# UNIVERSITI PUTRA MALAYSIA

# USE OF SELECTED PERIPHYTON SPECIES TO IMPROVE WATER QUALITY AND SHRIMP POSTLARVAL PRODUCTION

**HELENA KHATOON**

**FS 2006 56**

# USE OF SELECTED PERIPHYTON SPECIES TO IMPROVE WATER QUALITY AND SHRIMP POSTLARVAL PRODUCTION

By

**HELENA KHATOON**

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

**October 2006**

DEDICATION

To the memory of my late grandfather

*&*

To my parents and siblings who always inspire and encourage me to achieve my goal

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

**USE OF SELECTED PERIPHYTON SPECIES TO IMPROVE THE WATER QUALITY AND SHRIMP POSTLARVAL PRODUCTION**

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**Chairman: Professor Fatimah Md. Yusoff, PhD**

**Faculty : Science**

In marine shrimp larval rearing practices, a large amount of water has to be exchanged frequently in order to maintain good water quality. This procedure contributes to the eutrophication of aquatic environment due to flushing of nutrient-enriched waters from aquaculture facilities. Furthermore, the process of frequent water exchange will eventually result in lack of good water supply which can also increase the risk of diseases in the hatchery. To overcome eutrophication and the risk of diseases, an alternative eco-friendly method was investigated to decrease harmful compounds especially ammonia and nitrite by using periphyton grown on substrates.

Different periphyton species (*Oscillatoria, Navicula* sp.*, Cymbella* sp. and *Amphora* sp.) from marine shrimp culture ponds were isolated, purified and mass cultured in the laboratory and grown in Conway medium. The effects of salinity (0, 15, 20, 25, 30 and 35 ppt) on the growth of these genera under

laboratory condition was determined. The highest ($p<0.05$) growth was achieved at 25-35 ppt salinity.

Nutritional composition of different periphyton genera were analysed to determine their importance as shrimp feed. All periphyton genera contained high protein (*Oscillatoria* 42%, *Cymbella* 43%, *Navicula* 49% and *Amphora* 44% of dry wt.), lipid (*Oscillatoria 20*%, *Cymbella* 26%, *Navicula* 26% and *Amphora* 23% of dry wt.) and carbohydrates (*Oscillatoria* 24%, *Cymbella* 20%, *Navicula* 11% and *Amphora* 18% of dry wt.). The periphyton genera also contained of docosahexaenoic acid (DHA) (*Navicula* 2%, *Cymbella* 2%, and *Amphora* 3%, *Oscillatoria* 1% of total lipid) and ecosapentaenoic acid (EPA) (*Amphora* 15%, *Cymbella* 3%, *Navicula* 8% and *Oscillatoria* 1% of total lipid).

Periphyton colonization using different substrates (bamboo, polyvinylchloride pipe, plastic sheet, fibrous scrubber and ceramic tile) in intensive shrimp culture ponds were studied for a period of 60 days. Nineteen periphyton genera dominated by the Chlorophyceae colonized the substrates during the first 15 days. Periphyton colonization on bamboo showed the highest biomass ($p<0.05$) amongst all the substrates used. Biomass of periphyton in terms of chlorophyll-a varied from 179 to 1137 $\mu g\ m^{-2}$ with mean values of 1137 ± 0.6, 929 ± 0.6, 684 ± 1.2, 179 ± 0.6 and 658 ± 0.6 $\mu g\ m^{-2}$ on bamboo, polyvinyl chloride (PVC) pipe, plastic sheet, fibrous scrubber and ceramic tile respectively on first 15 days.

Effectiveness of different periphyton genera in reducing total ammonia nitrogen (TAN), nitrite nitrogen ($NO_2$–N) and soluble reactive phosphorous (SRP) in hatchery tanks without shrimp postlarvae were studied for a period of 16 days. It was found that *Oscillatoria* significantly reduced ($p<0.05$) TAN (90%), SRP (83%) and $NO_2$–N (91%) whereas diatom species decreased 60%, 74% and 78% of the same parameters respectively. In addition, *Oscillatoria* yielded the highest ($p<0.05$) biomass compared to other periphyton species. Results of this study showed that all the periphyton genera were able to significantly reduce TAN, SRP and $NO_2$-N concentrations in larval rearing tanks.

