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TREATMENT OF AQUACULTURE WASTE WATER BY USING THE MACROALGA (*Gracilaria* sp.) : A LABORATORY SCALE STUDY

Siti Noor Roslinda Binti Saibani

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Siti Noor Roslinda Binti Saibani
Programme of Aquatic Resources Science and Management
Faculty of Resources Science and Technology

Abstract

Effluent from the tiger shrimp pond usually contains high concentration of dissolved nutrients such as ammonia-nitrogen, nitrite, nitrate, orthophosphate and silicate compared to the influent water sources. Improvement of the effluent quality by using the macroalgae had been done in several studies. The aim of this laboratory scale study is to evaluate the effectiveness of the macroalga *Gracilaria* sp in treating the waste water of the tiger shrimp pond. Four treatments were carried out for three weeks: A (effluent stocks + *Gracilaria*), B (diluted effluent stocks + *Gracilaria*), C (effluent without *Gracilaria*), and D (influent without *Gracilaria*). The concentration of nutrients (ammonia-nitrogen, nitrite, nitrate, orthophosphate and silicate) was analyzed on Day 7, 14 and 21 using HACH Kit (2010). The nutrients water content was compared before (Day 0) and after the treatment done (Day 7, 14, and 21). As a result, nutrients uptake pattern for each nutrient parameters in all of the treatment tanks (A, B, C and D) were observed. Generally, there were nutrient uptake for ammonia-nitrogen, nitrite and orthophosphate by the macroalga. However, there was no significantly improvement in water quality for silicate and nitrate structures used by the *Gracilaria*.

Keywords: Shrimp pond effluent, Influent, *Gracilaria* sp., Orthophosphate, Ammonia-nitrogen,

Abstrak

Air kumbahan daripada kolam udang harimau kebiasaannya mengandungi kepekatan bahan nutrient terlarut yang sangat tinggi contohnya seperti ammonia-nitrogen, nitrit, nitrat, ortofosfat dan silikat jika dibandingkan dengan sumber air yang masuk (influen). Tujuan kajian makmal ini dijalankan adalah untuk mengkaji kecekapan makroalga Gracilaria sp dalam proses merawat air kumbahan daripada kolam udang harimau. Empat jenis rawatan yang telah dijalankan selama tiga minggu kajian iaitu: A (air kumbahan asal + Gracilaria*), B (air kumbahan yang telah dicairkan +* Gracilaria*), C (air kumbahan tanpa* Gracilaria*) dan D (air influen tanpa* Gracilaria*). Kepekatan nutrient (ammonia-nitrogen, nitrit, nitrat, ortofosfat dan silikat) telah dianalisis pada hari ke-7, 14 dan 21 dengan menggunkan HACH Kit (2010). Kandungan nutrient yang terkandung di dalam sampel air dibandingkan sebelum dan selepas rawatan dijalankan.Sebagai keputusannya, corak pengambilan nutrien untuk setiap parameter di dalam setiap jenis tangki rawatan (A, B, C,dan D)telah diperolehi. Secara amnya, terdapat corak pengambilan nutrient bagi parameter ammonia-nitrogen, nitrit dan ortofosfat oleh makroalga. Walau bagaimanapun,hasil kajian ini juga menunjukkan bahawa makroalga tidak memberikan kesan pengambilan nutrien secara signifikan untuk parameter nitrat dan silikat.*

Kata kunci: Air kumbahan kolam udang, influen, Gracilaria *sp., Ammonia-nitrogen, Ortofosfat,*

1.0 INTRODUCTION

The subject of waste management in general, and particularly the issue of effluents, has become an important issue in pond aquaculture. Ponds generally have overflow after heavy rains, water exchange is used in some types of aquaculture, and ponds maybe drained for harvest. These discharges contain nutrients, organic matter, and suspended solid that can be sources of pollution in receiving water.

In this recent year, increasing efforts have been made to protect the terrestrial and freshwater environments, especially along the coasts of the countries. Nutrient-rich waste waters, either treated or untreated, are still discharged directly into the sea. The direct discharge of waste water nutrients may add significantly to the nutrient budget locally and considerably alter the natural nutrient and productivity pattern of the recipient coastal ecosystem. Therefore, as the nutrients in the waste water effluents are a considerable burden and nuisance to the recipient terrestrial and aquatic environments, legislation has been enacted in the countries to reduce the nitrogen and phosphorus concentration of the treated waste water effluents.

