THE EFFECT OF NITRATE AND PHOSPHATE ON THE GROWTH OF HARMFUL ALGAE *Cochlodinium polykrikoides*

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ABSTRACT

The harmful algae species *Cochlodinium polykrikoides* was studied to investigate the effects of nitrate and phosphate concentrations on the growth and chlorophyll *a* production. The experiments were conducted at constant temperature 25°C, 100μmol$m^{-2}s^{-1}$ and under 12:12 light: dark photo cycle and the culture media used was the f/2 media with nitrate concentrations ranged from $2.21X10^{-4}$M to $1.09X10^{-3}$M and phosphate concentrations ranged from $9.06X10^{-6}$M to $5.07X10^{-5}$M. In the nitrate experiment, the cells density increase significantly ($P<0.05$) as the nitrate concentration increased. *C. polykrikoides* exhibited the highest maximum cell density (512$\pm$97cells/mL) at nitrate concentration 9.41X10-^{4}M with chlorophyll *a* 6.8708 mg/m^3. The growth of the *Cochlodinium polykrikoides* are inhibited at the highest nitrate concentrations ($1.09X10^{-3}$M) and the lowest nitrate concentrations ($2.21X10^{-4}$M). In the phosphate experiments, the growth of *C. polykrikoides* are also significantly affected ($P<0.05$) where the highest cell density (742$\pm$52cells/mL) was recorded at the lowest phosphate concentrations $9.06X10^{-6}$M with the highest concentration of chlorophyll *a* at 72.967 mg/m^3. The cell density decrease as the phosphate concentrations increases and their growth was inhibited at concentrations of $4.35X10^{-5}$M and $5.07X10^{-5}$M. The concentrations of chlorophyll *a* shows a positive relationship with the cell density.

ABSTRAK

Spesis alga bahaya *Cochlodinium polykrikoides* dikaji untuk menentukan kesan kepekatan nitrat dan fosfat terhadap pertumbuhannya dan jumlah kepekatan klorofil *a*. Eksperimen ini dijalankan pada suhu, cahaya dan fotokala yang tetap (25°C, 100µM dan 12: 12: terang: gelap) dan dikultur dengan mengunakan media f/2 dengan 5 kepekatan nitrat ($2.21X10^{-4}$M – $1.09X10^{-3}$M) dan 5 kepekatan fosfat ($9.06X10^{-6}$M – $5.07X10^{-5}$M). Eksperimen nitrat menunjukkan bahawa terdapat kesan signifikan ($P<0.05$) ke atas pertumbuhan sel dimana bilangan sel meningkat apabila kepekatan nitrat meningkat. *C. polykrikoides* mencapai bilangan sel tertinggi pada kepekatan nitrat $9.41X10^{-4}$M dengan kepekatan klorofil *a* 6.8708mg/m^3. Pertumbuhan *Cochlodinium polykrikoides* terencat pada kepekatan nitrat paling tinggi dan pada kepekatan nitrat paling rendah. Eksperimen fosfat juga menunjukkan kesan signifikan ($P<0.05$) dimana *C. polykrikoides* mencapai bilangan sel pertumbuhan tertinggi pada kepekatan fosfat paling rendah $9.06X10^{-6}$M dengan kepekatan klorofil direkodkan pada 74.9672 mg/m^3. Bilangan sel menurun dengan peningkatan kepekatan fosfat dan pertumbuhan adalah terencat pada kepekatan fosfat $4.35X10^{-5}$M dan $5.07X10^{-5}$M. Penghasilan klorofil *a* juga menunjukkan hubungan positif dengan dengan bilangan sel.

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LIST OF SYMBOLS

^oC	Degree Celsius
$\mu molm^{-2}s^{-1}$	Micro mol meter per second
H	Hour
mL	Milliliter
M	Molarity
DI	Distilled water
Mg	Milligram
Ppt	Part per thousand
G	Gram
µM	Micro mol
%	Percent
nm	nanometer
E	Absorbance
µg/l	Microgram per liter
Mg/m^3	Milligram per cubic

CHAPTER 1

INTRODUCTION

1.1 Harmful Algal Blooms (HABs)

All over the world, the coastal countries are seriously impact by the phenomena we now term "harmful algal blooms" or HABs. According to Anderson *et al.*(1996a), these phenomena were called red tides in the previous years because of the intense discoloration of the water by the algal pigments. The common threat is that they can cause harm by producing potent neurotoxins. Some shellfish feed on the harmful algae and the toxin accumulate in their body which can cause poisoning in human consumers called paralytic, diarrhetic, amnesic and neurotoxin shellfish poisoning (PSP, DSP, ASP, and NSP respectively) (Anderson *et al.*, 1996a).

The alteration of the marine ecosystem and function is the diverse effects of HAB toxins and also affecting fecundity and survival at all levels such as some toxic blooms kill wild and farmed fish populations, while some non-toxic algal species can cause problems through biomass effects – shading of submerged vegetation, disruption of food web dynamics and structure, and oxygen depletion as the blooms decay (Anderson *et al.*, 1996a).

