UNIVERSITI PUTRA MALAYSIA

INFLUENCE OF HUMIC ACID ON WATER QUALITY AND GROWTH OF ALGAE IN FRESHWATER ECOSYSTEM

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By

HAMID KHODA BAKHSH

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science in the Faculty of Agriculture Universiti Putra Malaysia

October 2001

DEDICATION

To my parents

Yahya Khoda Bakhsh

Mansoureh Kiamary

For their true love, favor and effort, now there is only memory of my father, he passed away during my study in Malaysia

To my wife

Sanaz

For her attention and cooperation during my study

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

INFLUENCE OF HUMIC ACID ON WATER QUALITY AND GROWTH OF ALGAE IN FRESHWATER ECOSYSTEM

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October 2001

Chairman : Dr. Anuar Abdul Rahim

Faculty : Agriculture

One of the approaches to improve aquaculture production is through improvement of fertilization program for phytoplankton production and water quality variables. There are serious problems with the use of raw organic fertilizer in ponds and environment. Oxygen depletion, degradation of water quality, reduced light penetration, and spread of diseases frequently occur after large doses of manure are added to a pond at irregular intervals. This study was conducted to investigate the influence of humic acid (HA) on primary productivity (phytoplankton production) and water quality variables in a freshwater aquatic ecosystem.

Two experiments were carried out to evaluate effects of humic acid alone or in combination with inorganic (urea and single super phosphate) or organic fertilizer (cow manure) on the phytoplankton productivity. Prior to the experiments, HA contents of cow manure (CM), chicken manure, compost and tropical peat were estimated to determine the suitable source (quantity) for HA extraction. The results showed that tropical peat contained 46.5% (dry weight basis) HA and 11.6-17.2 folds

higher than the other organic fertilizers. Humic acid from the tropical peat was used in the subsequent experiments.

An experiment was conducted at the Gharehsoo Station of Fisheries Research Centre of Mazandaran (FRCM) in North of Islamic Republic Iran (July and August 1999) to study the effects of HA and inorganic fertilizer (urea and single super phosphate) on growth of selected freshwater phytoplankton and water quality variables. The experiment was carried out in eighteen 1.5 Liter transparent plastic bottles (control, 25 ppm HA+UP, 50 ppm HA+UP, 100 ppm HA, 100 ppm HA+UP, 150 ppm HA+UP). Three species of green algae (*Chlorella vulgaria, Scenedesmus quadricauda,* and *Oocystis solitaria*) and a species of blue-green alga (*Oscillatoria agardii*) were selected for the study.

The results showed that highest population (bloom) was achieved in week 3 by 100 ppm HA. All treatments with a combination of HA and urea-phosphate led to a blue-green algal (*Oscillatoria agardii*) dominancy with a low phytoplankton bloom and low total nitrogen (TN) and total phosphorus (TP) ratio (0.7-1.03). The green algal dominance was associated with a high TN:TP ratio (16-17) in culture media. pH of HA treatment was within the suitable range (7.84-8.51) for phytoplankton production. Light penetration correlated well with blue-green algal population.

Another experiment was also carried out at the Aquatic Resource Technology Centre, Institute of Bioscience, Universiti Putra Malaysia (Oct. and Nov. 1999) to determine the effects of HA, CM and their combination on the growth of freshwater algae and water quality variables (unfertilized, 100 ppm HA, 1gram per liter CM, 70% HA + 30% CM). The experiment was conducted in twelve glass aquaria (60 cm x 30 cm x 30 cm). The results showed that the total phytoplankton population in fertilized aquaria was higher than that of unfertilized aquaria. The highest bloom of phytoplankton was observed when a combination of HA and CM was used. Liquid HA (100 ppm) encouraged growth of edible green algae and the highest population of Chlorophaceae and Bacillariophyceae were observed in the mixture of HA and CM. Cow manure treatment encouraged the growth of blue-green algae (Cyanophyceae). Chlorophaceae population showed positive correlation with NO_3-N, NH_4-N:PO_4-P ratio. The population of periphyton increased with time and the highest population increase was achieved with CM followed by the combination of HA and CM, humic acid alone and the control (unfertilized), respectively.