Waste water treatment is the removal of pollutants from waste water for safe and nuisance-free disposal. Waste water treatment can be divided into five generic stages (primary, secondary, tertiary, quaternary, quinary), their order reflecting the most feasible and economical progression in removing unsightly, infectious, and biologically disruptive materials. Waste treatment methods could be physical, chemical, biological or combinations of all threes. In certain places, where climate permits, the algal-bacterial systems can equal or exceed conventional waste water treatment and less expensive.

Although primary treatment consists of essentially physical processes, biological processes are the only economical way to remove a significant fraction of dissolved organics from waste water. In nutrient removal, intensive methods of tertiary waste treatment involved ammonium removal by ion exchange or air stripping, nitrate removal by anoxic reduction to nitrogen gas, and phosphate removal by lime precipitation or by anoxic reduction to phosphine.

The nutrient removal process is completed when the organic nitrogen is converted to ammonium or to nitrogen gas (N_2). The nitrogen gas is produced through heterotrophic nitrification and denitrification in the anaerobic bottom of the pond and escapes to the atmosphere along with methane. Ammonium is taken up by algal for growth. Surplus ammonium is converted to ammonia (NH_3), which escapes to the air during gentle mixing at high pH. Phosphorus is taken up by algae or precipitated at high pH as a calcium phosphate (Borowitzka and Borowitzka, 1983).

Various species of macroalgae can rapidly assimilate large quantities of dissolved organic and inorganic nutrients, usually with a preference for ammonium (NH_4^+) (Harlin, 1978; Ryther *et al.*, 1981). Rhodophyta (red algae) are particularly efficient at taking up nutrients rapidly and have mechanisms for storing large reserves or nutrients (Vergara *et al.*, 1993). For example the red macroalga *Gracilaria edulis* rapidly assimilates NH_4^+ (Jones *et al.*, 1996).

In this final year project study, macroalga *Gracilaria* sp. was chosen because of its ability to assimilates or taking up the nutrients in the water column. The aim of this study is to examine the effect of treatment with macroalga on the concentration of organic compounds and nutrients of the tiger shrimp pond effluent waste water. Five nutrient parameters (ammonia-nitrate, nitrite, nitrate, orthophosphate and silicate) were analyzed in order to check the water quality of aquaculture waste water after treated using *Gracilaria* sp. Simultaneously, the pattern of nutrient level treated with *Gracilaria* sp. was monitored for three weeks.

2.0 LITERATURE REVIEW

Quantitative comparisons of shrimp farm influent and effluent water have demonstrated that effluent can contain elevated concentrations of dissolved nutrients, phytoplankton, bacteria, and other suspended organic and inorganic solids (Ziemann *et al.*, 1992). The potential of adverse environmental impacts from untreated effluent have raised concerns about the sustainability of shrimp farming (Philips *et al.*, 1993; Primavera, 1994).

According to Boyd and Tucker (1998), the typical concentrations of water quality variables in effluent from intensive shrimp ponds are salinity (10-35 ppt), temperature (22-31°C), pH (7.5-9.0), total phosphorus (0.05-0.40 mg/l), total nitrogen (0.5-5.0 mg/l), total ammonia nitrogen (0.05-1.0 mg/l), dissolved oxygen (4-12 mg/l), biological oxygen demand (5-20 mg/l), chlorophyll a (20-250 mg/l) and total suspended solid (30-190 mg/l).

In aquatic ecosystems, the nutrients that control plant growth are nitrogen and phosphorus. Nitrogen is used by plants in a large number of chemical reactions and is an essential component of amino acids, the building blocks of proteins. Nitrogen is also most important as a nutrient in the forms of ammonia (NH_3) or ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and organic molecules (Gross, 1982). Nutrients that can be used by aquatic plants are in the forms of ions. If these elements are in the unsuitable molecular structures, they cannot be used by the plants. Furthermore, a very high concentration of nutrients can damage the plants and animals rather than stimulate primary productivity.

