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TREATMENT OF AQUACULTURE WASTE WATER USING BIOFILM : A LABORATORY SCALE STUDY

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Bachelor of Science with Honours
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TREATMENT OF AQUACULTURE WASTE WATER USING BIOFILM: A LABORATORY SCALE STUDY.

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This project is submitted in partial fulfillment of
the requirements for the degree of Bachelor of Science with Honours
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Treatment of Aquaculture Waste Water by Using Biofilm: A Laboratory Scale Study.

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ABSTRACT

Biofilm is a community of microorganisms that accumulated on the surface of a substratum. The main objective of this research is to study the effectiveness of biofilm in reducing the nutrient content of effluent from shrimp pond. High load of nutrient and organic matters in waste water from shrimp pond culture is discharge directly to the sea and there is a need to treat this water in order to prevent pollution in the receiving waters. The effluent is taken from the Lembaga Kemajuan Ikan Malaysia (LKIM) tiger shrimp (*Penaeus monodon*) culture pond at Telaga Air, Sarawak.The nutrients were analyzed by HACH Kit (DR 2010). The final result of this study had show that the biofilm only can treat the silicate (SiO_2) waste in the shrimp pond waste water. Others nutrient such as ammonia nitrogen (NH_3-N), nitrite (NO_2^-), nitrate (NO_3^-) and orthophosphate (PO_4^{3-}) were not successfully treated by the biofilms. It is recommended to find a better methodology in laboratory and analytical method. The research of the microorganism species in the biofilm community to treat the aquaculture waste water should be done.

Key words: Biofilm, treatment, aquaculture waste water.

ABSTRAK

*Biofilem adalah suatu komuniti mikroorganisma yang melekat pada permukaan sesuatu subratum. Objektif utama peneyelidikan ini ialah untuk mengkaji keberkesanan biofilem dalam mengurangkan kandungan nutrien dalam effluen dari kolam udang. Kandungan nutrien dan bahan organik yang tinggi dalam air buangan kolam ternakan udang adalah dibuang secara terus ke dalam laut dan tindakan perlu diambil untuk merawat air ini dalam usaha mengelakkan pencemaran dalam air persekitaran. Air buangan adalah diambil dari kolam ternakan udang harimau (*Penaeus monodon*) Lembaga Kemajuan Ikan Malaysia (LKIM) di Telaga Air, Sarawak. Nutrien-nutrien adalah dianalisis menggunakan HACH kit (DR 2010). Keputusan akhir kajian ini telah menunjukkan bahawa biofilem hanya boleh merawat buangan silikat (SiO_2) di dalam air buangan kolam udang. Kandungan nutrient lain seperti ammonia nitrogen (NH_3-N), nitrit (NO_2^-), nitrat (NO_3^-) dan ortofosfat (PO_4^{3-}) tidak berjaya dirawat oleh biofilem. Adalah dicadangkan untuk mencari kaedah yang lebih baik dalam prosedur makmal dan analitikal. Penyelidikan perlu dilakukan terhadap spesies mikroorganisma di dalam komuniti biofilem untuk merawat air buangan akuakultur.*

Kata Kunci: Biofilem, rawatan, air buangan akuakultur.

1. Introduction

Aquaculture waste water or effluent is one of the critical water pollutants that affected the natural water body. The effluent water from aquaculture ponds contains elevated concentrations of dissolved nutrients and suspended particulates compare to the influent water. The shrimp culture is chosen for this study because it has a higher concentration of nutrients compare to any other type of aquaculture (Nyanti *et al.*, 2005).

There are several commonly defined water quality parameters which the aquaculturist should be familiar: pH and alkalinity, salinity and hardness, temperature, dissolved oxygen, and nutrients (including nitrogen, phosphorus, and silicon) (Landau, 1992). In this study, the chosen parameter is nutrient by focusing in ammonia-nitrogen, nitrate, nitrite, orthophosphate and silicate.

Waste water treatment in aquaculture is important to control the effluent pollution. The methods for waste water treatment can be physical, biological, chemical, or combinations of all these three. However, the biological treatment is the most environmental friendly and has a lower cost (Landau, 1992). Nowadays, the biological treatments are practiced mostly by using bivalve and macroalgae (Jones, 2000). The biofilm recently had been applied in the technological innovation. Some of the technological innovations of biofilm were in an anaerobic waste water treatment (Rittmann, 1992). The usage of biofilm in this study is to find out an alternative and a more effective method for aquaculture waste water treatment.

The problem statement for this study is that high load of nutrient and organic matters in waste water from shrimp pond culture is discharge directly to the sea and there is a need to treat this water in order to prevent pollution in receiving waters.