Both experiments indicated that HA alone is suitable for freshwater algal production and with better water quality variables. Besides, humic acid has the capability of reducing the negative effect of high dosage of nitrogen (N) and phosphorus (P) in treatments. Humic acid act as supplier and storehouse for N and P for algae and phytoplankton in aquatic ecosystem. However an inclusion of an organic fertilizer (CM) seemed to speed up and improved the efficiency of HA on phytoplankton population (8410 cells mL^{-1}). The addition of liquid HA did not have undesirable effect on water quality variables (pH, dissolved oxygen, and light penetration).

Abstrak tesis dipersembahkan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN ASID HUMIK TERHADAP MUTU AIR DAN TUMBESARAN ALGA DI DALAM EKOSISTEM AIR TAWAR

Oleh

HAMID KHODA BAKHSH

Oktober 2001

Pengerusi: Dr. Anuar Abdul Rahim

Fakulti: Pertanian

Satu pendekatan untuk meningkatkan pengeluaran akuakultur adalah melalui perbaikan program pembajaan dan mutu air. Terdapat beberapa masalah serius akibat penambahan baja organik ke kolam dan persekitaran. Pengurangan oksigen, mutu air, pengurangan kemasukan cahaya, dan perkembangan penyakit sering berlaku setelah banyak kuantiti baja organik ditambah ke dalam kolam. Kajian ini telah dijalankan untuk menyelidik kesan asid humik (HA) terhadap penghasilan fitoplankton dan mutu air pada ekosistem akuakultur air tawar.

Dua eksperimen telah dijalankan untuk menilai kesan asid humik dan kombinasinya dengan baja organik atau bukan organik terhadap produktiviti fitoplankton. Sebelum kajian pembajaan dijalankan, kandungan asid humik di dalam tahi lembu, tahi ayam, kompos dan gambut tropika dinilai untuk menentukan sumber terbaik untuk pengekstratan asid humik. Keputusan kajian menunjukkan bahawa tanah gambut tropika mengandungi 46.5% (berat kering) asid humik dan 11.6 – 17.2 ganda lebih tinggi daripada sumber organan lain. Asid humik dari gambut tropika digunakan untuk eksperimen-eksperimen berikutnya.

Satu eksperimen telah dijalankan di Pusat Penyelidikan Perikanan Stesen Gharehsoo di Mazandaran (FRCM) di utara Iran (Julai 1999) untuk mengkaji kesan

asid humik dan baja bukan organik (urea dan fosfat) terhadap pertumbuhan fitoplankton air tawar dan mutu air. Eksperimen tersebut dijalankan mengguna lapan belas botol plastik lutsinar berukuran 1.5 liter (control, 25 bsj HA+UP, 50 bsj HA+UP, 100 bsj HA, 100 bsj HA+UP, 150 bsj HA+UP). Spesies-spesies alga hijau (*Chlorella vulgaria, Scenedesmus quadricauda, Oocystis solitaria*) dan satu spesies alga biru-hijau (*Oscillatoria agardii*) telah dipilih untuk dikaji. Keputusan menunjukkan bahawa puncak perkembangan populasi tertinggi dicapai pada minggu ke 3 dengan rawatan 100 bsj asid humik. Semua rawatan kombinasi asid humik dan urea-fosfat membawa kepada kedominan alga biru-hijau (*Oscillatoria agardii*) dengan perkembangan fitoplankton yang rendah. Kedominan alga biru-hijau berkait rapat dengan jumlah N: jumlah P (TN:TP, 0.7-1.03) yang rendah dalam media. Kedominan alga hijau berhubung kait dengan TN:TP (16-17) yang tinggi pada media kultur. pH pada rawatan asid humik adalah pada paras berkesesuaian (7.84-8.51) untuk penghasilan phytoplankton. Penebusan cahaya berkait rapat dengan populasi alga biru hijau.