For instance, nitrogen compounds can be toxic to plants and especially to animals, when these compounds are present at high concentrations. The most toxic of the nitrogen compounds is ammonium. Ammonia and ammonium are in equilibrium depending on the pH of the water. Acidic water contains more free hydrogen ions that will shift the equilibrium from NH_3 to NH_4^+ (Riley and Chester, 1971). The toxicity of ammonia is usually reduced when it is oxidized to nitrite and more dramatically when it is oxidized again to nitrate.

The chemistry of nitrogen is complex due to several oxidation stages such as ammonification, nitrification and denitrification. The relationships between various forms of nitrogen in a nitrogen cycle are shown in the Figure 1.

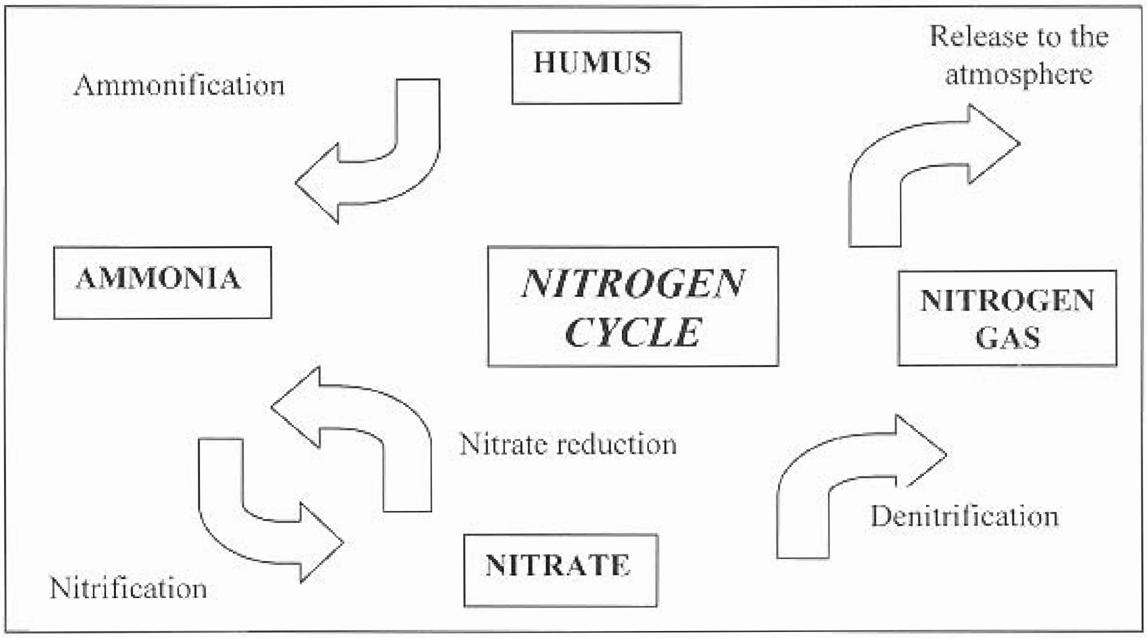

Figure 1: Nitrogen cycle in the tiger shrimp pond (Source: Huner and Brown, 1985)

The primary sources of nitrogenous compounds in aquaculture systems are from the organic materials such as detritus and uneaten feed. Most of the nitrogen in organic matter exists as amino groups in protein. Proteins are deaminated through the biological activity and ammonia nitrogen is produced by ammonification process (Alexander, 1961) which is a heterotrophic process. It may occur under either aerobic or anaerobic conditions. The oxidation of ammonia is primarily carried out by two genera of bacteria *Nitrosomonas* and *Nitrobacter*. Nitrification is optimum at pH 7 to 8 and at temperature of 25°C to 35°C. The oxidation of ammonia to nitrite is a potential source of acidity in aquatic ecosystem. Under anaerobic condition, nitrate and nitrite are both reduce by a process called denitrification. According to Patrick and Tusneem (1972), denitrification usually occurred in the hypolimnion zone of the eutrophic water column or when oxidized nitrogen compound diffuse into anaerobic layers of mud.

In the natural environments, ammonia-nitrogen is predominant which depends on the pH, temperature and salinity of the water. The water pH plays an important role that is the strongest influence for the form of ammonia. Referring to the Equation 1, under acidic condition, the reaction will shift to the right. Meanwhile Equation 2 shows the reaction that will shift to the left if the water pH is high. In other words, high pH and warmer temperatures increase the toxicity of a given ammonia concentration. High ammonia concentrations can stimulate excessive aquatic primary production and indicate pollution.

