DEVELOPMENT OF A PROTOCOL FOR THE IMMOBILIZATION OF PYRODINIUM BAHAMENSE VAR. COMPRESSUM FOR USE AS A BIOSENSOR FOR HARMFUL ALGAL BLOOMS

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THIS DISERTATION IS BEEN PRESENTED TO FULLFILL PART OF THE REQUIREMENT TO OBTAIN BACHELOR OF SCIENCE WITH HONOURS

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IJAZAH: Sarjana muda dgn kepujian

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I affirm that this paper is my own work effort, except for the references and summaries, which have been, cited clearly its source.

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ABSTRACT

Harmful Algal Bloom (HAB) algae species that is found in Sabah, *Pyrodinium bahamense var. compressum* was immobilized and cultured in 4 types of different culture media in order to determine the growth rate after it had been immobilized. *Pyrodinium bahamense var. compressum* cells were immobilized through gel-entrapment technique by using sodium alginate (3% w/v). Beads with the diameter from 4 ± 0.33 mm until 5 ± 0.33 mm is formed with 10 ml of algae-alginate mixture were able to produce 100 beads. These beads were cultured in 4 types of media that were f/2, f/50, L1 10 dan L1 50 at the temperature of 25 ± 3 ^{0}C and 14:10 light:dark cycle with light intensity of 3000 lux for 21 days. Difference between growth rate of free algal cells culture and immobilized algal cells culture was observed through cell density per day value and OD reading at 680 nm wavelenght per day value with the growth rate of immobilized algae cells was slower compared to the growth rate of free algal cells. Overall, cell density value is proportional to OD reading at 680 nm wavelenght value for all culture media for immobilized algal cells culture with the growth rate of immobilize algal cells were optimum when cultured in f/2 media.

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LIST OF ABBREVIATION

HAB	Harmful Algal Bloom
PSP	Paralytic Shellfish Poisoning
ASP	Amnesic Shellfish Poisoning
NSP	Neurotoxic Shellfish Poisoning
DSP	Diarrhetic Shellfish Poisoning
STX	saxitoxin
Na^+	sodium ion
Ca^+	calcium ion
MU	mouse unit
$MUmg^{-1}$	mouse units per milligram
HPLC	High Performance Liquid Chromatography
PSII	Photosystem II
APA	Alkaline phosphatase activity
Sub-ppb	sub parts per billion
BSA	Bovine serum albumin
rpm	rotation per minute
ml	millilitre
$mgml^{-1}$	milligram per millilitre
gml^{-1}	gram per millilitre
gL^{-1}	gram per litre
N	Normality

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$FeCl_3.6H_2O$	ferum (II) chloride hexahydrate
$Na_2EDTA.2H_2O$	sodium edetate
$NiSO_4.6H_2O$	Nickel (II) sulfate hexahydrate
Na_3VO_4	sodium orthovanadate
K_2CrO_4	potassium chromate

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CHAPTER 1

INTRODUCTION

1.1) Introduction

Harmful Algal Bloom (HAB), commonly known as "Red tide" is a phenomenon where certain phytoplankton species contain reddish pigments, and "bloom" such that the water appears to be coloured red. This term red tide is no longer used because it is a misnomer. The blooms are not always red but can be yellow, orange, brown, pink or reddish brown. Besides that, blooms also are not dependent on the tides. Therefore, the term HAB is used to describe a bloom phenomenon that contains toxins or a bloom that cause negative impacts to the environment (Anderson, 2000) In Malaysia, harmful algal blooms were first reported in 1976 in the west coast of Sabah. It is caused by a toxic dinoflagellates known as *Pyrodinium bahamense var. compressum* which produce toxin known as saxitoxin which are well known for causing paralytic shellfish poisoning (PSP) (Lim, 2002). PSP is a group of heterocyclic guanidines. In marine context, it is known as product of dinoflagellates red tides that accumulate in shellfish to cause PSP when contaminated shellfish are consumed. Saxitoxin cause PSP by inhibiting nerve conduction

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by blocking neuronal sodium channel thus causing oral facial numbness decreased arterial blood pressure, lost of voluntary muscle coordination and inhibition of respiration. By mass, saxitoxin is 1000 times more potent than cyanide and 50 times stronger than curare (Katırcıoğlu *et al*., 2004)

Harmful algal bloom occurs throughout the world, and Scandinavian and Japanese fisheries are occasionally drastically affected, but in Malaysian the damage to industry is not major. However, it had give an unhealthy impact to fishing sectors in affected areas such as Sabah especially to the fisherman and the fish mongers as the public demands towards seafood are affected every time the bloom outbreak occur (Utusan Malaysia, 4 April 2000).

