OPTIMUM GROWTH PARAMETERS FOR BOTH INDOOR AND OUTDOOR PROPAGATION OF MICROALGAE, *CHLORELLA VULGARIS* AND *ISOCHRYSIS GALBANA*

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By

SITTI RAEHANAH BINTI MUHAMAD SHALEH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy.

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Dedicated to

Ayah, Mummy, Sharudin,Husna, Nadia & Sofea

Thank you for their true love, support, understanding and their non-stopped devotions for making everything come true.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

Optimum Growth Parameters for Both Indoor and Outdoor Propagation of Microalgae, *Chlorella vulgaris* and *Isochrysis galbana*

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June 2004

Chairmain : Hishamuddin Bin Omar, Ph.D

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Chlorella vulgaris and *Isochrysis galbana* were grown under different growth conditions both in indoor and outdoor cultures. Best culture conditions such as light intensities, photoperiods, salinities and nutrient concentrations were determined as well as the biochemical compositions. Indoor cultures of *C. vulgaris* and *I. galbana*, when continuously illuminated, grew optimally at 100 $\mu molm^{-2}s^{-1}$ and *4*0 $\mu molm^{-2}s^{-1}$, respectively. However, under 12 hour light, *C. vulgaris* grew optimally at 200 $\mu molm^{-2}s^{-1}$. The $chlorophyll_a$ concentrations in both algae were lower at higher light intensity and higher at lower light intensity. Total protein and total lipid contents of both species were also significantly higher ($p<0.05$) at lower light intensity. *I. galbana* contained more lipid than *C. vulgaris* in those conditions. In contrast to protein and lipid, carbohydrate content increased with increasing light intensity. Similar to *C. vulgaris*, the lowest carbohydrate content of *I. galbana* was obtained at l0 $\mu molm^{-2}s^{-1}$ conditions for both photoperiods. Although the protein contents of *C. vulgaris* varied under different light intensity and photoperiod, the relative

proportions of constituent amino acids were found to be almost similar. Total essential amino acids under continuous illumination was significantly higher ($p<0.05$) at the lowest and the highest intensities while in *I. galbana*, it was significantly higher ($p<0.05$) only at the lowest intensity. It was surprising that *C. vulgaris* contained high proportions of ω-3 highly unsaturated fatty acids at continuous illuminations although both eicosapentaenoic acid (20:5ω-3) and docosahexaenoic acid (22:6ω-3) were absent at 12 hours light. EPA in *I. galbana,* was absent at 12 hours light, while the DHA was present in relatively smaller quantities than in continuous light.

In indoor conditions, at full media concentration, the best salinity for *C. vulgaris* was at 30 ppt and 25 ppt for *I. galbana. C. vulgaris* grown in full media concentration contained more chlorophyll$_a$ relative to that in half media concentration. For *I. galbana* grown in full media concentration, chlorophyll$_a$ increased as salinity increased. Protein contents decreased as salinity increase both at full and half strength media for *C. vulgaris* and *I. galbana*. The cellular lipid contents of both algae were significantly higher ($p<0.05$) in half media concentration. In full media concentration, total lipid contents in *C. vulgaris* decreased at higher salinity but in *I. galbana* no clear trend in lipid contents in relation to salinity observed. As salinity increased, higher carbohydrate contents were found in *C. vulgaris* in full media concentration, while in half media concentration an inverse trend was detected. *I. galbana* also revealed higher proportions of total carbohydrates in half media concentration compared to full media concentration. The amino acid profile of both algae exhibited minor differences at different salinity and media

concentrations. Most of the essential amino acid in *C. vulgari*s and *I. galbana* were significantly higher ($p<0.05$) at lower salinity. In *C. vulgaris,* the EPA was absent while only trace amount of DHA were detected in cells grown under full media concentration. However in *I. galbana* the amount of EPA was very high at higher salinity and in contrary the, DHA was higher at lower salinity.

