EFFECTS OF DIFFERENT SALINITIES ON GROWTH RATE, CHLOROPHYLL *a*, TOTAL PROTEIN AND LIPID CONTENT OF *CHLORELLA VULGARIS*.

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DECLARATION

I hereby declare that the thesis is based on my original work except for certain citations, quotations and summaries, which have been duly acknowledged.

22nd March 2005

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ABSTRACT

Chlorella vulgaris, a freshwater microalgae has a high potential as feed in the aquaculture industry. *C. vulgaris* was cultured outdoors under different salinities at 0, 5, 15 and 30ppt. The study intended to determinine salinity's effect on growth, chlorophyll $_a$, total protein and lipid content of *C. vulgaris*. *C. vulgaris* cultured in 5ppt attained the highest growth rate, chlorophyll $_a$ and protein content at 13.83 x 10^6 mL^{-1}, 0.33mgL^{-1} and 63.07% of dry weight respectively. *C. vulgaris* cultured in 15ppt has the highest total lipid content of 20.24% of dry weight. It was observed that total lipid content had decreased with increasing protein content in control (0ppt) and 5ppt treatments. *C. vulgaris* cultured in 30ppt had the lowest growth rate and biochemical composition, indicating that salinity beyond the tolerance level could cause stress in *C. vulgaris*, thus resulting in disruption of cellular metabolism. Changes in salinity have shown a significant difference on the growth rate, chlorophyll $_a$, total protein and lipid content of *C. vulgaris* ($P<0.05$).

ABSTRAK

KESAN PERBEZAAN SALINITI TERHADAP KADAR TUMBESARAN, KANDUNGAN KLOROFIL $_a$, PROTEIN DAN LIPID *CHLORELLA VULGARIS*

Chlorella vulgaris, sejenis mikroalga air tawar mempunyai potensi yang tinggi sebagai makanan ternakan dalam industri akuakultur. *C. vulgaris* telah dikultur dalam keadaan semulajadi, di luar makmal di dalam saliniti yang berbeza iaitu 0, 5, 15 dan 30ppt. Ini telah dilakukan untuk menentukan kesan saliniti terhadap kadar tumbesaran, kandungan klorofil $_a$, protein dan lipid dalam *C. vulgaris*. *C. vulgaris* yang dikultur dalam 5 ppt telah mencapai kadar tumbesaran, kandungan klorofil $_a$ dan protein yang tertinggi, iaitu masing-masing sebanyak 13.83×10^6 mL^{-1}, 0.33 mgL^{-1}, dan 63.07% daripada berat kering. *C. vulgaris* yang dikultur dalam 15ppt mempunyai kandungan lipid yang tertinggi iaitu 20.24% daripada berat kering. Kandungan lipid telah menurun apabila terdapat peningkatan pada kandungan protein dalam rawatan kawalan (0ppt) dan 5ppt. *C. vulgaris* yang dikultur dalam 30ppt telah menunjukkan kadar tumbesaran dan komposisi biokimia yang terendah. Ini menunjukkan bahawa saliniti yang melebihi tahap toleransi akan menyebabkan stres kepada *C. vulgaris* dan seterusnya akan mengganggu metabolisma sel. Perbezaan dalam saliniti telah menunjukkan perbezaan yang signifikan terhadap kadar tumbesaran, kandungan klorofil $_a$, protein dan lipid dalam *C. vulgaris* ($P<0.05$).

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CHAPTER 1

INTRODUCTION

1.1 An Introduction of *Chlorella vulgaris*

Microalgae are minute organisms that are suspended in natural waters, drifting or swimming within the currents and usually remaining near the surface of the water where many of these species derive their energy through photosynthesis (Firth, 1969). The microalgae community are the primary producers in the natural waters. Much of this microalgae are consumed by zooplanktons that are in turn eaten by larger animals (Krauss, 1962). Microalgae play an important role in aquaculture as food for many molluscs, crustaceans and some fish (Gatenby *et al.*, 2002). A functional definition of microalgae might be "photosynthetic singe-celled or colonial microorganisms", however most of these microbes are able to grow without light if dissolved sugars are provided (Wilkfors, 1984).

The microalgae being proposed in this study is *Chlorella vulgaris*. *C. vulgaris* is a freshwater microalgae and is commonly referred as the green algae. These green algae belong to the class Chlorophyceae. The shape of *C. vulgaris* cell is somewhat globose to ellipsoidal and bounded by a true cellulosic wall. The cell dimension of *C. vulgaris* is 2-10μm (Maruyama *et al.*, 1997).