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1.2 Research Scope

This project scope is to immobilize the toxic-producing algae based on the immobilization technique that is available for immobilizing cells.

1.3 Objective

The research objective is to immobilize *Pyrodinium bahamense var. compressum* growth based on the immobilization technique that been used for immobilizing cells such as cell entrapment. From this project, it is hope that we will be able to:

- To immobilize the toxic-producing algae in gel
- To compare the growth condition of the immobilized algae grown in two types of culture media, f/2 media and L1 media in various concentration.
- To determine the algae viability after immobilization.

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CHAPTER 2

LITERATURE REVIEW

2.1 Dinoflagellates

The dinoflagellates were firstly defined by Otto Bűtschli in 1885. At that time it was defined as the flagellate order Dinoflagellida. Botanists classified them under the division of Pyrrophyta and class of Dinophyceae. They are an important group of phytoplankton in marine and fresh water because of their roles in the aquatic food chain. Their adaptation to wide variety of environment is reflected by a tremendous diversity in form and nutrition and an extensive fossil record dating back few hundreds million years (*Dinoflagellate*, Wikipedia).

Some dinoflagellates produce toxins that are dangerous to man, marine mammals, fish, seabirds, and other components of the marine food light. Besides that, the dinoflagellates are large group of flagellate protests. Most of them are marine plankton but they are common in fresh habitats as well. About half of them are photosynthetic, thus making them the largest group of algae aside from diatoms (Hacket *et al.,* 2004).

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Most dinoflagellates are unicellular forms with two dissimilar flagella. One of these extends towards the posterior, known as the longitudinal flagellum, while the other forms a lateral circle, called the transverse flagellum. The transverse flagellum provides most of the force propelling the cell and other imparts to it a distinctive whirling motion, which is what gives the dinoflagellates refers to. In Greek, *dinos* means whirling (*Dinoflagellate*, Wikipedia).

The dinoflagellates are photosynthetic organisms that are known to the capability of bioluminescence behaviour. It is widely accepted that the dinoflagellates account for much of the planktonic bioluminescence in the ocean (Kelly *et al.*, 1978). The physiology and the biochemistry of dinoflagellate are relatively well understood. The bioluminescence system of dinoflagellate consists of enzyme luciferase, its substrate luciferin, and a protein that binds luciferin. Nearly all luminescent organisms in the ocean emit light with a peak wavelength near 490 nm, and dinoflagellates are no exception (Hacket *et al.*, 2004).

Most dinoflagellates are haploid and reproduce primarily through fission, but sexual reproduction also occurs. This takes place by fusion of two individuals to form a zygote, which may remain mobile in typical dinoflagellate fashion or may form a resting cyst, which later undergoes meiosis to produce new haploid cells (*Dinoflagellate*, Wikipedia).

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Dinoflagellates sometimes bloom in concentration of more than a million per cells per millilitre. Some species produce toxins that in such quantities will kill fish and accumulate in filter feeders such as shellfish, which in turn may pass them on people who consumed the affected fish or shellfish. This phenomenon is called a red tide or harmful algal bloom that will be discussed further.

2.2 *Pyrodinium bahamense var. compressum*

Pyrodinium bahamense var. compressum is one of two variety of *Pyrodinium bahamense*. Another variety of *Pyrodinium bahamense* is *Pyrodinium bahamense var. bahamense*. *Pyrodinium bahamense* in both varieties are bioluminescent. Except for the appearance, *Pyrodinium bahamense var. compressum* is longitudinally compressed and forms long chains of cells while *Pyrodinium bahamense var. bahamense* is round and found individually or in pairs (Refer Figure 2.1 and Figure 2.2)

Pyrodinium bahamense var. compressum can be found in the Indo-Pacific Ocean. In Malaysia, *Pyrodinium bahamense var. compressum* is found only the South China Sea in the coastal area of West Sabah, where it cause harmful algal bloom almost every year since it first outbreak reported in 1976 (Anton. *et al.*, 2000).