Equation 1:

$$2NH_3 + 3O_2 \leftrightarrow 2NO_2^- + 2H^+ + 2H_2O$$

(ammonia) (nitrite)

Equation 2:

$$2NO_2^- + O_2 \leftrightarrow 2NO_3^-$$

(nitrate)

(Sources: Huner and Brown, 1985)

The second important nutrient for plant growth is phosphorus in the form of orthophosphate (PO_4^{-3}). It is usually found in a lower concentration and is needed in lesser quantities compared to the nitrogen. Another nutrient that is measured as water quality parameter is silicate. Silicate in the form of orthosilicic acid ($Si\ (OH)_4$). It is normally found in high concentrations that is enough to sustain all life in the water especially diatoms which need this compound for building their frustules.

Consequently, a biological treatment might be a potential alternative to treat the effluent using macroalgae to remove the nutrients such as ammonia-nitrate, nitrite, nitrate, orthophosphate and silicate. Besides improving the water quality of shrimp pond effluent water, macroalga also can provide an additional source of income for shrimp farmers. Retamales *et al.*, 1994, reported that tank culture of *Gracilaria chilensis* supplied with salmon seawater effluent had demonstrated production rates of four times compared to those in wild beds and doubled the agar content. The incorporation of macroalga into a polyculture system may also provide additional income. However, these macroalga have specific requirements and management for growth. For example, *Gracilaria* sp. requires sufficient water flow and periodic harvesting to ensure rapid growth and nutrient removal.

A simple removal of nutrients from treated sewage effluent could be important in preventing unwanted eutrophication of coastal and lake waters. This has been experimented, where the waste water effluent was run through a serial polyculture system at the Harbor Branch Oceanographic Institution in Florida (Ryther *et al.*, 1981) to see if the water could be effectively stripped of its nutrients.

Effluent was mixed with seawater and used to grow phytoplankton that was harvested and fed to oysters, and the water from the oysters culture was shunted into a culture of the *Gracilaria*. About 95% of the inorganic nitrogen was taken up by the phytoplankton. The oysters consumed about 85% of the algae, although they regenerated some of the nitrogen and returned it to the system in the form of ammonia. The regenerated ammonia was completely taken up by the macroalga. This system showed about 95% effective at nitrogen removing, and 45% to 60% efficient at removing phosphorus (Williams *et al.*, 1977). Besides that, *Gracilaria* showed a very high yield of 12 to 17 grams dry weight/ m^2/ day in this trial.

Gracilaria belongs to the class of Rhodophyceae (red algae). It falls in the order of Gigartinales and family of Gracilariaceae. There are about 100 species in this genus, found in temperate and tropical waters, but only a few are important culture species.

Gracilaria does not have "leaves" and the hold-fast appear nonexistent. It tends to just float around the sump, rarely taking hold to a substrate. They are all branching filaments and fleshy seaweeds with a bushlike appearance (Sze, 1997). *Gracilaria* grow best in low-wave-action environments with salinities of 8 to 25 ppt (Trainor, 1978).

This macroalga divides by fragmentation process. Like many other red algae, *Gracilaria* display a complex life history which includes the alternation of generation stages (Lobban and Wyne, 1981). The alga is light red or rust colored and very "rubbery". When the nutrients content (especially nitrogen), in the water is low, the plants lose their normal reddish-brown hue and begin to take on a straw color. The dark color is a reflection of the amount of phycoerythrin in the plant. It is believed that this pigment is the site of nitrogen storage by *Gracilaria* (Corwin *et al.*, 1982).

Gracilaria has some important commercial values. First, it can produce agar which can be extracted by using hot water. The extracted agar forms a gel that will vary in strength with the structure. The principle user of agar is the food industry (Nisizawa *et al.*, 1987) in making jams and jelly. Second, agar is also very important in the microbiological industries (medical supply industry), where it is used in laboratory (culture of bacteria). Third, it can be used as diet to the young abalones when their size reach about 0.5 cm (Hahn, 1988).

3.0 MATERIALS & METHODS

3.1 Sources of Effluent Water and Macroalga (*Gracilaria* sp.)

The effluent water samples was obtained from Lembaga Kemajuan Ikan Malaysia (LKIM) tiger shrimp *Penaeus monodon* farm, which is located at Telaga Air, Kuching and the macroalga (*Gracilaria* sp.) was collected from floating cages of Inland Fisheries, Semariang Batu, Kuching, Sarawak.