C. vulgaris is commonly cultured as feed in aquaculture. Many cultured animals consume it as food source especially in their early stages of development (Leornados *et al.*, 1999). Apart from being a food source, *C. vulgaris* could also be used to grow small invertebrates such as rotifers and brine shrimp that are fed to crustaceans and finfish during its larval stages and are used to enrich these small invertebrates with nutritional compounds required by the larval crustaceans and finfish (Wilkfors *et al.*, 1984).

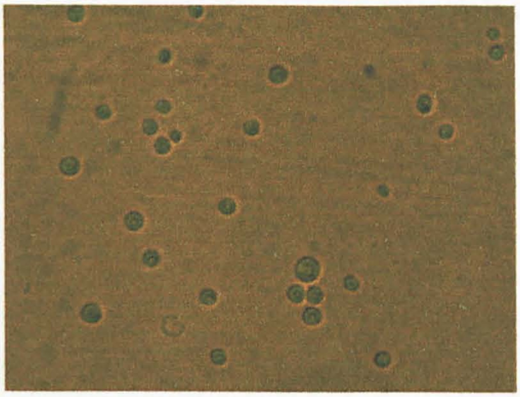

Photo 1.1 *Chlorella vulgaris* cells under magnification of X40.

(Source : Kamala, M., 2005)

C. vulgaris contains 55.0% of total protein, 10.2% of total lipid and 23.2% of total carbohydrate, 18 amino acids and 6 fatty acids (Maruyama *et al.*, 1997). Apart from the nutrients above, it also contains mineral and vitamins. The high biochemical composition of *C. vulgaris* is said to be essential for both human and cultured animals (Moore, 2001).

Aside from used as feed in aquaculture, *C. vulgaris* is also used as health food for human in the East Asia. According to Gottfried Mende, director of Klötze Plant in

Germany, a daily dose of 3g of *C. vulgaris* can boost the immune system and cardiovascular system of humans (Moore, 2001). The boost that *C. vulgaris* provides to the immune system is said to be due to the branched chain of polysaccharides that are known to be antigenic whereas the cardiovascular benefits derive from antioxidants and omega unsaturated fatty acids. These are the same nutrients that are found in some fishes, which were acquired through the consumption of microalgae (Moore, 2001). In the medical field, the first record of an antibacterial product from algae was of a substance known as chlorellin, from *Chlorella* (Round, 1973). Microalgae are also applied in the removal of heavy metals contamination from water and soil. Due to this, dentists in Germany have started prescribing patients with *Chlorella* tablets to absorb the mercury released during the replacement of mercury amalgam fillings (Moore, 2001).

In culturing *C. vulgaris*, it is important to maintain growth conditions in the optimal range to reduce the duration of cultivation and to ensure the greatest yield. For a species acclimatised to a particular set of environmental conditions, the optimal growth could be achieved using a specific combination of salinity and irradiance. For large-scale outdoor operations, salinity and irradiance are two environmental factors that can be manipulated in inexpensive ways (Wong and Chang, 1999).

Salinity is considered as one of the major constraints on species diversity and productivity of natural population of microalgae because changes in salinity of water often affect their growth, metabolism and photosynthetic activity (el-Sheekh, 2004). Salinity causes physiological events in microalgae by changing the movements of water molecules and ions across the cell membrane. Salt might have direct affect upon processes involved in electron transport and photophosporylation (el-Sheekh, 2004). This might result in an increase or decrease in the quantum efficiency of photosynthesis in microalgae.

The main objective of this study is to culture *C. vulgaris* under different salinities at 5ppt, 15ppt and 30ppt, respectively. The culturing shall be conducted outdoor, in natural environment condition. Therefore, the culture system holds no control over the temperature and light intensity available to the culture. Previous studies on this microalgae have focussed on the use of organic carbon to enhance the growth and biochemical composition of *C. vulgaris*. Using the optimum salinity for the same purpose can be a cheaper and easier alternative than using organic carbon.

1.2 Objectives

The objectives of this study are:

1) To determine the growth rate of *Chlorella vulgaris* under different salinities.
2) To observe the difference in the chlorophyll $_a$, total protein and total lipid content of *Chlorella vulgaris* cultured in different salinities.
3) To determine the optimum salinity that promotes maximum growth and highest biochemical composition in *Chlorella vulgaris*.

1.3 Justification of study

The present study endeavours to improve the nutritional value and also the growth of *C. vulgaris* through an affordable and easier culturing method.

CHAPTER 2

LITERATURE REVIEW

2.1 General Characteristics of *Chlorella vulgaris*

Phytoplankton are free floating, microscopic plant that are unicellular, filamentous or chain forming species inhabiting surface waters (photic zone) of open oceanic and coastal environments (Kennish, 1994). They form the first link in the food chain and are used either directly as food for farmed animals such as molluscs, first larval stages of crustacean or fed to animals which themselves form the prey of farmed fish and crustaceans (Barnabé, 1990). Microalgae have also colonised a great variety of environments, from hot-water springs to artic condition, and some species have shown great resistance to arid, high salinity and low light (Feuga *et al.*, 2003).