Blooms of *Cochlodinium polykrikoides* have caused mass mortality of aquacultured fish of the western coast of the island of Kyushu, Japan, and the southern coast of Korea. The *Cochlodinium polykrikoides* are also bloom in the Harima Nada of the Seto Inland Sea in the summer of 1986 (Yuki and Yoshimatsu, 1989) and in Uragami Bay, Wakayama Prefecture, in 1996. Along the coast of the island of Kyushu, red tides recently occurred in the Ariake Sea off Kumamoto Prefecture, Imari bay off Nagasaki Prefecture and Inokushi Bay off Oita Prefecture. In the summers of 2000, a massive bloom of this organism in the Yatsushiro Sea caused damage costing ~US$36.4 millions (RM 123.76 millions) (Kim *et al.*, 2004).

In Korea, the first red tide was recorded in the southern Korea in 1982 and the blooms have occurred frequently since 1989 (Kim, 1998). An extensive red tide in summer of 1995 caused particularly heavy mortalities of aquacultured fish, which amounted to a loss of ~US$95.5 million (RM 324.7 millions) (Kim, 1997). Blooms of *C. polykrikoides* have also occurred in Quanshou Bay in China (Du *et al.*, 1993) and in Manzanillo Bay (Morales-Blake and Hernandez-Becerrill, 2001) and the Gulf of California in Mexico (Gárate-Lizárraga *et al.*, 2004).

During late 2003, dinoflagellates blooms, caused by the *Cochlodinium polykrikoides*, were noticed in Sabah (Northern Borneo) (NOWPAP CEARAC, 2006). In Japan and Korea the *Cochlodinium polykrikoides* has cause severe damage to fisheries during summer and fall. Studies in eastern Asia found that the optimum conditions for

Cochlodinium bloom which is at salinity 32-34 psu, temperature 25-28°C, and good sunlight (NOWPAP CEARAC, 2006), which are very similar to the oceanographic condition in Borneo.

1.2 The General effect of HABs.

According to Volterra and Kerr (1992), the fertilization increase primary production which can bring benefits for fishery yield but uncontrolled eutrophication of formerly productive systems can lead to undesirable consequences such as algae blooms, macroalgae overgrowth, hypoxia, anoxia, fish kills, transformation of sediments, disappearance of macrophytes, etc.

Such effects, in turn, have a consequence for the economies of those regions for which the sea represents a valuable resource. The economic losses from reduced tourism and fisheries potential have been particularly relevant. Tourism requires a seashore experience that is both pleasing and safe for bathing and related water sports. Reduction in water transparency, colored waters, mucilage production by algae in eutrophication area crample these values. Moreover, the human health consequences of such associated factors as pathogens present in algal mucilage and marine aerosols produced during toxic blooms; cutaneous irritations caused by some algae (Volterra and Kerr, 1992). Cultural eutrophication can also have a serious economic consequence for fisheries, as has been shown in many parts of the world. Fish eggs and larvae, being unable to avoided anoxic or toxic zones, are particularly vulnerable although their loss is not an immediately occurred. Loss of fish spawning grounds due to algae overgrowth or smothering due to sedimentation, is a further fisheries cost of eutrophication.

Steidinger *et. al* (1966) reported that the longest series of studies of harmful algal blooms regarding to the outbreaks of red tide have been recorded along the west coast of Florida since 1940s and this phenomenon was caused by the high concentrations of the unarmoured dinoflagellates *Gymnodinium breve*.

This species has been associated with discoloration of the water, fish kills, shellfish poisoning and the human-related ailments associated with aerosols (Steidinger *et al*., 1966). National Research Council (1999) reported that there are many problems associated with the ingestion of seafood contaminated by toxic algal blooms which affect fishes, seabirds, porpoises, whales and humans. The red tide incidences have been reported from Tolo Harbor, Hong Kong (Lam and Ho, 1989) and Inland Sea in Japan (Okaichi, 1997).

The mass fish kills in the Seta Inland Sea has been associated with the increase incidences of *Chatonella* blooms (Okaichi, 1997). Burkholder and Glasgow (1997) also reported that the dinoflagellates *Pfiesteria* has been associated with the mass fish kills along the eastern coast of the United States.

1.3 Significance of the Study

The study about the harmful algae species *Cochlodinium polykrikoides* is to understand the effect of certain parameter such as nitrate concentration and phosphate concentration on growth of this *Cochlodinium polykrikoides*. When there is an optimum concentration of nutrient such as nitrate and phosphate, it can trigger this *Cochlodinium polykrikoides* to blooms and thus can give an adversely effects to the environment, human health, economic loss and also the tourism industry.

From this study, we can determine the optimum concentration of nitrate and phosphate for the optimum growth of the *Cochlodinium polykrikoides*. Data obtained from this study, can be use to establish and to improve the culture study and the data from this study also can be used as a future references to anyone who want to further study about the *Cochlodinium polykrikoides*.