3.2 Laboratory Methods

One hundreds and fifty litres of effluent and ninety litres of influent water sources were obtained from LKIM tiger shrimp farm. About 1 kg of *Gracilaria* sp. was collected from the floating cage culture of Inland Fisheries. This macroalga attached to the net of the cage. Once collected, the algae were transported to the University Malaysia Sarawak (UNIMAS) laboratory, cleaned (to separate/eliminate the rubbish and other organisms) and placed in several well-aerated pails.

The initial (Day 0) nutrient contents (ammonia-nitrate, nitrite, nitrate, orthophosphate and silicate) of effluent stock were measured and recorded. This experiment was carried out in glass tanks (44 cm × 29 cm × 30 cm) and consisted of four different treatments (Figure 2):

A= Effluent stock with *Gracilaria* sp.

B= 50% diluted effluent stock with *Gracilaria* sp.

C= Effluent stock without *Gracilaria* sp. (positive control)

D= Influent without *Gracilaria* sp. (negative control)

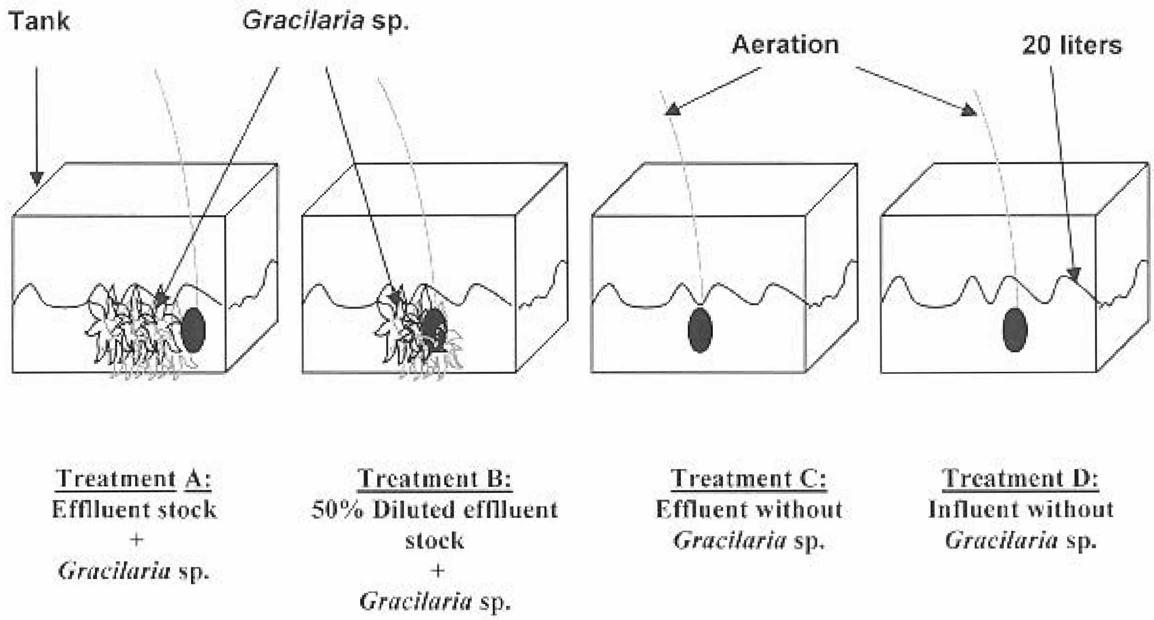

Figure 2: Four different treatment in this study

Twenty litres of effluent stock, 50% diluted effluent or influent was poured into each tank according to the treatment, respectively. Then, about 100 g (wet weight) of *Gracilaria* sp. was placed inside each treatment A and B. All experiments were done in 3 replicates. This experiment also was run for 3 weeks and 150 ml of the water samples were taken from each tank once a week. These samples were kept in acid washed bottles and stored at -20°C (can last for 6 months) for further analysis. Prior to analyzing the nutrient contents, the frozen samples were thawed and brought to room temperature. The value of all nutrient concentrations in the controls tank were compared with the value recorded in treatment A and B to indicate the effectiveness of *Gracilaria* sp. in waste water treatment. The above experiments were carried out in laboratory where the air conditioner and lights were switched on for 24 hours. During this experiment, the temperature, salinity, dissolved oxygen and pH of water inside each tank were also recorded.