Therefore, the main objective for this study is to study the effectiveness of biofilm in reducing the nutrient content (ammonia-nitrogen, nitrate, nitrite, orthophosphate and silicate) of effluent water from tiger shrimp pond.

2. Literature Review

Aquaculture Waste Water

Effluent is discharged from an aquaculture pond into adjacent water body through routine water exchange, overflow after heavy rain and draining during harvesting. Pond effluent contain nutrients, organic matter and suspended solids that could result in eutrophication, sedimentation and other pollution problems in receiving waters. There are many methods used to reduce environmental impacts of effluents such as biological filters, hydroponics, irrigation, culture medium for other aquatic organisms, sedimentation ponds, fludized-bed filters and wetlands (Landau, 1992). The effluent give an impact to the environmental quality of adjacent water bodies by proportional to the discharge volume and nutrient concentration. The typical concentration of water quality variables in effluent from intensive shrimp ponds are salinity (10-35 ppt), temperature (22-31^{o}C), pH (7.5-9.0), total phosphorus (0.05-0.40mg/l), total nitrogen (0.5-5.0mg/l), total ammonia nitrogen (0.05-1.0mg/l), dissolved oxygen (4-12mg/l), biological oxygen demand (5-20mg/l), chlorophyll a (20-250mg/l) and total suspended solid (30-190mg/l) (Nyanti *et al.*, 2005).

The concentration of nutrients in waste water produced in aquaculture farms are different in the quality and quantity of the components depending on the species cultured and the culture practices applied. The main types of wastes in hatcheries or production farms are residual food and faecal matter, metabolic by product and residues of biocides (Pillay, 1992). In an intensive aquaculture farm, the leftovers of feed will reduce the water quality significantly. The composition of commercially-available feeds contains protein, carbohydrate and fat, and also some additives such as vitamins and pigments (Pillay, 1992). All of the feed components and the by-products of metabolism could become a waste product containing organic carbon and organic nitrogen, ammonium, urea bicarbonate, phosphate, vitamins, therapeutants and pigments. However, the most nuisance component of waste food and faeces are organic carbon and nitrogen compounds which settle down into the sediment. The production of faecal and excretory wastes obviously depends on the stocking density in farms (Pillay, 1992). Ammonium in effluents exceed 100 times the quantity supplied in the feed, which supports the view that ammonium is mainly an excretion product (Pillay, 1992).

Biofilm

Biofilm is a surface with accumulation of microorganisms such as the bacteria, microalgae and the benthic diatoms, frequently characterized by large amounts of organic polymers of microbial origin that bind cells and other organic and inorganic materials together onto the substratum (Characklis, 1989). Biofilms form on available surfaces in virtually all aquatic ecosystems that can support microbial growth (Lappin-Scott, *et al.*, 1993). In the past, biofilm is less studied compared to other suspended growth of planktonic microbial systems. However, recently researchers have become conscious of the almost universal association of microorganisms with surfaces and with each other. Biofilm has a function in natural and also in technology. The biofilm can be found almost at any surface exposed to water irrespective of the prevailing trophic state. Biofilms accumulating on a river bed or on suspended particles in rivers, lakes, and in the marine environment are often considered advantageous since they contribute largely to the removal of contaminants from water (Characklis, 1989). In waste water or sewage treatment, biofilms filter and remove organic and inorganic pollutants.

The biofilm properties can be determined as physically, chemically and biologically. Physical properties of biofilm was mainly about the attachment of the microbial community on the substratum; chemical properties include the biochemistry process that involve in the biofilm metabolism while biological properties of biofilm were including the biofilm itself and the biofilm system. All the properties above are dependent on particular environment in which the biofilm accumulates. The environmental factor of abiotic and biotic does count in the biofilm properties. Factors of importance are the organisms themselves, their presence and activity, and the interactions between the various populations forming the biofilm community are also an important factor. It is the combination of the environmental factors which determines the properties and functions of the biofilm system (Characklis, 1989).

Biological Treatment

Biological treatment in waste water mostly will focus in the aspect of biological filter. The biological treatment is filtration that involves the active microbial growth in contact with waste water so that they can consume the impurities as food. Bacteria, benthic diatom and microscopic green algae of the biofilm community used nutrients for their growth. Biofilters is being used to maintain water quality in recirculating or closed loop systems (Landau, 1992). Biofilters are also used to improve water quality before water is discharged from a facility. There are many different methods for maintaining good water quality and biofiltration is a very important and essential component especially for recirculating aquaculture system.

