the growth of Tetraselmis sp.

CONCLUSIONS

1. Tetraselmis sp. can tolerate the large range of salinity (10 - 45ppt). The suitable range of salinity was from 35- 45 ppt, corresponding with maximum densities of 200 –266 x 10^4 cells/ml on day 16 of culture. At salinity of 10 ppt, the algae grew very slowly and took a long time to adapt. The optimum salinity for the growth of the algae was 35 ppt.

2. High temperature was not suitable for the growth of Tetraselmis sp. At temperature of 34^{o}C, the algae collapsed completely. The suitable range of temperature for the growth of the algae was from 22–31^{o}C. The optimum temperature for the Tetraselmis sp. growth was 28^{o}C.

3. In small culture volumes (500ml), the suitable initial densities for stable growth of Tetraselmis sp. were 10 -20 x 10^4 cells/ml. The optimum initial density for the growth of the algae was 20 x 10^4 cells/ml.

4. In fluorescent light condition with light intensities from 30-200µm photon.s^{-1}.m^{-2}, the density of the algae increased while the stationary phase decreased according to increase of light intensity. The suitable fluorescent light intensities for growth of Tetraselmis sp. were from 150-200µm photon.s^{-1}.m^{-2}. In addition, the growth of the algae was also affected by light quality. Comparing with the same light intensity and culture volume, under natural light condition, Tetraselmis sp. grew better than under fluorescent light condition.

5. The growth of Tetraselmis sp. depended largely on the variety of nitrogen and phosphate concentration in culture medium. In $F/_2$ medium, the critical range of N content for growth of the algae was from 7.36 – 22.36 mg/l. Out of the critical range, both very high or low nitrogen concentration were not good for the growth of Tetraselmis sp. Similarly, the optimum phosphate concentration for growth of Tetraselmis sp. were from 0.77 –3.27 mg/l.

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REFERENCES

1. Dang Van Giap, 1997. Analyze scientific data by MS-EXCEL. (Especially for the use of postgraduate students and trainees). Education Publishing House, 95pp.

2. Diep Luc Minh, 1999. Study on the effects of N, P, Si and harvesting scales to the growth of mixed natural algae and experimental culture of Nannochloropsis occulata (Droop) Hibber - 1981. Master thesis. University of Fisheries, Nhatrang.
3. Fulks. W. and Main. K. L., 1991. Rotifer and microalgae culture systems: Microalgal production systems. The Oceanic Institute Makapuu Point, Honolulu, Hawaii, pp. 5-22.
4. Guillard. R. R. L., 1975. Culture of phytoplankton for feeding marine invertebrates. Plemm Publishing Corporation. Massachusetts, pp. 29-60.
5. Kongkeo. H., 1991. An overview of live feeds production system design in Thailand. Rotifer and microalgae culture systems. Proceedings of U.S. Asia Workshop. The Oceanic Institute. Honolulu, Hawaii. pp, 175-186.
6. Lim. L. C., 1991. An overview of live feeds production system in Singapore. Rotifer and microalgae culture systems. Proceedings of U. S. Asia Workshop. The Oceanic Institute. Honolulu, Hawaii. pp, 203-220.
7. Okauchi. M., 1991. The status of phytoplankton production in Japan. Rotifer and microalgae culture systems. Proceedings of U.S. Asia Workshop. The Oceanic Institute. Honolulu, HI, 247-256.
8. Pham Thi Lam Hong, 1999. Study on the effects of salinity, light and harvesting scale to some biological characteristics, biochemistry composition of two micro-algal species Nannochloropsis occulata (Droop) Hibber, 1981 and Chaetoceros muelleri Lemmerman, 1898 in laboratory. Master thesis. University of Fisheries, Nhatrang.
9. Reintan. K. I., 1994. Nutritional effects of algae in first feeding of marine fish larvae. University of Trondheim, Norway.
10. Reitan. K. I., Rainuzzo. J. R., Oie. G. and Olsen. Y., 1997. A review of the nutritional effects of algae in marine fish larvae. Aquaculture. ELSEVIER. Norway. 155: 207-221.
11. Renaud. S. M., Thinh. L. V. and Parry. D. L., 1999. The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. Aquaculture 170. ELSEVIER. Australia, 147-159.
12. Tamaru. C. S., Ako. H., Lee. C. S. and Bass. P., 1993. Fatty acid composition of rotifers cultured on various combinations of phytoplankton and baker's yeast and its effect on the growth and survival of larval milkfish (Chanos chanos). TML Conference Proceedings. Finfish hatchery in Asia. Proceedings of finfish hatchery in Asia '91. Tungkang Marine laboratory, TFRI, Taiwan. The Oceanic Institute, Hawaii, USA. 3:97-108.
13. Tamaru. C. S., Fitzgerald. W. J. and Vernon Sato, 1993. Hatchery manual for the artificial propogation of Striped Mullet (Mugil cephalus L.): Chapter 4: Phytoplankton culture. Christine Carlstrom-Trick. Guam, pp, 45-68.
14. Throndsen. J., 1997. Chapter 5: The planktonic marine Flagellates (of Identifying marine phytoplankton). Academic Press, Inc. pp 591-699.
15. Ukeles. R., 1971. Nutritional requirements in shellfish culture. In: K.S. Price and D.L. Maurer (Eds.). University of Delaware, Newark, Delaware, pp. 43-64.
16. Volkman. J. K., Brown. M. P., Dunstan. G. A. and Jeffrey. S. W., 1993. The biochemical composition of marine microalgae from the class Eustimatophyceae. Phytochemistry. CSIRO. Australia, Vol. 29, pp. 69-78.
17. Zhukova. N. and Aizdaicher. N. A., 1995. Fatty acid composition of 15 species of marine microalgae. Phytochemistry. Elsevier Science Ltd. Britain Vol. 39. No. 2, pp. 351-356.