1.4 Objectives of the Study

a) To determine the effects of nitrate and phosphate concentration on the growth of *Cochlodinium polykrikoides*.

b) To determine the effects of nitrate and phosphate concentration on the production of the chlorophyll *a*.

c) To determine the optimum concentration of nitrate and phosphate for *Cochlodinium polykrikoides* growth and chlorophyll *a* production.

CHAPTER 2

LITERATURE REVIEW

2.1 The Definitions of nutrient and HAB (Red Tide)

2.1.1 Nutrient

Nutrient can be defined as the elements that the organism need as a food and can be divided into two group; macronutrient (element are that need in high amount) and micronutrient (element are that need in less amount) (Lawrence, 1995). However, these definitions are general. In this research, the nutrient definition that been proposed by Barreta-Bekker *et al.* (1992) and Kennish (1994) are more appropriate to use because they define that nutrient is in form of non dissolve organic substances (like nitrate and phosphate) or complex organic compound like vitamins.

2.1.2 HAB (red tide)

Some of the older literature used water discoloration as a definition of an algal bloom. Another definition included the concept of high numbers of cells and low species diversity (Fryxell and Villac, 1999). Anderson (1996b) includes the concept of blooms which is the concentrations of one or more species that cause accumulation of toxins in a way that causes harm to those who consume the toxic species. Many algal specialists consider a bloom to be defined as a population density in excess of 1×10^6 cells L^{-1}. This definition leads to other complications regarding which measurement should be use in the bloom definition; cell numbers, biomass, cell volume, or indices calculated on the basis of chlorophyll *a* concentration (Fryxell and Villac, 1999).

2.2 Phytoplankton Blooms and the HAB Concept

The estuarine and coastal phytoplankton depend on a fine balance of various factors, and these aspects of phytoplankton ecology need to be concerned and viewed within the twin aspects of spatial heterogeneity and seasonal/interannual variation. Of the (approximate) total numbers of marine phytoplankton species (5000), some 300 species are known to occur at numbers high enough to discolor seawater (Sournia *et al*., 1991).

About 40 or 50 of these species produce toxins that can affect natural marine populations of plants and animals as well as human beings (Hallegreaff *et al*., 1995). These are so-called harmful algal blooms or HABs have become the main focus of increase research over the past decade as part of the perception that these blooms are increasing in both frequency and severity of impact on world basis. Although the HAB concept may be refined (Smayda, 1997), there is a general agreement that an understanding of the causes of such bloom and bloom dynamics with respect to impact on secondary production has become the principal research interest in aquatic systems.

HABs take three general forms (Hallegreafff *et al*., 1995):

1) Nontoxic blooming population reaching concentrations that will affect important environmental factors such as dissolved oxygen with the resulting hypoxia/anoxia ending in debilitation and/or extirpation of other populations.

2) Toxic bloom species that introduce toxic agents into associated food webs to the extent that upper trophic levels (including humans) are adversely affected.

3) Toxic bloom species that produce and release substances having direct and/or indirect effects on associated population. These species are usually not harmful to humans but are known to affect other aquatic life plant and animals species adversely.

2.3 Environmental factors that contribute to the harmful algae bloom.

2.3.1 Nutrient

The nutrient that will be manipulated in this experiment is the nitrogen element; nitrate (NO_3-N) and phosphate (PO^-_4) because these nutrients are needed by the algae to form atoms for growing. Milero (1996) propose that the nitrogen and phosphorus are the important nutrient because they aid in phytoplankton growth. Nitrogen is essential for its role as a key component of all amino acids and hence protein, the nitrogenous bases (purines, pyrimidines) of nucleotides (and some toxins), the porphyrin ring of chlorophyll, and cytochromes. Some of the phospholipids also contain nitrogen.

Nitrate is the nitrogen inorganic compound that has the most high in oxidation state (+5). The symbol that common use is NO^-3. Nitrate is the final product in the decomposition of aerobic nitrogen organic compound (Baretta-Bekker *et al*., 1992). Nitrate can be converted to nitrite through nitrification process or oxidize into free nitrogen, N_2 through denitrification by the microorganism (Kaplan, 1983; Carpenter and Capone, 1983; O'Neili, 1995). Nitrate is the nutrient-limited and always easy to deplete in the surface of the seawater in the most of the oceans world (Carpenter and Capone, 1983). In the ocean, the oxygen influences the nitrate distribution. In the research that has been conducted in the north and south Atlantic, the maximum level of nitrate is in the layer that have low dissolve oxygen (Redfield *et al*., 1963; Broecker, 1974).

Dinoflagellates are capable to store nitrate internally, even though they lack a large cell vacuole like diatoms, but tropical, oceanic species of *Pyrocyctis* was an exception (Bhovichitra and Swift, 1977). Internal concentrations of inorganic nitrogen in *A. carterae* and *Protogonyaulax tamarense* grown on sufficient nitrate (1.8 mM NO^-_3, 8.2 mM NH^+_4 and 49 mM NO^-_3, 3.7 mM NH^+_4 respectively) and ammonium (NO^-_3 0 mM, 92 mM in *A. carterae*) support the view that they can store inorganic nitrogen (Dortsch *et al*., 1984).

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