Nitrification

In the natural environment, the dead organic material is broken to ammonium by the bacteria. Ammonium and other forms of ammonium-nitrogen in the aquaculture originated from the decomposition of the urea, shrimp faeces and uneaten food pellets. The ammonia-nitrogen (NH_3-N) have a higher toxicity level compared to other two forms that are the ammonium (NH_4^+) and ammonia (NH_3). In anoxic environments, most of the ammonium is reassimilated by bacteria and plants. While in aerobic environments where oxygen is present, the ammonium is oxidized by nitrifying bacteria to nitrate (Blackburn, 1983). Nitrification is one of the process occurs in the nitrogen cycles. The bacteria which perform this oxidation are usually autotrophic lithotrophs, but heterotrophic nitrification can also occur, resulting in transient accumulation. Nitrification is important in preventing the persistence or accumulation of high ammonia-nitrogen levels in the effluent. The nitrification has a reaction that convert the ammonia-nitrogen turned into nitrite and lastly into nitrate. The overall reaction for nitrification is:

$$NH_4^+ + SO_2 \rightarrow 2H^+ + NO_3^- + H_2O$$

The nitrification occurs in two steps, involving primarily two bacterial genera, the *Nitrosomonas* which forms nitrite as an intermediate and *Nitrobacter* which produced nitrate as the final product (Bababunmi *et al.*, 1986). The first step is an oxidation to nitrite (NO_2^-) by *Nitrosomonas* :

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

The nitrite is then oxidized in the second step to nitrate by *Nitrobacter*:

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

The rate of nitrate production was proportional to the number of nitrifying bacteria, since for many ammonium-oxidizing bacteria, the maximum activity per cell is relatively constant

(Blackburn, 1983). The process of nitrification also depends on many factors, including the amount of dissolved oxygen, temperature, pH, the microbial population, and the nitrogen forms present. Nitrification is also an oxygen-consuming process which may lead to a depletion of dissolved oxygen and acidification. As a result the microbial activity may be inhibited.

The Microbial Silica Cycle

Silicon is biologically transferred and altered in the silica cycle and it influences microbial systems including the biofilms as well as plants and animals (Krumbein and Werner, 1983). Silicon forms tetrahedral configurations similar to phosphorus and double bonds similar to carbon are possible though perhaps under different temperature and pressure regimes (Krumbein and Werner, 1983). The similarity of the silicon to the carbon in its capacities and potential, and also that it is one of the least reactive elements in terms of its compounds presently important for geochemical cycles. Quartz which is the dioxide of silicon, SiO_2, is one of the most stable compounds on earth. The important factor that involved both biological and physico-chemical parameters of the silica cycle is the solubility of silicon, silica and silicates. In contrast to many other chemical compounds of the earth's crust below a pH 9, silicon is present in solution only as silicic acid in the monomeric stage (H_4SiO_4). At pH above 9, it dissociates to $H_3SiO_4^-$ and $H_2SiO_4^{2-}$. Since the pH of most natural environments is below pH 9, most of the reactions involving silicon are ruled by the chemistry of silicic acid. The physico-chemical solubility of silica is as followed reaction:

$$SiO_2\text{ (amorph)} + 2\,H_2O \leftrightarrow H_4SiO_4\text{ (aqueous)}$$

All steps and stages of low-temperature reactions of silicon in nature are controlled by biological and mainly microbial pathways and interactions. Diatoms (Bacillariophyta) contribute about 20 to 25 % of biological world (land and ocean) primary production. In the most productive areas of the oceans, diatoms are the most important organisms in the aquatic silica cycle because they use silicate in the formation of their cell walls, the frustules (Krumbein and Werner, 1983).

Phosphorus Cycle

Phosphorus is an important content in protoplasm that involve in this cycle where the organic matter is decompose into phosphate which is once again used by the plants. However the largest reserve pool for phosphorus is not in the atmosphere but in rock. The phosphorus enters into the aquatic ecosystem through erosion of phosphate rock on land and through waste water input (Welch, 1980). Phosphorus is initially utilized through plant and microbial uptake of dissolve inorganic phosphorus that involves with the process of photosynthesis, chemosynthesis and decomposition (Welch, 1980). Therefore, plants and phytoplankton is the main organisms that have stimulated growth with the supply of phosphorus. However, phosphorus is often the limiting nutrient in aquatic ecosystems. The growth of algae and other aquatic microorganism will be quickly stimulated with the presence of phosphorus (Walker, 2005).