The use of periphyton coated substrate (periphyton grown on polyvinylchloride pipes) for improving water quality and survival of shrimp postlarvae in hatchery without water exchange was studied for a period of 16 days. Periphyton species significantly reduced ($p<0.05$) TAN in shrimp culture tanks as compared to the control (without periphyton coated substrate). Amongst the treatments, tanks with *Oscillatoria* had the lowest mean TAN ($0.09 \pm 0.00$ mg $L^{-1}$) compared to tanks with diatoms ($3.77 \pm 0.17$ mg $L^{-1}$) and the control ($5.17 \pm 0.08$ mg $L^{-1}$). Similarly, $NO_2$–N ($0.04 \pm 0.00$ mg $L^{-1}$) and SRP ($0.22 \pm 0.00$ mg $L^{-1}$) concentrations were significantly ($p<0.05$) lower in the shrimp culture tanks with periphyton species than the control ($4.13 \pm 0.24$ mg $L^{-1}$). Shrimp cultured with periphyton coated substrate showed significantly higher survival (51% - 60%) than those without periphyton (37%). In addition, the shrimp postlarvae produced in this system showed high resistance to reverse salinity stress test (37% - 43%) compared

to the control (26%). This study illustrated that beneficial Periphyton species could improve water quality, provide live feed and serve as refugium for the shrimp postlarvae.

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

**PENGGUNAAN SPESIES PERIFITON TERPILIH UNTUK MEMPERBAIKI KUALITI AIR DAN PENGELUARAN PASCALARVA UDANG**

Oleh

**HELENA KHATOON**

**October 2006**

**Pengerusi: Professor Fatimah Md. Yusoff, PhD**

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Dalam aktiviti penternakan larva udang, kuantiti air yang banyak terpaksa diganti dengan kerap untuk mengekalkan kualiti air yang bersih. Proses ini menyumbang kepada eutrofikasi persekitaran akuatik disebabkan pelepasan air yang kaya dengan nutrien dari fasiliti akuakultur. Tambahan pula, proses pertukaran air yang kerap akan menyebabkan kekurangan sumber kualiti air yang bersih yang boleh meninggikan risiko serangan penyakit di dalam kolam penternakan. Untuk mengatasi masalah eutrofikasi dan risiko serangan penyakit, cara alternatif yang mesra alam sekitar dengan penggunaan perifiton yang dibiak pada subsrat telah dikaji dalam mengurangkan komponen yang berbahaya seperti ammonia dan nitrit.

Spesies perifiton yang berbeza (*Oscillatoria, Navicula* sp., *Cymbella* sp. dan *Amphora* sp.) dari kolam penternakan udang telah diasingkan, ditulinkan dan dikultur secara besaran di dalam makmal dangan menggunakan medium

Conway. Kesan saliniti (0, 15, 20, 25, 30 dan 35 ppt) terhadap pembiakan genera ini dalam makmal telah dikaji. Pembiakan paling tinggi ($p<0.05$) telah didapati pada saliniti 25-35 ppt.

Komposisi nutrien spesies perifiton yang berlainan telah dianalisis untuk menentukan kepentingannya sebagai makanan udang. Semua spesies perifiton mengandungi protein yang tinggi (*Oscillatoria* 41%, *Cymbella* 43%, *Navicula* 49% dan *Amphora* 44% berat kering), dengan lipid (*Oscillatoria 20*%, *Cymbella* 26%, *Navicula* 26% dan *Amphora* 23% berat kering) dan karbohidrat (*Oscillatoria* 24%, *Cymbella* 20%, *Navicula* 11% dan *Amphora* 18% berat kering). Spesies perifiton juga mengandungi asid docosahexaenoic (DHA) (*Oscillatoria* 1%*, Cymbella* 2%, *Navicula* 2%*,* dan *Amphora* 3% jumlah lipid) dan asid eicosapentaenoic (EPA) (*Oscillatoria* 1%*, Cymbella* 3%, *Navicula* 8%*,* dan *Amphora* 15% jumlah lipid).