The growth performances of *C. vulgaris* outdoor cultures were relatively better than *I. galbana* under all growth conditions studied. *C. vulgaris* grown both in unsheltered and sheltered areas demonstrated rapid increased in cell density after four days of culture with no lag phases observed. Unlike *C. vulgaris*, cultures of *I. galbana* grown under unshaded conditions demonstrated increase in cell numbers for 2 days but cells under sheltered place demonstrated no increased at all. The highest specific growth rate in *C. vulgaris* was in lower salinity at half media concentration while the maximum cell density was achieved by cells in full media concentration of the same salinity. For *I. galbana* cells density of half media concentration was higher then in full media concentration. The highest total protein content of *C. vulgaris* was obtained in culture under shaded conditions and generally total lipid and carbohydrate were found to be higher in unshaded conditions. However, it was unexplainable why less carbohydrate was found in older cells at both conditions. *I. galbana* was a fast growing algae and the protein as well as lipid contents were significantly higher ($p<0.05$) at sheltered condition while no significant changes to carbohydrate content was recorded. The carbohydrate content in *C. vulgaris* was higher in older cultures in full

and half media concentration while at early culture age, the lipid content was higher. In the early culture age of *I. galbana,* protein content was insignificantly affected ($p>0.05$) by salinity and media concentration. However, total lipid and carbohydrate were found higher at 15 ppt compared to higher salinity in half media concentration.

In the bioeconomic study the production cost of *C. vulgaris* was found to be directly influenced by labour cost. From the production forecast, it was discovered that the high production cost was due to underutilized labour force which can be reduced by having bigger scale production. In order to break-even for one cycle of 100 L cultures, the minimum culture volume should be 1,902 litres.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

Parameter Pertumbuhan Optima untuk Propagasi Mikroalga *Chlorella vulgaris* dan *Isochrysis galbana* dalam Keadaan Terlindung dan Terdedah.

Oleh

SITTI RAEHANAH BINTI MUHAMAD SHALEH

Jun 2004

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Fakulti : Sains

Pengkulturan di dalam makmal dan di luar makmal telah dijalankan ke atas *Chlorella vulgaris* dan *Isochrysis galbana*. Tindakbalas pertumbuhan unggul alga terhadap faktor cahaya, fotokala, saliniti air dan kepekatan nutrien dikenalpasti di samping komposisi biokimia. Dalam keadaan makmal iaitu pada cahaya berterusan, didapati intensiti cahaya optimumt bagi *C. vulgaris* dan *I. galbana* adalah masing-masing pada paras 100 $\mu molm^{-2}s^{-1}$ dan 40 $\mu molm^{-2}s^{-1}$. Manakala dalam keadaan 12 jam bergelap, kedua-dua mikroalga tumbuh secara optimum pada paras 200 $\mu molm^{-2}s^{-1}$. Kepekatan klorofil$_a$ dalam kedua-dua alga juga serupa, iaitu rendah pada keadaan cahaya tinggi. Kandungan protien dan lipid kedua-duanya juga berbeza dengan ketara ($p<0.05$) iaitu tinggi dalam keadaan cahaya terhad. Namun *I. galbana* mengandungi lebih lipid berbanding *C. vulgaris.* Berbeza dengan protien dan lipid, karbohidrat meningkat dengan peningkatan intensiti cahaya. Seperti *C. vulgaris*, kandungan karbohidrat dalam *I. galbana* rendah pada cahaya terhad dalam kedua-dua fotokala. Sungguhpun kandungan

protien *C. vulgaris* berbeza pada intensiti cahaya dan fotokata berbeza, profil asid amino adalah hampir serupa. Dalam cahaya berterusan, jumlah asid amino perlu ketara lebih tinggi ($p<0.05$) pada cahaya terendah dan tertinggi, manakala perlakuan sama dalam *I. galbana* adalah pada cahaya yang rendah. *C.vulgaris* mengandungi asid lemak ω-3 yang tinggi pada cahaya berterusan walaupun EPA dan DHA tiada pada keadaan 12 jam bergelap. Dalam keadaan 12 jam bergelap, EPA juga tidak didapati dalam *I. galbana* manakala DHA pula tinggi berbanding dalam cahaya berterusan.