3. Materials and Methods

3.1 Sources of Effluent Water

The shrimp culture effluent was taken from the Lembaga Kemajuan Ikan Malaysia (LKIM) tiger shrimp (*Penaeus monodon*) culture pond at Telaga Air, Kuching, Sarawak (Figure 1).

3.2 Formation of Biofilm

The formation of biofilm was done in an aerated tank containing 500 litres of seawater (Figure 2). The waving PVC plates (8cm x 22cm with total surface area of 560 cm^2) were used as a substratum and immersed inside the tank. Prior to the immersion, 12 pieces of plates were arranged into a basket (Figure 3). The seawater in the tank was changed once a week. After two weeks, the biofilm plates were collected and used in the waste water treatment experiment in UNIMAS laboratory.

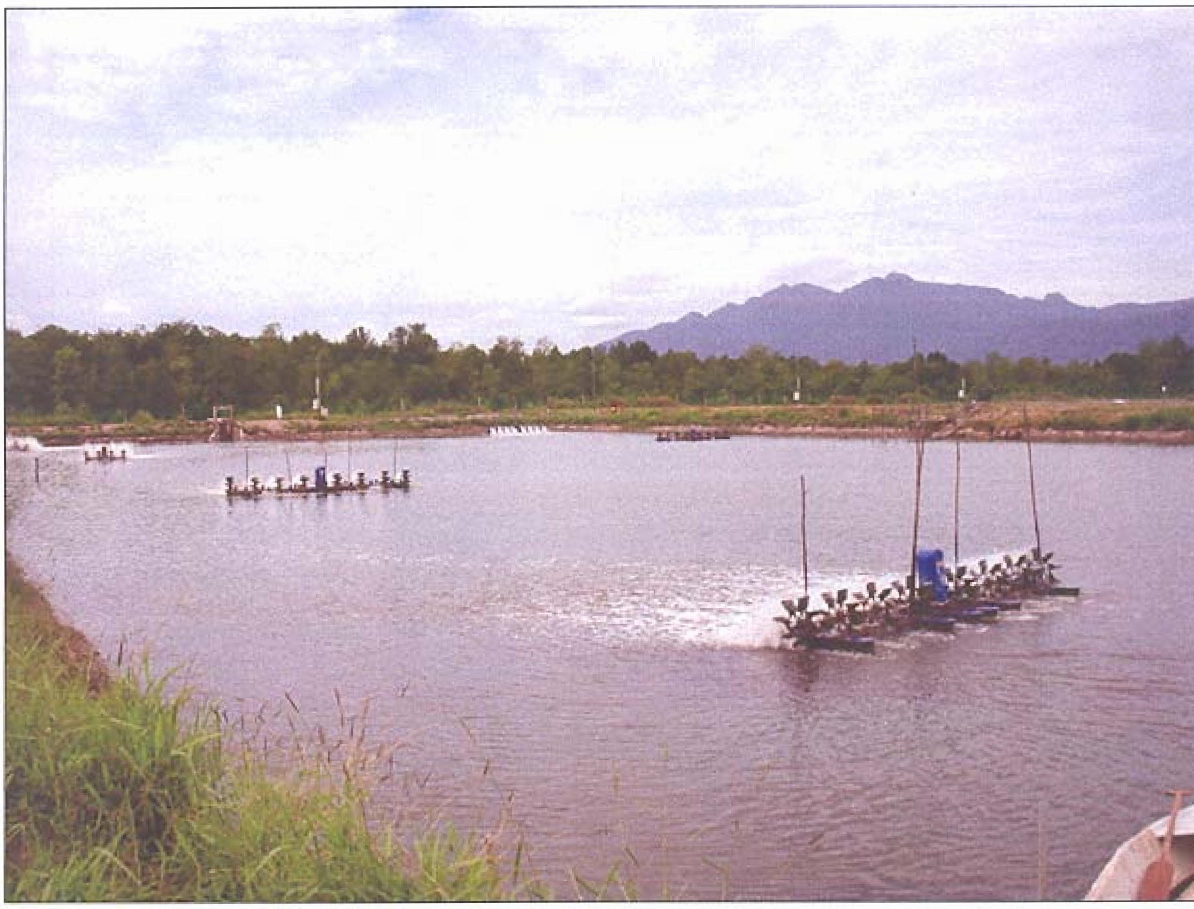

Figure 1: Tiger Shrimp pond in the LKIM.