3.3 Nutrients Analysis

The nutrients content such as ammonia-nitrate, nitrite, nitrate, orthophosphate and silicate were analyzed by using the HACH Kit (DR 2010). The ammonia nitrogen content was analyzed using the Standard Method 8038 based on Nessler Method. Whereas nitrite was analyzed using the Diazotization Method 8507. The nitrate content was measured according to the Standard Method 8192. The content of orthophosphate was analyzed using the Standard Method 8038. Water samples were filtered before stored in -20°C freezer. Silicate content was analyzed using the Heteropoly Blue Method 8186. All procedures were referred to the DR 2010 Spectrophotometer Procedures Manual.

3.4 Basic Water Parameter

Ambient water parameters include pH, dissolved oxygen, salinity and temperature are measured by using the Hydrolab SVR3- DL Surveyer 3 Water Quality Logging System equipment and ATAGO-28 hand refractometer or salinometer and recorded.

3.5 Statistical Methods

The concentration level of each nutrient (ammonia-nitrate, nitrite, nitrate, orthophosphate and silicate) for each treatment was analyzed. The concentration values were compared to the initial day samples concentration level (before treatment). Data were statistically analyzed using the General Linear Model (GLM) Multivariate Test in a level of significant of 0.05 in the SPSS software. If there is a significant observes, means were compared by the multiple range Tukey test.

4.0 RESULTS

4.1 Nutrients

4.1.1 Initial concentration of nutrients

The initial concentration (Day 0) of five nutrients (ammonia-nitrate, nitrite, nitrate, orthophosphate and silicate) in the influent and the effluent water samples before the treatment was done is shown in Table 1. For the effluent water sample, the mean concentration for ammonia-nitrogen, nitrite, nitrate and orthophosphate were much higher than the mean concentration of the influent water sample. However, the mean concentration of the silicate in the effluent water sample was lower compared to influent water sample.

Table 1: The mean concentration (mg/l) of ammonia-nitrate, nitrite, nitrate, orthophosphate and silicate in effluent and influent water samples on Day 0.

SAMPLES/ NUTRIENTS	Influent (mg/l)	Effluent (mg/l)
Ammonia-nitrogen	0.5±0.05	1.5±0.05
Orthophosphate	0.4±0.05	1.4±0.05
Nitrite	1.01±0.05	13.5±0.05
Nitrate	1.3±0.05	1.5±0.05
Silicate	0.7±0.05	0.6±0.05

4.1.2 Ammonia-nitrogen

Figure 3 showed the ammonia-nitrogen mean concentration after various treatments for day 7, 14 and 21. The influent and effluent mean concentration for the initial day (Day 0) were 0.5 mg/l and 1.5 mg/l, respectively. Moreover, the mean concentration of the treatment A (effluent stock + *Gracilaria*) were 0.46 mg/l on the Day 7, 0.17 mg/l for the Day 14 and 0.6 mg/l for the Day 21. This showed a reduced pattern of the mean concentration from the Day 7 to the Day 14. However, from the Day 14 to the Day 21, the mean concentration of the ammonia-nitrogen was increased (0.17 mg/l to 0.60 mg/l).

Apart from that, the mean concentrations for treatment B (diluted effluent stock + *Gracilaria*) were 0.2 mg/l (Day 7), 0.27 mg/l (Day 14) and 0.37 mg/l (Day 21). The changes pattern of this sample (treatment B) was increased from the Day 7 to the Day 21. In addition, the mean concentrations of the ammonia-nitrogen for the effluent control (treatment C) water sample within the three weeks were 0.8 mg/l (Day 7), 0.16 mg/l (Day 14) and 0.27 mg/l (Day 21). Meanwhile, for the influent water sample, the mean concentrations were 0.86 mg/l (Day 7), 0.5 mg/l (Day 14) and 0.47 mg/l (Day 21).

In general, the mean concentrations of the ammonia-nitrogen in all treatments (A, B, C, D) within three weeks experiment showed a decreased pattern compared to the initial day mean concentrations for influent (0.5 mg/l) and effluent (1.5 mg/l) water samples.