Kolonisasi perifiton menggunakan substrat berbeza (buluh, paip polivinilklorida (PVC), lembaran plastik, penggosok selulosa dan kepingan seramik) dalam kolam kultur udang intensif telah dikaji dalam tempoh masa 60 hari. Sembilan belas genus perifiton yang didominasi oleh Chlorophyceae telah mengkolonisasi substrat-substrat tersebut pada 15 hari pertama. Kolonisasi perifiton pada buluh menunjukkan biojisim yang paling tinggi ($p<0.05$) di antara kesemua substrat yang telah digunakan. Biojisim perifiton dari segi kandungan klorofil-a menunjukkan variasi dari 179 ke 1137 μg $m^{-2}$ dengan nilai min purata 1137 ± 0.6, 929 ± 0.6, 684 ± 1.2, 179 ± 0.6 dan 658

± 0.6 µg $m^{-2}$ pada buluh, paip polivinilklorida (PVC), lembaran plastic, penggosok selulosa dan kepingan seramik masing-masing.

Keberkesanan spesies perifiton yang berbeza di dalam mengurangkan jumlah amonia nitrogen (TAN), nitrit nitrogen ($NO_2$-N) dan keterlarutan fosforus reaktif terlarut (SRP) dalam kolam penternakan tanpa larva udang telah dikaji selama 16 hari. *Oscillatoria* telah didapati ($p<0.05$) mengurangkan TAN (90%), SRP (83%) dan $NO_2$-N (91%) manakala spesies diatom telah mengurangkan sebanyak 60%, 74% dan 78% masing-masing bagi parameter yang sama. Tambahan pula, *Oscillatoria* menghasilkan biojisim yang tertinggi ($p<0.05$) berbanding spesies perifiton yang lain. Hasil kajian mendapati kesemua spesies perifiton dengan dapat mengurangkan kepekatan TAN, SRP dan $NO_2$-N dengan signifikan di dalam tangki penternakan larva udang.

Penggunaan substrat yang dibiakkan dengan perifiton untuk memperbaiki kualiti air dan kemandirian pascalarva tanpa pertukaran air telah dikaji dalam tempoh 17 hari dengan menggunakan perifiton yang biak pada paip PVC. Perifiton telah mengurangkan TAN ($p<0.05$) di dalam air dari tangki kultur udang berbanding kawalan (tanpa perifiton). Di antara hasil kajian, tangki yang mengandungi *Oscillatoria* mempunyai nilai min yang terendah (0.09 ± 0.00 mg $L^{-1}$) berbanding tangki yang mengandungi diatom (3.77 ± 0.17 mg $L^{-1}$) dan kawalan (5.17 ± 0.08 mg $L^{-1}$). Selain itu, kepekatan $NO_2$-N (0.04 ± 0.00 mg $L^{-1}$) dan SRP (0.22 ± 0.00 mg $L^{-1}$) di dalam tangki kultur udang yang mengandungi perifiton adalah lebih rendah dengan signifikan ($p<0.05$) dari

tangki kawalan (4.13 ± 0.24mg$L^{-1}$). Udang yang telah dikultur dengan substrat yang telah dibiakkan dengan perifiton telah menunjukkan kemandirian yang tinggi (51%-60%) ($p<0.05$) berbanding rawatan kajian tanpa perifiton (37%). Tambahan pula, penghasilan larva udang dalam sistem ini menunjukkan daya ketahanan rintangan yang tinggi terhadap ujian tekanan saliniti terbalik (37%-43%) berbanding dengan kawalan (26%). Kajian ini menunjukkan spesies perifiton yang berfaedah boleh meningkatkan mutu air, menyediakan makanan hidup yang berfungsi sebagai tempat persembunyian pascalarva .

## ACKNOWLEDGEMENTS

Thanks to Almighty Allah for giving me mental peace, health and strength to pursue this study.

I am truly indebted to my advisory committee chairperson Prof. Dr. Fatimah Md. Yusoff, who provided for every academic need I had; for her valuable advice, intelligent counsel, counseling motivation, helpful comments, suggestions and encouragement. Under her guidance, I successfully overcame all the difficulties and learned a lot about my work and far beyond. My sincerest appreciation and thanks to my advisory committee members, Prof. Dato' Dr. Mohamed Shariff Mohamed Din, Assoc. Prof. Dr. Japar Sidik Bujang and Prof. Dr. Suhaila Mohamed for providing all the expertise and critical suggestions that I required. Thank you for seeing me through until the end. My sincere thank goes to Dr. Ekramul Azim and Dr. Wahab for providing the literatures and comments, which helped me a lot to complete this thesis.