Dalam kepekatan media penuh iaitu dalam kultur terkawal, saliniti unggul untuk *C. vulgaris* adalah 30 ppt dan bagi *I. galbana* pada 25 ppt. *C. vulgaris* dalam media berkepekatan penuh didapati mengandungi lebih klorofil$_a$ dan kepekatan klorofil$_a$ dalam *I. galbana* meningkat dengan peningkatan saliniti. Protien dalam *C. vulgaris* dan *I. galbana* menurun apabila saliniti air meningkat dalam kedua-dua media. Kandungan lipid kedua-dua alga ketara lebih tinggi ($p<0.05$) dalam media berkepekatan separuh. Dalam media berkepekatan penuh pula, lipid *C. vulgaris* menurun apabila saliniti meningkat manakala dalam *I. galbana* tiada corak perlakuan yang jelas terhadap kesan saliniti. Apabila saliniti meningkat, kandungan karbohidrat dalam *C. vulgaris* meningkat dalam media berkepekatan penuh manakala keadaan sebaliknya ditunjukkan dalam media berkepekatan separuh. *I. galbana* juga mengandungi karbohidrat yang tinggi dalam media berkepekatan separuh berbanding media berkepekatan penuh. Profil asid amino bagi kedua-dua mikroalga mengalami sedikit perbezaan terhadap kesan saliniti dan kepekatan media. Kebanyakan asid amino perlu ketara

lebih tinggi ($p<0.05$) dalam *C. vulgaris* dan *I. galbana* pada saliniti rendah. Dalam *C. vulgaris*, tiada EPA ditemui manakala DHA amat sedikit pada sel yang tumbuh dalam media berkepekatan penuh. Walau bagaimanapun, dalam *I. galbana*, EPA didapati sangat tinggi pada saliniti tinggi dan DHA pula secara songsang tinggi pada saliniti rendah.

Perlakuan pertumbuhan *C. vulgaris* di luar makmal yang tidak terkawal secara perbandingan dengan *I. galbana* adalah sangat memuaskan dalam semua keadaan kultur. *C. vulgaris* dalam cahaya semulajadi dan terlindung telah menunjukkan peningkatan kepadatan sel selepas empat hari pengkulturan. Manakala *I. galbana* pula menunjukkan peningkatan selama dua hari dan tiada sebarang peningkatan dalam kultur terlindung. Pertumbuhan spesifik tertinggi *C. vulgaris* adalah pada saliniti rendah iaitu dalam media separuh dan kepadatan sel maksimum direkodkan dalam media penuh. Sebaliknya kepadatan sel *I. galbana,* tinggi dalam media berkepekatan separuh. Kandungan protin tertinggi bagi *C. vulgaris* ditemui dalam kultur terlindung manakala lipid dan karbohidrat umumnya tinggi dalam keadaan cahaya semulajadi. Sesuatu yang tidak dapat dijelaskan mengenai karbohidrat yang berkurangan dengan peningkatan masa pengkulturan iaitu dalam sel-sel tua telah ditemui dalam *C. vulgaris*. Kandungan protin dan lipid dalam *I. galbana* direkodkan ketara lebih tinggi ($p<0.05$) dalam kultur terlindung. Dalam keadaan media berkepekatan penuh dan separuh, kandungan karbohidrat dalam sel-sel tua *C. vulgaris* juga lebih tinggi manakala sel-sel mudanya mengandungi lebih lipid. Protien pula dalam sel-sel muda *I. galbana* tidak menunjukkan perubahan ketara ($p>0.05$)

terhadap kesan saliniti dan kepekatan media. Tetapi lipid dan karbohidrat didapati tinggi pada saliniti rendah dan lebih banyak tersintesis dalam keadaan media berkepekatan separuh.

Dalam kajian bioekonomi, kos pengeluaran *C. vulgaris* dipengaruhi secara langsung oleh kos buruh. Melalui unjuran pengeluaran, didapati bahawa kos pengeluaran tinggi yang disumbangkan oleh kos buruh dapat dikurangkan melalui penghasilan yang lebih tinggi dalam skala kultur yang lebih besar. Untuk mendapatkan semula modal pengeluaran untuk satu pusingan bagi setiap 100 L alga, isipadu minimum yang diperlukan ialah sebanyak 1,902 liter.

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I certify that an Examination Committee met on 15th June 2004 to conduct the final examination of Sitti Raehanah binti Muhamad Shaleh on her Doctor of Philosophy thesis entitled "Optimum Growth Parameters for Both Indoor and Outdoor Propagation of Microalgae, *Chlorella vulgaris* and *Isochrysis galbana*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This Thesis submitted to the Senate of Universiti Putra Malaysia and was accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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SITTI RAEHANAH BINTI MUHAMAD SHALEH

Date:

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