

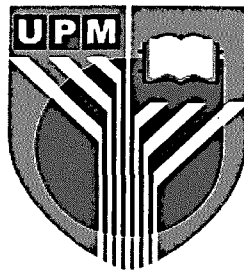


UNIVERSITI PUTRA MALAYSIA

***EFFECTS OF SALINITY AND TEMPERATURE ON GROWTH AND
ULTRASTRUCTURE OF *Tetraselmis tetraathele* (WEST)
BUTCHER, 1959***

DORA LAI JANG ING

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UPM
UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI

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ULTRASTRUCTURE OF *Tetraselmis tetrathele* (WEST)
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By

DORA LAI JANG ING

**Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in fulfilment of the Requirements for the
Degree of Master of Science**

May 2013

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**EFFECTS OF SALINITY AND TEMPERATURE ON GROWTH AND
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May 2013

Chair: Fatimah Md. Yusoff, PhD

Faculty: Faculty of Agriculture

The effects of salinity and temperature on the growth and ultrastructure of *Tetraselmis tetraathele* were evaluated in this study. Seven salinity levels (0, 10, 20, 30, 40, 50 and 60 ppt) were examined with a 12h:12h light:dark cycle under light intensity of $67.5 \mu\text{mol}/\text{m}^2 \text{ s}$. Effects of temperature on the growth and ultrastructure of *T. tetraathele* was carried out at 25°C and 30°C at the salinity of 30 ppt with other culture conditions similar (12h:12h L:D; $67.5 \mu\text{mol}/\text{m}^2 \text{ s}$) to those used in salinity experiment. After execution of the studies with salinity and temperature, the selected salinity (30 ppt) and temperature, $26 \pm 2^\circ\text{C}$ were used to up-scale *T. tetraathele* using annular photobioreactors with different culture medium (f/2 and Conway medium) in an indoor environment.

The specific growth rate of *T. tetrahele* was lowest ($p < 0.05$) at 0 ppt. However, the growth rates of *T. tetrahele* did not differ significantly ($p > 0.05$) in 10, 20, 30, 40, 50 and 60 ppt. The study revealed that *T. tetrahele* thrived best in 40 ppt. Upon exposure for 30 minutes, transmission electron micrographs of *T. tetrahele* revealed that cells were surrounded with a few layers of thecae and chloroplast of the cells were filled with starch and lipid. After 4 days of cultivation, layers of thecae, amount of lipid and starch decreased in all the treatments.

The specific growth rate of *T. tetrahele* at 30°C (0.24/day) was significantly lower to those grown in 25°C (0.63/day). Numerous starch plates and lipid globules observed in cultures exposed for 30 minutes at 25°C and 30°C. Starch plates and lipid globules were depleted after 4 days of both culture. Electron micrographs of *T. tetrahele* grown at 30°C revealed that chloroplast structure was loosely stacked in the microalgal cells indicating that cells were unable to withstand high temperatures (30°C).

Culture medium did not affect ($p > 0.05$) the growth and biomass production of *T. tetrahele* grown in indoor photobioreactors. Protein content was affected by growth phases ($p < 0.05$) with a significantly higher percentage ($p < 0.05$) during the exponential phases compared to the stationary phases in both culture medium. Carbohydrate and lipid contents increased during the stationary phase of both f/2 and Conway enriched cultures. Conway enriched *T. tetrahele* produced significantly higher ($p < 0.05$) lipid content as

compared to f/2 enriched cultures. Polyunsaturated fatty acids (PUFA) were found to be the major proportion in the fatty acid profiles of *T. tetrathele*.

Fatty acids that were found to be major constituents of *T. tetrathele* were palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2 n-6) and α -linolenic acid (C18:3 n-3). The results of the present study showed that low salinity (0 ppt) and high temperature (30°C) decreased the growth of *T. tetrathele*. Both f/2 and Conway medium can be used for the cultivation of *T. tetrathele* as growth and biomass were comparable in both medium ($p > 0.05$). Besides that, protein was found to be a major composition (16.00 to 39.00%) in *T. tetrathele* as compared to carbohydrate (16.00 to 23%), lipid (21.00 to 35.00%), ash (15.00 to 23.00%) and moisture (0.10 to 0.30%).

