UNIVERSITI PUTRA MALAYSIA

NUTRIENT UPTAKE AND GROWTH OF IMMOBILIZED FILAMENTOUS MICROALGAE Stigeoclonium nanum (DILLWYN) KÜTZING 1849 FOR IMPROVEMENT OF WATER QUALITY

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164109

This project report is submitted in partial fulfillment of the requirements for the degree of Bachelor of Agriculture (Aquaculture)

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CERTIFICATION OF APPROVAL

DEPARTMENT OF AQUACULTURE

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This is to certify that I have examined the final year project report and all corrections have been made as recommended format stipulate in the AKU 4999 project guidelines, Department of Aquaculture, Faculty of Agriculture, and Universiti Putra Malaysia.

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ABSTRACT

In aquaculture 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 microalgae. In this study, filamentous green microalgae *Stigeoclonium nanum* was used. The first objective of this project was to study growth of the filamentous microalgae (*S. nanum*) immobilized in alginate beads and in free suspension condition. Four experimental treatments, control 1 (no algae and no beads), control 2 (beads with free algae), algae in free suspension and beads with algae were tested. The parameters that were examined included *S. nanum* growth in terms of chlorophyll-a content, and ammonium loading for the microalgae culture. The ammonia loading is ammonia which was added up during experiment when it has zero reading. From the study, significantly higher ($p < 0.05$) chlorophyll-*a* content was observed in alginate beads (5.27 ± 0.63 mg/l) on the final day of the experiment than the microalgae cultured in normal suspension culture (0.62 ± 0.20 mg/l). Significantly higher ($p < 0.05$) total ammonium loading was also observed in the culture of immobilized microalgae. The second objective of the experiment was to measure the uptake of the ammonia-N (NH_4-N) and nitrate-N (NO_3-N) by immobilized filamentous microalgae. Five culture media with different concentrations of NO_3-N and NH_4-N were tested in this experiment, treatment A was Bold's Basal Medium (BBM), treatment B was zero concentration of NO_3-N and NH_4-N (control), treatment C was zero concentration of NO_3-N with 10 mgL^{-1} concentration of NH_4-N, treatment D was 10 mgL^{-1} of NO_3-N with zero concentration NH_4-N, and treatment E was 10 mgL^{-1} of NO_3-N with 10 mgL^{-1} of NH_4-N. The parameter examined was *S. nanum* uptake of the NH_4-N and NO_3-N. This study illustrated that NH_4-N was preferred than NO_3-N as nitrogen source by the immobilized *S. nanum.*

ABSTRAK

Dalam amalan akuakultur, sejumlah besar air perlu ditukar kerap untuk mengekalkan kualiti air yang baik. Prosedur ini menyumbang kepada eutrofikasi air di dalam habitat akibat curahan air dengan nutrien diperkaya daripada kemudahan akuakultur. Tambahan pula, proses pertukaran air kerap akhirnya akan mengakibatkan kekurangan bekalan air yang baik yang juga boleh meningkatkan risiko penyakit di tapak penetasan . Untuk mengatasi eutrofikasi dan risiko penyakit, eko- mesra kaedah alternatif telah diselidiki dalam mengurangkan sebatian berbahaya terutamanya ammonia dan nitrat dengan menggunakan mikroalga. Dalam kajian ini, mikroalga hijau berfilamen *Stigeoclonium nanum* telah digunakan. Objektif pertama projek ini adalah untuk mengkaji pertumbuhan mikroalga yang berfilamen (*S. nanum*) bergerak di dalam manik alginat dan dalam keadaan kultur bebas. Empat rawatan eksperimen telah digunakan, kawalan 1 (tiada alga dan tiada manik), kawalan 2 (manik dengan tiada alga), alga dalam penggantungan percuma dan manik dengan alga telah diuji. Parameter yang dikaji adalah pertumbuhan *S. nanum* segi kandungan klorofil-a dan muatan ammonium di dalam didalam kultur mikroalga. Muatan ammonia adalah ammonia yang ditambah semasa eksperimen apabila bacaan ammonia adalah kosong. Hasil kajian tersebut, didapati kandungan klorofil-a lebih tinggi dan ketara ($p < 0.05$) di dalam manik alginat (5.27 ± 0.63 mg/l) pada hari terakhir daripada mikroalga dikultur dalam kultur penggantungan biasa (0.62 ± 0.20 mg/l). Muatan ammonium lebih tinggi juga dilihat dalam kultur mikroalga tidak bergerak. Objektif kedua eksperimen ini adalah untuk mengukur pengambilan ammonia-N (NH_4-N) dan nitrat-N (NO_3-N) dengan mikroalga berfilamen di dalam manik alginat. Lima media kultur dengan kepekatan yang berbeza NO_3-N dan NH_4-N telah diuji dalam eksperimen ini , rawatan A adalah Bold Basal Media (BBM) , rawatan B adalah kepekatan sifar NO_3-N dan NH_4-N (kawalan), rawatan C kepekatan sifar NO_3-N dengan kepekatan 10 mgL^{-1} NH_4-N, rawatan D adalah 10 mgL^{-1} NO_3-N dengan kepekatan sifar NH_4-N, dan rawatan E adalah 10 mgL^{-1} NO_3-N dengan 10 mgL^{-1} NH_4-N. Parameter yang dikaji adalah pengambilan NH_4-N dan NO_3-N daripada *S. nanum*. Dalam eksperimen ini, kita boleh menyimpulkan bahawa NH_4-N adalah pilihan utama berbanding NO_3-N sebagai sumber nitrogen oleh *S. nanum* didalam manik alginat.