The microalgae species that is proposed in this study is *C. vulgaris*. It is also referred to as the green algae, belonging to the class Chlorophyceae (Kennish, 1994). *C. vulgaris* is a unicellular non-motile alga (Photo 2.1). Their cells are bound by a true cellulosic wall. Each of the *Chlorella* cell has a bell-shaped or cup-shaped parietal chloroplast with or without a pyrenoid (Photo 2.2). There may be a hyaline cavity toward one side of the chloroplast and in this cavity, or in the colourless central cytoplasm, are located the single nucleus, the mitochondria and the Golgi bodies (Kumar and Singh, 1971).

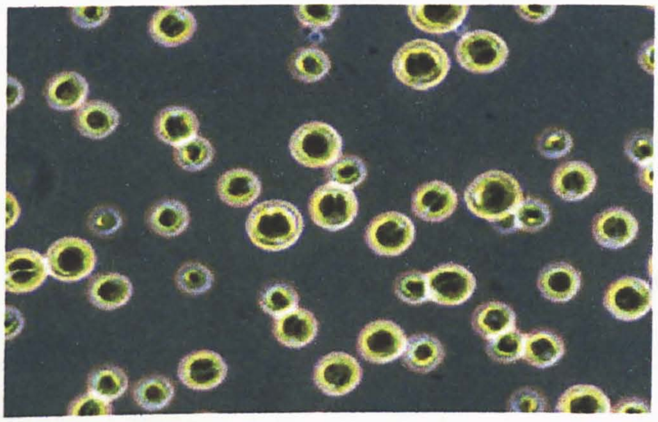

Photo 2.1 *Chlorella vulgaris* cells at a high density. Cells are round to ellipsoidal in shape and are non motile.

(Source : University of Stuttgart)

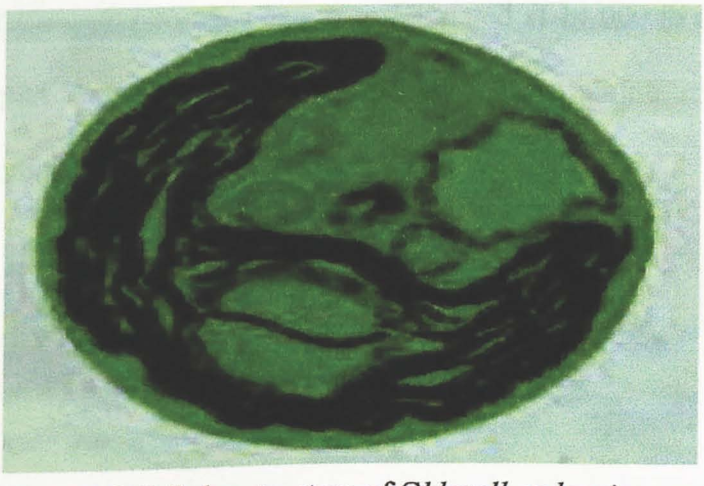

Photo 2.2 Cellular structure of *Chlorell .vulgaris*. The cell contains many organelles that function in the cell metabolism.

(Source : Univeristy of Oslo)

2.2 The Importance of *Chlorella vulgaris*

Probably the best-known use of *C. vulgaris* is as feed for aquaculture animals. *C. vulgaris* has been used as rotifer food since 1982. Because of its high growth rate and the ability to grow regardless of culture method, *C. vulgaris* could be an ideal candidate for mass culture. This highly nutritious microalgae is used to enrich rotifers that would later be fed to juvenile fishes. Total production of the juvenile fish, which require rotifers during their early larval stages has steadily increased since the 1980's and reached about 200 million fries per year as, reported by the Japan Sea-Farming Association (Maruyama, 1997).

Protein is the most limiting item in the average diet in many parts of the world. The possibilities of growing algae for food become even more impressive. In Japan, production of *Chlorella* cultures has been estimated at 14 000 lb of protein per acre per year. Apart from that, *Chlorella* contains all the amino acids recognized as essential in human nutrition (Maruyama, 1997).