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai mematuhi keperluan untuk ijazah Master Sains

**KESAN KEMASINAN DAN SUHU KE ATAS PERTUMBUHAN DAN
ULTRASTRUKTUR *Tetraselmis tetrathele* (WEST)
BUTCHER, 1959**

Oleh

DORA LAI JANG ING

Mei 2013

Pengerusi: Fatimah Md. Yusoff, PhD

Fakulti: Fakulti Pertanian

Kesan kemasinan dan suhu terhadap pertumbuhan dan ultrastruktur *Tetraselmis tetrathele* diuji dalam kajian ini. Tujuh tahap kemasinan (0, 10, 20, 30, 40, 50 dan 60 ppt) digunakan dengan jangkamasa cahaya 12h:12h siang:malam di bawah kesan cahaya $67.5 \mu\text{mol}/\text{m}^2 \text{ s}$. Kesan suhu pula dikaji pada 25°C dan 30°C dengan tahap kemasinan 30 ppt. Parameter eksperimen (jangkamasa cahaya dan keamatan cahaya) adalah sama seperti yang digunakan dalam kajian tahap kemasinan. Selepas kajian tahap kemasinan dan suhu, kemasinan (30 ppt) dan suhu ($26 \pm 2^\circ\text{C}$) dipilih untuk pengkulturan *T. tetrathele* dalam media berlainan (f/2 dan Conway media) dengan menggunakan fotobioreaktor dalam bilik.

Kadar pertumbuhan spesifik *T. tetrahele* adalah paling rendah ($p < 0.05$) pada tahap kemasinan 0 ppt. Namun demikian, kadar pertumbuhan spesifik *T. tetrahele* pada 10, 20, 30, 40, 50 dan 60 ppt tidak menunjukkan perbezaan yang signifikan ($p > 0.05$). Keputusan kajian menunjukkan tahap kemasinan 40 ppt adalah paling baik untuk pertumbuhan *T. tetrahele*. Selepas didedahkan selama 30 minit dalam tahap kemasinan masing-masing, mikrograf mikroskop elektron menunjukkan sel-sel diselaputi beberapa lapisan dinding sel dan kloroplast sel dipenuhi dengan kanji dan lipid. Selepas 4 hari, lapisan dinding sel, jumlah lipid dan kanji semakin berkurang dalam semua rawatan.

Kadar pertumbuhan spesifik *T. tetrahele* adalah 0.24/hari pada 30°C dan jauh lebih rendah ($p < 0.05$) daripada sel yang dikultur pada 25°C (0.63/hari). Banyak kanji dan lipid yang didapati dalam sel yang dikultur selama 30 minit pada 25°C dan 30°C. Kanji dan lipid didapati berkurangan selepas 4 hari dalam kedua-dua rawatan suhu. Namun, mikrograf elektron *T. tetrahele* yang dikultur pada 30°C menunjukkan struktur kloroplast disusun dengan longgar. Ini menunjukkan sel tidak dapat bertahan dalam suhu yang tinggi (30°C).

Pertumbuhan dan penghasilan biomas *T. tetrahele* yang dikultur menggunakan fotobioreaktor dalam bilik tidak dipengaruhi ($p > 0.05$) oleh kultur media yang digunakan (Conway dan f/2). Kandungan protein dipengaruhi oleh fasa pertumbuhan ($p < 0.05$) dengan peratusan yang lebih

tinggi pada fasa eksponen berbanding dengan fasa pegun untuk kedua-dua media yang digunakan. Kandungan karbohidrat dan lipid meningkat semasa fasa pegun dalam kedua-dua kultur media f/2 dan media Conway. *Tetraselmis tetrathele* yang dikultur dalam media Conway menghasilkan lipid yang lebih tinggi ($p < 0.05$) berbanding dengan kultur menggunakan media f/2. Asid lemak tak tepu (PUFA) adalah paling banyak didapati dalam profil asid lemak *T. tetrathele*.

Kandungan asid lemak yang banyak dalam sel adalah asid palmitik (C16:0), asid oleic (C18:1), asid linoleik (C18:2 n-6) dan α -asid linolenik (C18:3 n-3). Keputusan kajian menunjukkan tahap kemasinan yang rendah (0 ppt) and suhu yang tinggi (30°C) melambatkan pertumbuhan *T. tetrathele*. Kedua-dua media f/2 dan media Conway didapati sesuai untuk pengkulturan *T. tetrathele* dengan pertumbuhan dan penghasilan biomas yang standing ($p > 0.05$). Selain itu, protein merupakan kandungan nutrien yang paling tinggi dalam *T. tetrathele* (16.00 hingga 39.00%) berbanding dengan kandungan karbohidrat (16.00 hingga 23.00%), lipid (21.00 hingga 35.00%), abu (15.00 hingga 23.00%) dan kelembapan (0.10 hingga 0.30%).

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Microalgae are known as the baseline feed for young stages of crustaceans (Fabregas *et al.*, 1996; D'Souza and Kelly, 2000; D'Souza *et al.*, 2002), mollusks (Gallardo *et al.*, 1992; Ponis *et al.*, 2006) and fishes (Juario and Storch, 1984; Reitan *et al.*, 1997). Their high nutritional contents are important for enrichment of live feed organisms such as rotifers, *Artemia* and copepods (Barclay and Zeller, 1996; Ferreira *et al.*, 2008, 2009; Seychelles *et al.*, 2009; Matias-Peralta *et al.*, 2012) where the nutritional profiles of the fed organisms reflected those of their feed (Barclay and Zeller, 1996; Ferreira *et al.*, 2008, 2009; Seychelles *et al.*, 2009). Hence, microalgae are crucial as one of the main food items in hatcheries for aquaculture.