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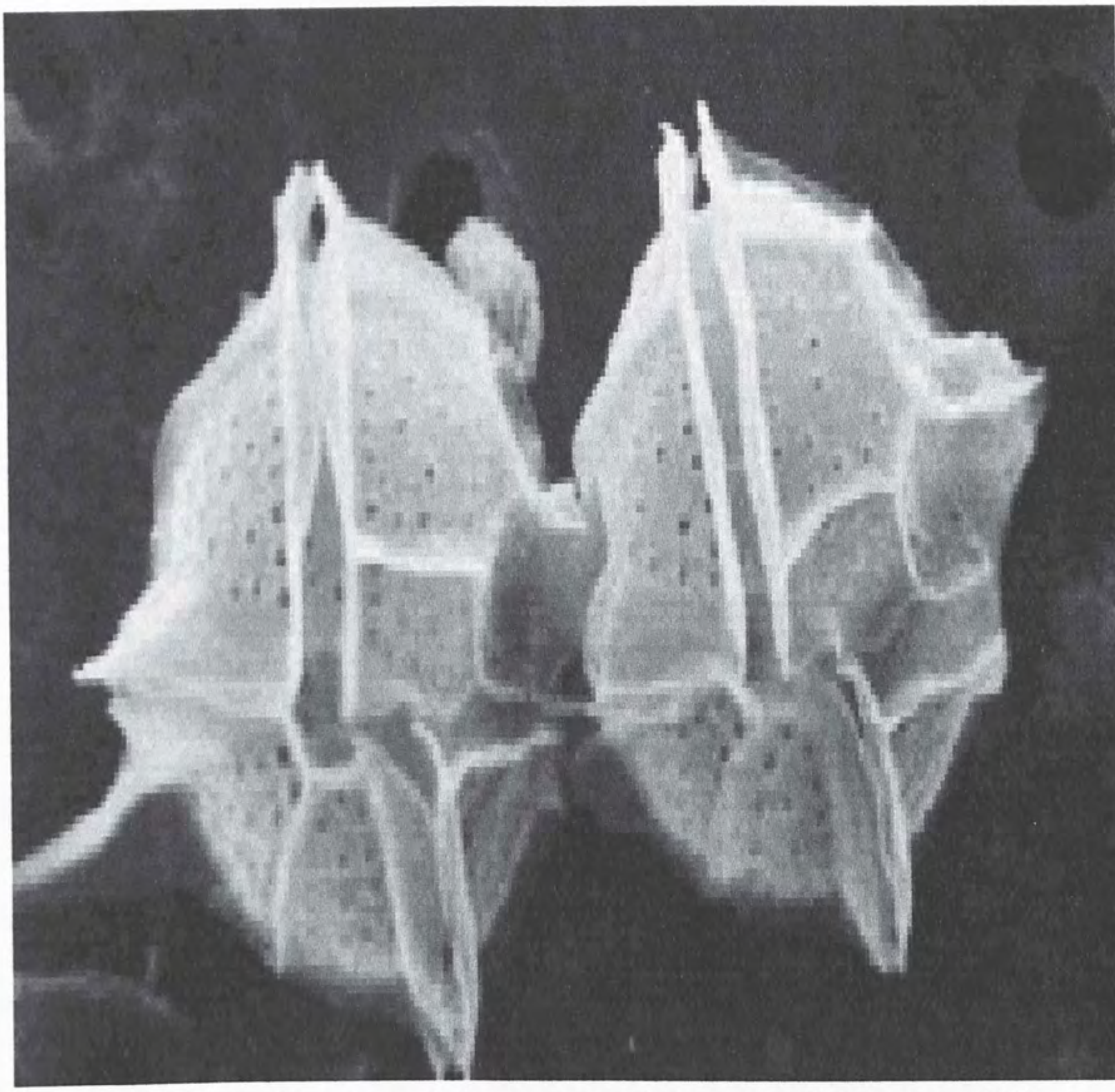

Figure 2.1 *Pyrodinium bahamense var. bahamense* (Phlips *et al.,* 2004)