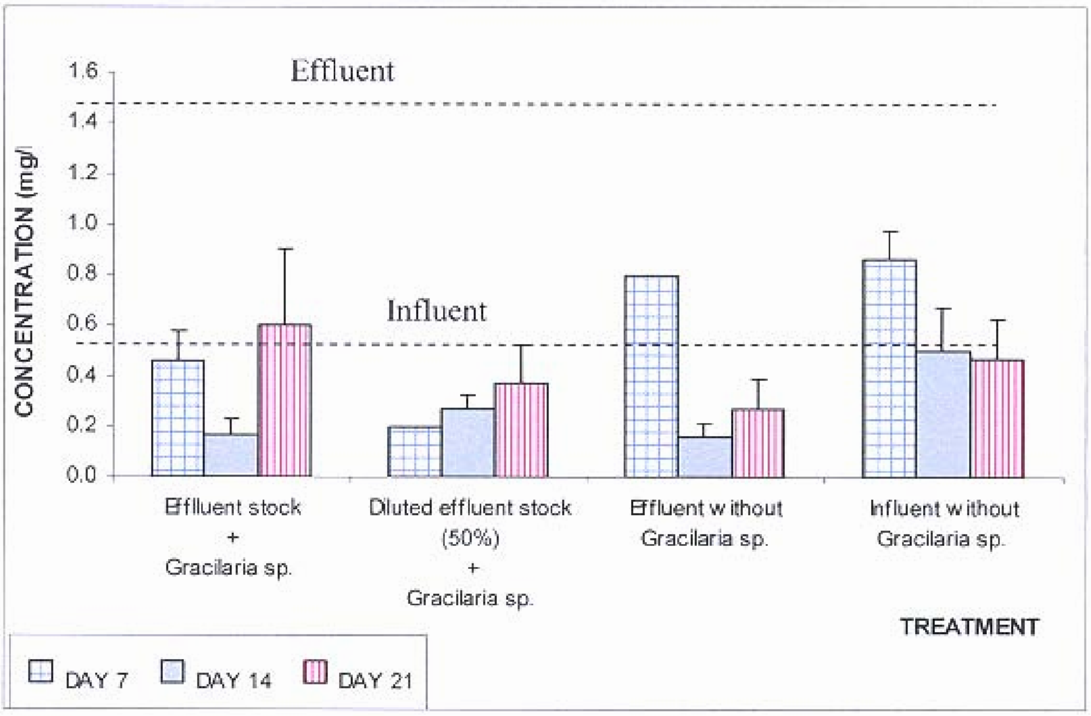

Figure 3: Ammonia-nitrogen mean concentration (mg/l) depending on specific treatment within three weeks. The dotted lines represent the concentration level of influent and effluent for Day 0.

4.1.3 Nitrite

The mean concentration of the nitrite parameter for the initial day (Day 0) can be divided into two samples which were influent and effluent water samples (Figure 4). Both of these mean concentrations were represented by the dotted line. For the influent water sample, the nitrite mean concentration was 1.01 mg/l. besides, the mean concentration for the effluent water sample was 13.5 mg/l.

After treatment, the nitrite mean concentration for treatment A (effluent stock + *Gracilaria*) water samples were 14.3 mg/l (Day 7), 8.59 mg/l (Day 14) and 10.57 mg/l (Day 21). Meanwhile, the nitrite mean concentrations for samples B (diluted effluent stock + *Gracilaria*) were 14.02 mg/l (Day 7), 1.97 mg/l (Day 14) and 8.36 mg/l (Day 21). The pattern of changes for treatment A and B was similar to each other, where reduction of nitrite occurred from the Day 7 to the Day 14 and increased again from the Day 14 to the Day 21. Overall, both of these samples were much lower than the effluent mean concentration on Day 0.

The nitrite mean concentration for the effluent control (tank C) were 21.02 mg/l (Day 7), 27.89 mg/l (Day 14), 28.2 mg/l (Day 21) and 20.31 mg/l (Day 7), 16.5 mg/l (Day 14), 38.72 mg/l (Day 21) for the influent control (tank D). After treatment, the mean concentration of nitrite for the effluent control and influent control was much higher than the initial day value.