Eksperimen juga telah dijalankan di Pusat Sumber Teknologi Akuatik, Institut Biosains, Universiti Putra Malaysia (Oktober and November 1999) untuk menentukan kesan asid humik, tahi lembu dan kombinasinya terhadap tumbesaran alga air tawar dan mutu air (kawalan, asid humik, tahi lembu (CM), HA + CM). Eksperimen tersebut dijalankan mengguna dua belas akuarium kaca (60cm x 30cm x 30cm). Keputusan menunjukkan bahawa jumlah populasi fitoplankton di dalam akuarium yang dibaja adalah lebih tinggi berbanding dengan tanpa pembajaan. Perkembangan fitoplankton yang terpantas didapati apabila kombinasi asid humik dan tahi lembu digunakan. Asid humik cecair (100 bsj) membantu pertumbuhan alga hijau dan populasi tertinggi bagi Choloraphaceae dan Bacillariophyceae didapati pada campuran asid humik dan tahi lembu. Baja tahi lembu menggalakkan pertumbuhan alga biru-hijau. Chlorophaceae berkait secara terus dengan nisbah NO_3-N, NH_4-N:PO_4. Populasi perifiton meningkat dengan peningkatan masa, dan peningkatan populasi tertinggi dicapai dengan tahi lembu diikuti, kombinasi asid humik dan tahi lembu, asid humik sahaja dan kawalan (tanpa baja).

Kedua-dua eksperimen menunjukkan asid humik sesuai untuk penghasilan alga air tawar dan mutu air. Asid humik juga berupaya mengurangkan kesan negatif kadar N dan P yang tinggi. Asid humik bertindak sebagai membekal dan penyimpan baja lepas perlahan dengan nitrogen dan fosforus untuk alga dan microorganisma pada sistem akuatik (8410 cells mL^{-1}). Penambahan asid humik cair tidak memberi kesan negatif terhadap mutu air (pH, oksigen terlarut, dan kemasukan cahaya).

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I certify that an Examination Committee met on 19th October 2001 to conduct the final examination of Hamid Khoda Bakhsh on him Master of Science thesis entitled "Influence of Humic Acid on Water Quality and Growth of Algae in Freshwater Ecosystem" 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 has been accepted as fulfilment for the requirement for the degree of Master of Sciences.

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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.

HAMID KHODA BAKHSH

Date: 30 NOV 2001

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA= Analysis of variance

DO= Dissolved oxygen

DOC= Dissolved organic carbon

DOM= Dissolved organic matter

Chl. *a*= Chlorophyll *a*

L.P= light penetration

FW= Freshwater

HA= Humic acid

CM= Cow manure

HA + CM= Humic acid and Cow manure

se= Standard Error

ppm= Part per million

ppt= Part per thousand

TN= Total nitrogen

TP= Total phosphorus

TN:TP= Total nitrogen and total phosphorus ratio

UP= Urea and single supper phosphate

DNMRT= Duncan New Multiple Range Test

CHAPTER I

INTRODUCTION

At present aquaculture is practiced in almost every country of the world. The extent of aquaculture depends on several factors such as availability of expertise, technology, water, land, government policy and others (Wheaton, 1977).

One approach to improve aquaculture production is through improvement of basic aquaculture practices such as the management of water quality, selection of fast growing and disease resistant species, reliable and adequate seed supply, formulation of better artificial feed, enhancement of the production of natural food, post-harvest handling and others (Landau, 1992). Although there are commercial intensive fish farms, the majority still practices semi-intensive farming which involved fertilization of ponds using cow manure, inorganic fertilizers (such as urea and phosphate) and little feeding.

It is very difficult to persuade fish farmers to change from semi-intensive farming to intensive farming due to their limited knowledge, labour and capital. One practical way to enhance production is through the improvement of existing semi-intensive farming. One of the problems commonly encountered in semi-intensive farming is the use of huge amount of cow manure, which lead to the degradation of water quality (oxygen depletion and reduced light penetration), spread of diseases (bacterial and parasitic), lower hygiene and production (Lin *et al.*, 1997).