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LIST OF ABBREVIATIONS AND SYMBOLS

ml	milliliter
g	gram
µl	microliter
%	percent
°C	degree centrigrade
ml L^{-1}	milligram per liter
µmol	micro mole
TAN	total ammonia nitrogen
NO_3-N	nitrate nitrogen
NH_4-N	ammonia nitrogen

CHAPTER 1

1.0 INTRODUCTION

The aquaculture sector has recorded an annual growth rate of about 10 percent in the last 5 years and has now grown into a profitable and sustainable industry (FAO, 2013). The rapid expansion of intensive aquaculture industries, are often accompanied by waste production mainly from uneaten feed, feces and organic residues. These wastes increase nutrients in water, in particular nitrogen and phosphorous, and water quality can rapidly deteriorates as a result (Beveridge 1996). Microbial decomposition of organic matter leads to increased levels of total ammonia nitrogen (TAN) and nitrite-N, which are very harmful to fish even at low concentrations (Meade, 1985; Jiménez-Montealegre et al., 2002; Torres-Beristain et al., 2006). The TAN present in the water may be transformed into nitrite, nitrate and gaseous nitrogen. Bacteria present in the water and sediment carry out these nitrogen transformations by nitrification and denitrification processes.

In general, both TAN and nitrate can be assimilated by microalgae, present in the water column. The microalgae can be consumed by cultured organisms (Turker et al., 2003). In stagnant ponds TAN tends to accumulate within the system due to insufficient nitrification activity (Grommen et al., 2002). Therefore, presence of microalgae may be helpful to reduce nitrogen in the water. There are many groups of microalgae which can be found in aquatic ecosystem for example green algae (Chlorophyta), red algae (Rhodophyta), blue-green algae (Cyanobacteria) and diatoms (Bacillariophyta). In this research, *Stigeoclonium nanum*, a green filamentous fresh water microalga, was studied. Immobilization of microalgae in alginate beads was studied because in this form, they are easy to manage, microalgae biomass can be maintained at certain volume, and algae bloom can be prevented in the culture system.

This research investigated the growth of *S.nanum* culture in normal suspension and *S.nanum* immobilized in alginate beads. Futhermore, this research investigated the efficiency of immobilized *S.nanum* to utilize ammonium-N and nitrate-N in water.

1.1 Research objectives

i. To study the growth of the filamentous microalgae (*Stigeoclonium nanum*) immobilized in alginate beads and in free suspension condition
ii. To measure the uptake of the ammonia-N and nitrate-N, by immobilized filamentous microalgae (*Stigeoclonium nanum*)

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