In the recent years, microalgae had extended their potentials and applications into the realm of food supplement industries for aquaculture (Muller-Feuga, 2000) and human diets (Lubian *et al.*, 2000; Olaizola, 2003). Applications in cosmetics (Spolaore *et al.*, 2006), pharmaceuticals (Spolaore *et al.*, 2006), CO₂ sequestration to reduce global warming effects (Dibenedetto, 2011; Kumar *et al.*, 2011) are emerging and of late, microalgae are metabolically engineered as a single cell factories for fine chemical productions such as astaxanthin (He *et al.*, 2007), β -carotene (Hejazi *et al.*, 2004; Garcia-Gonzalez *et al.*, 2005; Tafreshi and

Shariati, 2006) and biofuel production (Hirano *et al.*, 1997; Beer *et al.*, 2009; Radakovits *et al.*, 2010).

Chlorella, *Chaetoceros*, *Isochrysis*, *Nannochloropsis*, *Pavlova*, *Phaeodactylum*, *Skeletonema*, *Tetraselmis* and *Thalassiosira* are common genera of microalgae frequently used in aquaculture industries (Muller-Feuga, 2000). These microalgae were used in feeding zooplankton (Habib *et al.*, 2003; Lora-Vilchis *et al.*, 2004; Farhadian *et al.*, 2008), bivalves (Chotipuntu, 2005; Reitan, 2011), shrimps (Ju *et al.*, 2009), sea urchin (Carboni *et al.*, 2012), juvenile worms of *Sabellastarte spectabilis* (Tamaru *et al.*, 2011) and fish (Zaki and Saad, 2010). Chrysophytes such as *Isochrysis galbana* and *Pavlova lutheri* are used in feeding the molluscan larvae as they are high in DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) (Lora-Vilchis *et al.*, 2004; Hu *et al.*, 2008). *Nannochloropsis* is usually used in growing rotifers, corals and filter feeders due to their high EPA levels (Ferreira *et al.*, 2009; Hemaiswarya *et al.*, 2011) while diatoms such as *Chaetoceros* and *Thalassiosira* are used in shrimp farms (Hemaiswarya *et al.*, 2011). Combinations of these microalgae as feed were found to be more superior in nutritional values than monospecies diet (Spolaore *et al.*, 2006; O'Connor *et al.*, 2011). Microalgae monocultures can be used as feed, but it is not as better-quality as mixed microalgae formulation (Asha and Muthiah, 2006) in terms of biochemical composition and dietary energy (Phatarpekar *et al.*, 2000). A large number of investigations (Fabregas *et al.*, 2001; Lora-Vilchis *et al.*, 2004; O'Connor *et al.*, 2011) were conducted and are still ongoing to elucidate the

nutritional values of microalgae for aquaculture species. Usage of some species from the genus *Tetraselmis* is summarized in Table 1.

Table 1. Application of microalgae from genus *Tetraselmis*.

Microalgae species	Use	References
<i>T. suecica</i> <i>T. gracilis</i> <i>T. tetraathele</i> <i>T. chuii</i>	Common aquaculture feed	Asha and Muthiah (2006), Ronquillo <i>et al.</i> (2006)
<i>T. suecica</i> <i>T. chuii</i> PLY 429 <i>T. striata</i> PLAT-P <i>Tetraselmis</i> sp. MC:2 <i>T. chuii</i> UW.445 <i>T. chuii</i> PLY 429S	Bivalve production	Wikfors <i>et al.</i> (1996), Jo <i>et al.</i> (2004)
<i>T. suecica</i>	Heavy metal removal and biomonitoring	Perez-Rama <i>et al.</i> (2002), Moreno-Garrido <i>et al.</i> (2005)

Even though much work has been carried out to replace microalgae with commercial feed due to high operational cost and unpredictable culture conditions (Coutteau and Sorgeloos, 1993), results of the commercial feed is still not as superior as feeding with live or dried microalgae (Robert and Trintignac, 1997). The study conducted by Øie *et al.* (1994) revealed that rotifer fed with microalgae had better survival rate when tested at low temperature (8.0°C) and high salinity (34 ppt) while lower growth rates and higher mortality rates were observed in cultures that were grown on non-algal feeds. Apart from that, growth and survival of *Penaeus monodon* postlarvae (PL) were found to be significantly higher when diatoms were supplied as supplement feed (Khatoun *et al.*, 2009). The authors reported that survival of the postlarvae was 56.3% in treatments supplemented with diatoms while only 36.0% was achieved in treatments fed with

commercial feed only. Besides that, *Litopenaeus vannamei* fed with *Diaphanosoma celebensis* enriched with *Chaetoceros calcitrans* showed a higher specific growth rate and survival rate from those fed with *Artemia* only (Khatoon *et al.*, 2013).