Since there is no precise guideline on the application of cow manure or organic fertilizer into a pond (unaerated), there is a strong tendency to over-fertilize a pond that leads to excessive organic matter, high biological oxygen demand, low dissolved oxygen (particularly in the morning), reduced light penetration necessary for photosynthesis, and the formation of anaerobic conditions at the bottom of the pond (Lin *et al.*, 1997). For these reasons, some farmers exclusively use inorganic fertilizers as they are more convenient. In fish ponds, chemical fertilizers stimulate phytoplankton production which indirectly increases fish yield. They contain nitrogen (N), phosphorous (P) and potassium (K) that are needed by phytoplankton (Boyd, 1979). Even though the use of inorganic fertilizers is more convenient, the availability of resulted natural food is limited.

Commercial pond managers use a combination of organic and inorganic fertilizers. A combined application of both fertilizers stimulates pond productivity largely through autotrophic pathways and also through heterotrophic pathways (Green *et al.*, 1989). The quantity of zooplankton, benthos and detritus can be indirectly increased through such fertilization regime. With fertilization, the fish and prawn production does not have to start at the bottom of the food chain but can be started at a level closer to the fish and prawn with less energy loss (Schroeder, 1978; Geiger, 1983). Many research have shown the benefits of fertilizers on fish and prawn production (Ling, 1967; Wohlfarth and Schroeder, 1979; Teichert-Coddington *et al.*, 1990). Mortimer (1954) reported that the carp production in fertilized ponds was 2 to 10 times higher than those of unfertilized ponds, while tilapia production in fertilized ponds (5,135-9000 kg ha^{-1}) was 2 to 4 times higher than in unfertilized ponds (2,240 kg ha^{-1}).

Studies have indicated that the use of humic acid (organic acid) can improve the production of terrestrial plants (Sladky, 1959; Stevenson, 1982; Steinberg and Baltes, 1984; Levinsky, 1998). Some studies have also shown that humic acid can increase the productivity of phytoplankton (Prakash and Rashid, 1968; Prakash *et al.*, 1973; Lee and Bartlett, 1976; Graneli and Moreira, 1990; Vrana and Votruba, 1995; Faust, 1999) in environment and laboratory experiments. Most of these reports have involved marine phytoplankton growth. Prakash and Rashid (1968) indicated that the biologically active and ingredients of humic substances stimulate growth of marine phytoplankton. However, little work has been done on the influence of humic substances on the growth of freshwater algal species, a major purpose of this investigation. It is necessary to know whether humic acid as natural organic fertilizer can improve production of selected phytoplankton in the freshwater pond.

According to Schroeder (1978) and Lin *et al*. (1997) it is well recognized that pond fertilization with animal manure stimulates production of bacteria, phytoplankton, zooplankton, and benthos. The nutrient availability and efficiency of animal manures to phytoplankton production have long been treated as a black box in pond dynamics. There are also some negative aspects of using animal manures such as oxygen depletion, unsuitable for high-yield culture, cost of gathering and transportation (ICAAE, 1996a). Therefore, results of this study can be compared to effectiveness of humic acid and another organic fertilizer (cow manure) on phytoplankton population and water quality variables in an aquatic ecosystem.

As noted, no work has been reported on the influence of tropical peat humic acid on algal growth and also influence of humic acid on water quality variables. There is no information available regarding the toxic efficacy of this source of humic substances.

Objectives

The aim of this study was to investigate the potential use of humic acid in improving productivity (phytoplankton) and provide more insight on water quality variables in a freshwater ecosystem. In this context, the specific objectives of this study were as follows:

1. To evaluate the different sources of humic acid content.
2. To evaluate the effect of humic acid and its combination with inorganic fertilizer on freshwater phytoplankton population and water quality variables.
3. To evaluate the productivity of phytoplankton and water quality variables in aquatic ecosystem fertilize with cow manure and liquid humic acid.