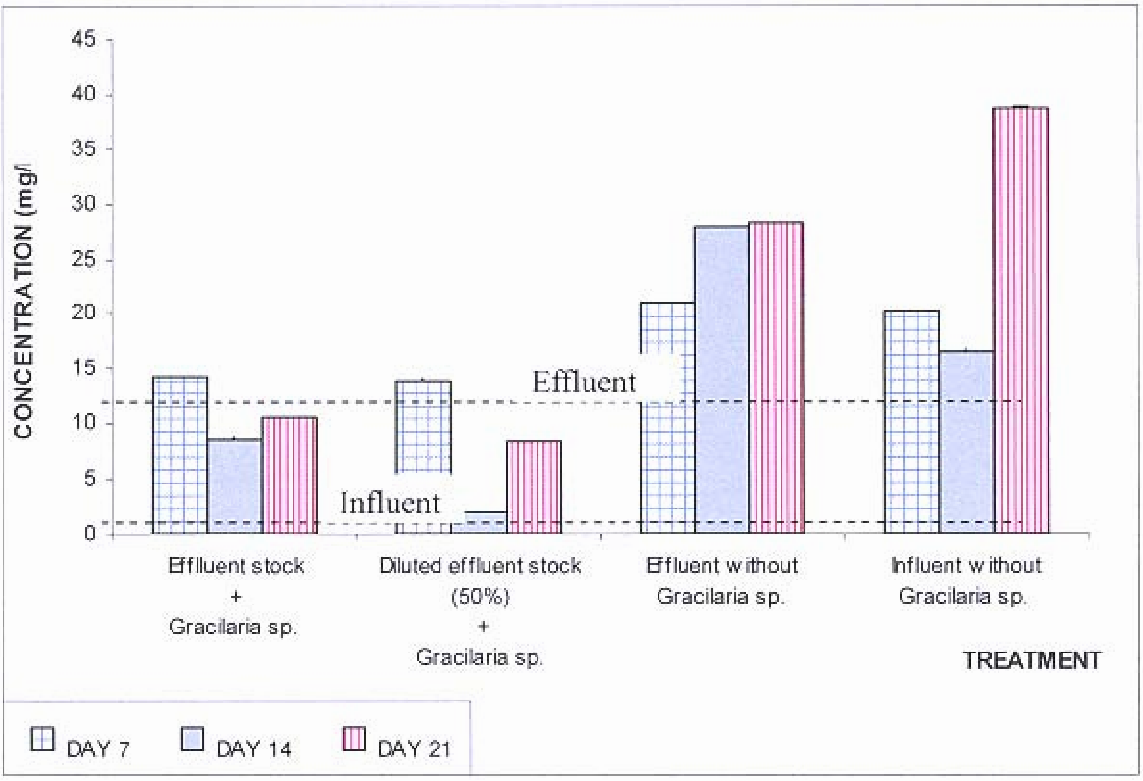

Figure 4: Nitrite mean concentration (mg/l) depending on specific treatment within three weeks. The dotted lines represent the concentration level of influent and effluent for Day 0.

4.1.4 Nitrate

The third nutrient parameter was nitrate (Figure 5). The histogram showed the changes of the nitrate mean concentration for the initial day (Day 0), Day 7, Day 14 and Day 21. The nitrate mean concentration for the initial day was 1.3 mg/l for the influent and 1.5 mg/l for the effluent water samples. Nitrate mean concentration for the treatment A within the three weeks study period were 7 667 mg/l (Day 7), 31 867 mg/l (Day 14) and 49 200 mg/l (Day 21). Besides, the mean concentrations for the tank B were 4 300 mg/l (Day 7), extremely increased to 51 000 mg/l on the Day 14 and decreased to 23 500 mg/l (Day 21).

For the effluent control (tank C) mean concentrations were 7 500 mg/l (Day 7), 40 533 mg/l (Day 14) and 38 133 mg/l (Day 21). Meanwhile, for the influent control (tank D), the mean concentration were 4 300 mg/l (Day 7), increased to 39 600 mg/l on the Day 14 and slightly decreased to 36 533 mg/l on the Day 21. Both of the influent and effluent control samples showed an increased pattern from the Day 7 to the Day 14 and a small portion decreased from the Day 14 to Day 21. However, the mean concentration for all of the treatments (A, B, C and D) were increased significantly within the three weeks experiment compared to the initial day mean concentration.