In this regard, microalgae production with various culture systems and techniques are equally important since culture conditions are known to change or modify the nutritional values of microalgae (Rousch *et al.*, 2003; Rodolfi *et al.*, 2009). Changes in nutritional value will in turn influence the organisms fed using microalgae (Seychelles *et al.*, 2009; Ferreira *et al.*, 2011). Besides that, culture systems such as photobioreactor enable high production of microalgae biomass from usual tank production (Zhang and Richmond, 2003; Briassoulis *et al.*, 2010). With the availability of suitable culture systems, culture conditions such as nutrient availability, light intensity, pH, temperature and salinity can then be optimized for high quality and maximum biomass production (Olaizola, 2003; Zhang and Richmond, 2003; Rodolfi *et al.*, 2009). For example, by optimizing the growth parameters of a helical-tubular photobioreactor, *Nannochloropsis* sp. cultured was able to achieve productivity of biomass that range between 1.10-3.03 g/L/day (Briassoulis *et al.*, 2010).

1.2 Statement of Problem

There has been a marked increase in aquaculture activities in Asia with China as the largest producer, accounting for 62.3% of the world production in 2008. In Malaysia, there was an increase of 96,140 t aquaculture production from 2007 to 2008 (FAO, 2010). Artificial diet such as yeast is developed as alternative feed due to their suitable particle size, stability in water column, high protein content and low operational cost (Coutteau and Sorgeloos, 1993). Øie *et al.* (1994) demonstrated that yeast and microalgae both offers equal protein content to rotifers though survivability of rotifers were better in those enriched with microalgae. Then again, preserved microalgae in commercial diets may lose their nutritional value during processing and storage. An investigation on the growth and survival of juvenile hard clam, *Mercenaria mercenaria* using fresh *Isochrysis* and commercial feeds added with *Isochrysis* showed that those fed with fresh diet had higher growth and survival (Espinosa and Allam, 2006). In addition, the same study also revealed that growth and survival of the controls (unfed clams) were similar to those fed with commercial diets indicating the loss of nutritional value in the commercial diets added with live algae. Therefore, sufficient high quality feed is vital for the development of this industry while maintaining low production cost and production reliability (Duerr *et al.*, 1998; Lopez-Elias *et al.*, 2005).

In the wild, environmental conditions alter growth, nutritional values and morphological characteristics of microalgae (Cai and Duan, 2007; He *et al.*, 2007; Converti *et al.*, 2009; Guedes *et al.*, 2010). With the knowledge that culture parameters are important for high quality microalgae production, these parameters need to be optimised for the selected microalgae species. Apart from that, many hatcheries in tropical countries are situated outdoors, hence, are subjected to fluctuations of temperature, light intensity and rainfall (Mandeep *et al.*, 2011).

For marine microalgae, salinity and temperature changes are two important factors that alter their growth, biochemical composition and morphology (Pfehofer and Belton, 1975; Villareal and Fryxell, 1983; Lewis *et al.*, 1993; Rousch *et al.*, 2003; Balzano *et al.*, 2011; Gu *et al.*, 2012). Ultrastructural changes in microalgae provide information at a cellular level which is important for understanding cellular feedback mechanisms of microalgae under different environments (Kirst and Kramer, 1981; Tchernov *et al.*, 2004). Feedback mechanisms are crucial for the adaptation and survivability of microalgae (Richardson *et al.*, 1983; Kirst, 1989; Davison, 1991). At the same time, it can serve as a basis of supporting evidence on the biochemical composition of microalgae, either for starch or lipid accumulation and to be utilized in consideration for mass culture harvesting (Damiani *et al.*, 2010).

Tetraselmis tetrathele is selected as the study organism because of its hardy characteristic, ease of culture, fast growing, good in nutrition and is isolated indigenously. Through selection of suitable salinity level and temperature,

nutrient composition of different media can be further investigated. In addition to that, biomass production of *T. tetraathele* with the selected salinity and temperature by upscaling in photobioreactor provide information for mass production of this species which is important for development of live feed in the aquaculture industry. Therefore, the study was undertaken with the following objectives:

1. To determine the effects of different salinity levels on the growth and ultrastructure of *T. tetraathele*
2. To determine the effects of different temperature levels on the growth and ultrastructure of *T. tetraathele*
3. To determine the growth and biochemical composition of *T. tetraathele* cultured with different media in 120 L annular photobioreactors

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