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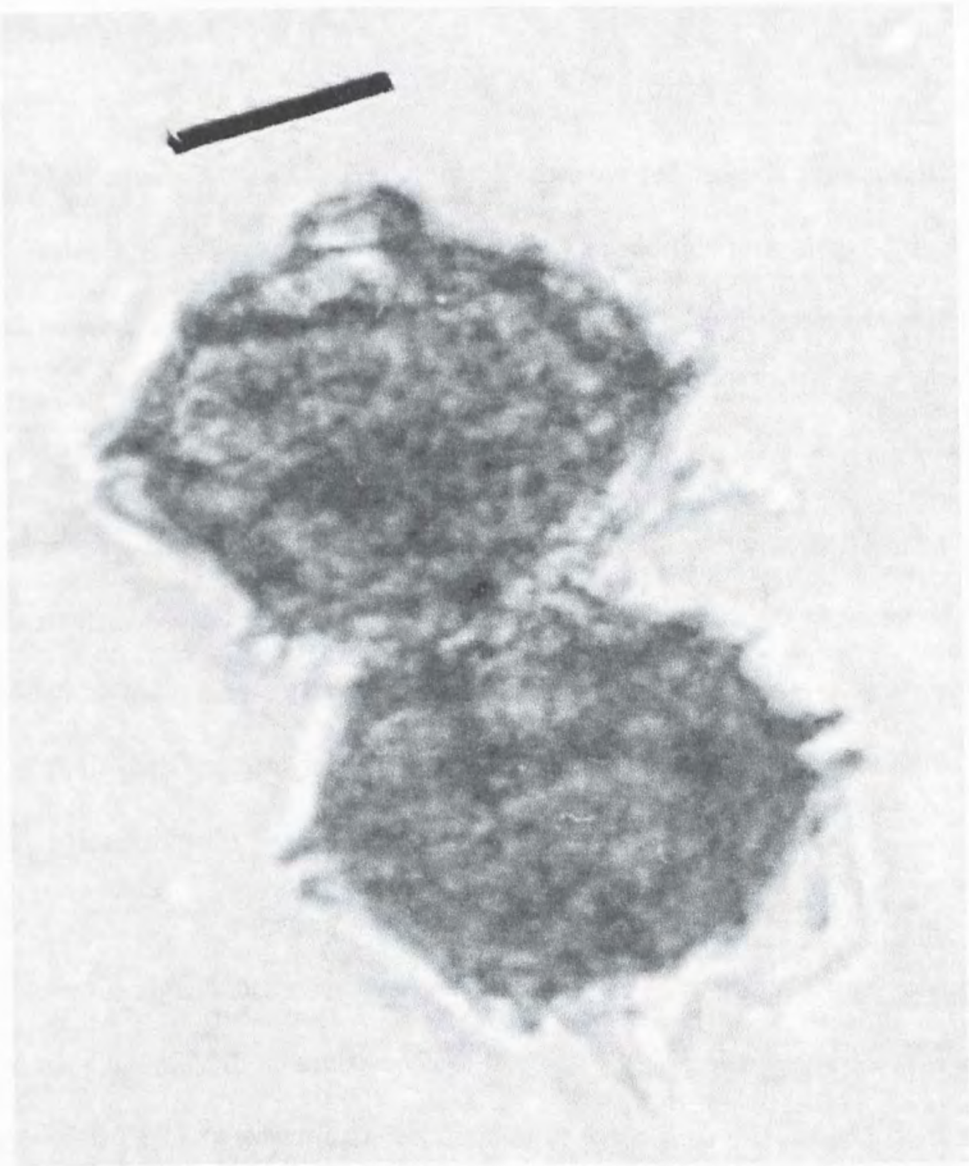

Figure 2.2 *Pyrodinium bahamense var. compressum* (UMS, 2005)

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2.3 Harmful algal bloom (HAB)

Harmful algal bloom (HAB) is commonly known as red tide. It is associated with a massive multiplication or "bloom" of algae cells. The red tide term derived from species by which phytoplankton reproduce a high concentration of cell that made water visibly turns a red or dark brown colour (*Algal Bloom*, Wikipedia).

However, this term is not so correct to define the phenomena as red coloured water can also be caused by many non-toxic algae species. Besides that, most occurrences of toxic marine algae are not accompanied by any visible change in the colour of the water and also the occurrence of red tides has almost nothing to do with ocean tides (Baeir, 2000).

Harmful algal bloom are characterized by one important feature, which is they cause harm. Generally there are two ways that algal bloom can cause harm. Firstly, the algal bloom cause harm through the production of toxin such as paralytic symptom toxin (PST) or secondly, in non-toxic means that is physical bumping into organisms that cause damage, depletion of oxygen, and etc. Most algal blooms cycle are "investigate" by some limiting nutrient that is the growth of an algal bloom will be halted by the consumption of the available supply of the limiting nutrient. In freshwaters, this limiting nutrient tends to be phosphorous meanwhile in salt-water environment; the limiting nutrients that prevent the formation of an algal bloom are usually nitrogen and iron (Anderson *et al.*, 2002). It can rapidly increase to very high concentrations. Some blooms of this phytoplankton can

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