I cannot find appropriate words to express my heartfelt gratitude to Dr. Sanjoy Banerjee for his untiring guidance, support and encouragement. I also extend my sincere and deep appreciation to Prabath, Edward and Altab for their moral support and encouragement all the time in striving towards the completion of this study. My heartfelt thanks go to Hazel for having a hard time to read the first draft of this thesis and her useful suggestions to improve the quality of the thesis. I am very grateful to Dr. Md. Shahidul Islam, Kyoto University, Japan to whom I enjoyed open access during the entire writing

period. His in depth criticisms always encouraged me to improve my writing skill.

I would like to give my special thanks to Mr. Perumal Kuppan for helping in the laboratory and field sampling. I give similar appreciation to all of my friends, Rozhan, Omid, Said, Natrah, Anarita, Jamal, Jimmy, Liza, Shila and Sha. The joy and happiness of being able to share friendship, love and time with all of them throughout my stay in UPM is simply indescribable. My special thanks to Mr. Jakir Hossen, Godhuli, Bukky and Zuriyati for providing me with moral and spiritual encouragement.

The financial support provided by Third World Organization for Women in Science (TWOWS) is gratefuly acknowledged.

Last, but not least, I am thankful to all of my well-wishers who helped me in different forms.

I certify that an Examination committee met on 16th October, 2006 to conduct the final examination of Helena Khatoon on her Doctor of Philosophy thesis entitled "Use of Selected Periphyton Species to improve the Water Quality and Shrimp Postlarval Production" 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 Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement 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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**HELENA KHATOON**

Date: 18/1/ 07

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| | |
|---|---|
| AFDM | ash free dry matter |
| ANOVA | analysis of variance |
| $DDH_2O$ | double distilled water |
| DHA | docosahexaenoic acid |
| DM | dry matter |
| DO | dissolved oxygen |
| E | east |
| EPA | eicosapentaenoic acid |
| $KH_2PO_4$ | anhydrous potassium dihydrogen phosphate |
| $LC_{50}$ | lethal concentration 50 |
| MT | metric tonne |
| MUFA | monounsaturated fatty acid |
| N | north |
| $NaNO_2$ | anhydrous sodium nitrite |
| $NH_3^+$ | ammonia |
| $NH_3$-N | ammonia-nitrogen |
| $NH_4^+$ | ammonium |
| $(NH_4)_2SO_4$ | anhydrous ammonium sulphate |
| $NO_2^-$ | nitrite |
| PL | postlarvae |
| PUFA | polyunsaturated fatty acid |
| PVC | polyvinyle chloride |
| rpm | rotation per minute |

| | |
|---|---|
| SAS | statistical analysis system |
| SFA | saturated fatty acid |
| SGR | specific growth rate |
| SRP | soluable reactive phosphorous |
| TAN | total ammonia nitrogen |

# CHAPTER I

# INTRODUCTION

## Background of the Study

Periphyton refers to microfloral community attached to surfaces of substrates submerged in water (Wetzel, 1983). Periphytic organisms are often closely intertwined with other attached organisms such as fungi, bacteria, protozoans and other attached invertebrates (Figure 1). Nowadays, the word periphyton is often used synonymously as biofilm or `aufwuchs' to describe the total assemblage of attached organisms on submerged substrates, including non-attached organisms and detritus (Azim *et al*., 2005). However, in specific applications such as wastewater treatment, the term `biofilm' is used mainly with reference to attached bacteria and protozoans, that help to speed up the mineralization process and improve the water quality. In this context, periphyton community comprises of not only the microflora, but also bacteria, fungi, protozoans, benthic microinvertebrates, detritus and a range of other larger aquatic invertebrates and their larvae (Azim *et al*., 2005). Periphyton is thus a complex mixture of autotrophic and heterotrophic organisms and cannot simply be regarded as an attached equivalent of phytoplankton, although it certainly performs similar functions in ponds, such as oxygen production and the uptake of inorganic nutrients (van Dam *et al*., 2002). In addition, periphytons are able to trap and process suspended organic materials in the water. Between autotrophic and heterotrophic components of