2.3 The Growth and Life Cycle of *Chlorella vulgaris*

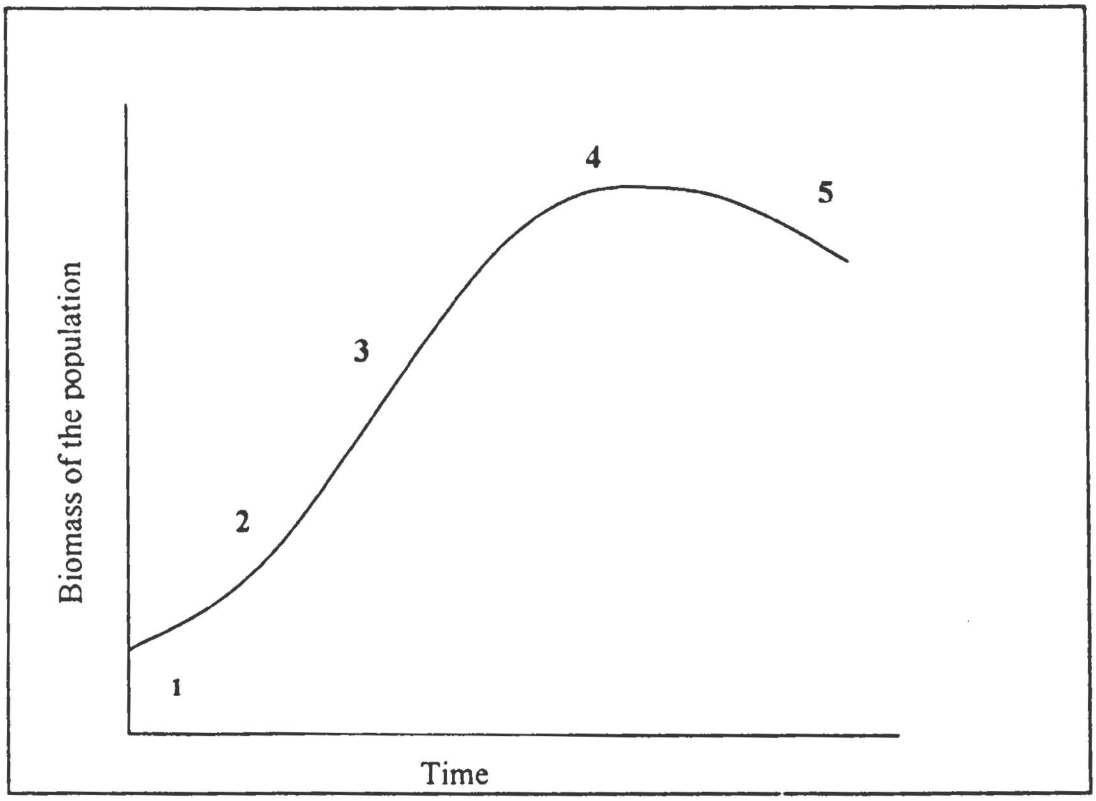

Figure 2.1 Growth and life cycle of *Chlorella vulgaris*, (Fogg, 1965)
1, 2, 3, 4 and **5** refers to the phases of the cells throughout their life cycle.

The growth of *C. vulgaris* is the result from both the increase in size and the division of the cells composing it (Feuga *et al.*, 2003). Therefore, it is important to understand the growth dynamics of *C. vulgaris* to achieve sustainable production. The growth of *C. vulgaris* can be described in five phases as shown in Figure 2.1.

The first phase (1), is referred to as the lag phase. Usually, the lag is "real" and can be attributed to several factors such as deactivation of enzymes in inoculum, increase in cell size, decrease in metabolite level in inoculum and introduction of inoculum into a medium containing high concentrations of some particular substance (Smith *et al.*, 1993). The second phase (2) is referred to as the exponential phase.

This phase is characterized by constant and rapid cell division. The third phase (3) is known as the phase of declining relative growth. The decline in growth at this phase can be caused by several factors that are depletion of a particular nutrient, change in pH due to preferential absorption, light limitation due to shading and rate of supply of carbon dioxide and oxygen (Fogg, 1965). The fourth phase (4) of the stationary phase is when the declining growth occurs rapidly, with a balance formed between growth rate and also the limiting factor. The final phase (5) is the death phase caused by depletion of nutrients (Smith *et al.*, 1993)

2.4 Factors Affecting the Growth of *Chlorella vulgaris*

There are many kind of environmental factors that could affect the growth and production of *C. vulgaris*. These factors can be categorized into physical environment and chemical environment. The main aspects of physical and chemical environments are discussed below and the detailed aspects needed for the culturing of *C. vulgaris* are discussed in section 2.7.

2.4.1 Physical Parameter

a. Light

Light energy has been deemed to be a limiting factor controlling the distribution of phytoplankton. Phytoplanktons utilize chlorophyll and also its accessory pigments to absorb all wavelengths of light within the range of photosynthetically active radiation,

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