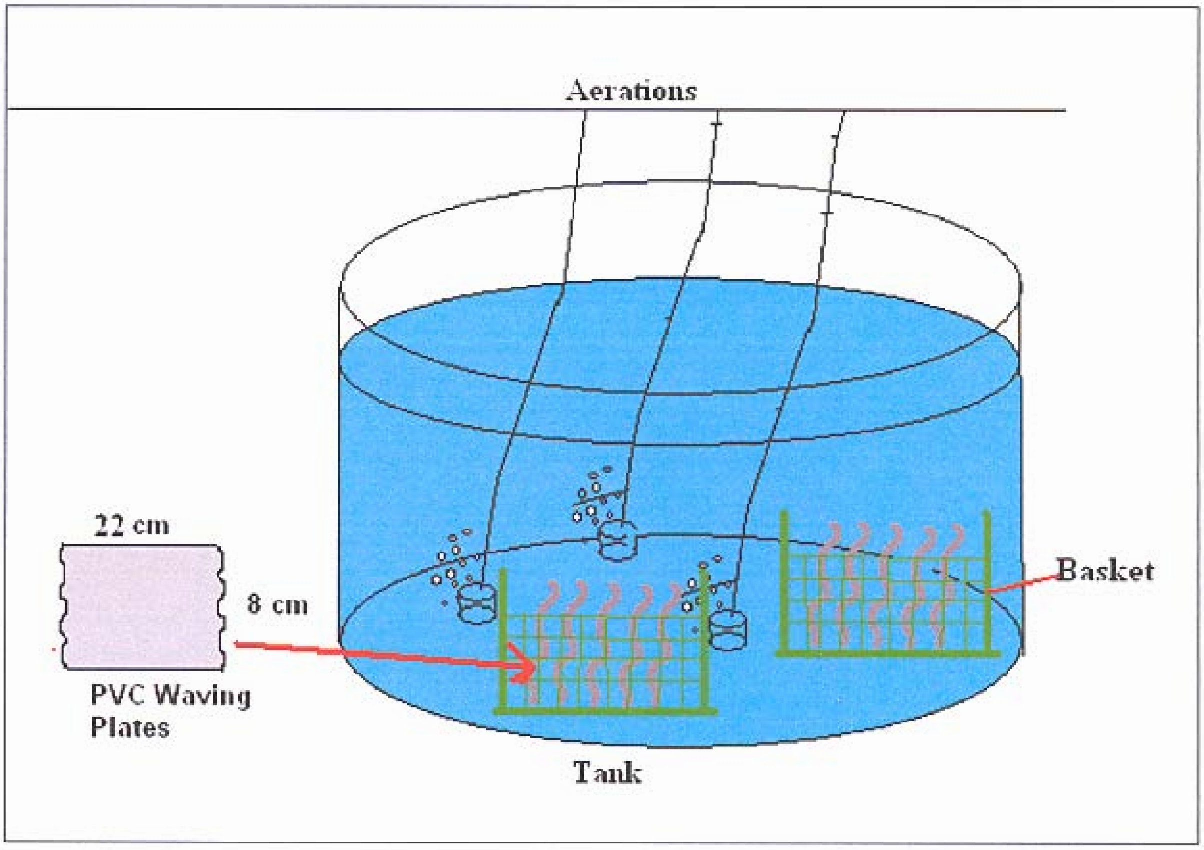

Figure 2: Formation of biofilm

(a) Biofilm plates before immersion

(b) Biofilm plates after 1 week of immersion

(c) Biofilm plates after 2 weeks of immersion and used for the experiment

Figure 3: Formation of biofilm before immersion, week 1 and week 2

3.3 Waste Water Treatment by Biofilm

Two hundred liters of tiger shrimp pond effluent and one hundred liters of influent were obtained from the Lembaga Kemajuan Ikan Malaysia (LKIM) in Telaga Air, Kuching, Sarawak. The initial (Day 0) nutrient (ammonia-nitrogen, nitrate, nitrite, orthophosphate and silicate) contents of influent and effluent stock were measured and recorded.

This experiment was carried out in glass aquariums (44 cm x 29 cm x 30 cm) and consisted of four different treatments (Figure 4):

A – Effluent stock with biofilm plates

B – 50% diluted effluent stock with biofilm plates

C – Effluent stock without biofilm plates (positive control)

D – Influent without biofilm plates (negative control)

Twenty liters of effluent stock, 50% diluted effluent or influent were poured into each aquarium according to the treatment, respectively. Then, 5 pieces of biofilm plates were placed inside each treatment A and B. All experiments were done in 3 replicates. This experiment was run for 3 weeks and 150 ml of water samples were taken from each aquarium 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 the room temperature. In this experiment, the value of all nutrient concentration were determined and used to compare the effectiveness of biofilm in waste water treatment.

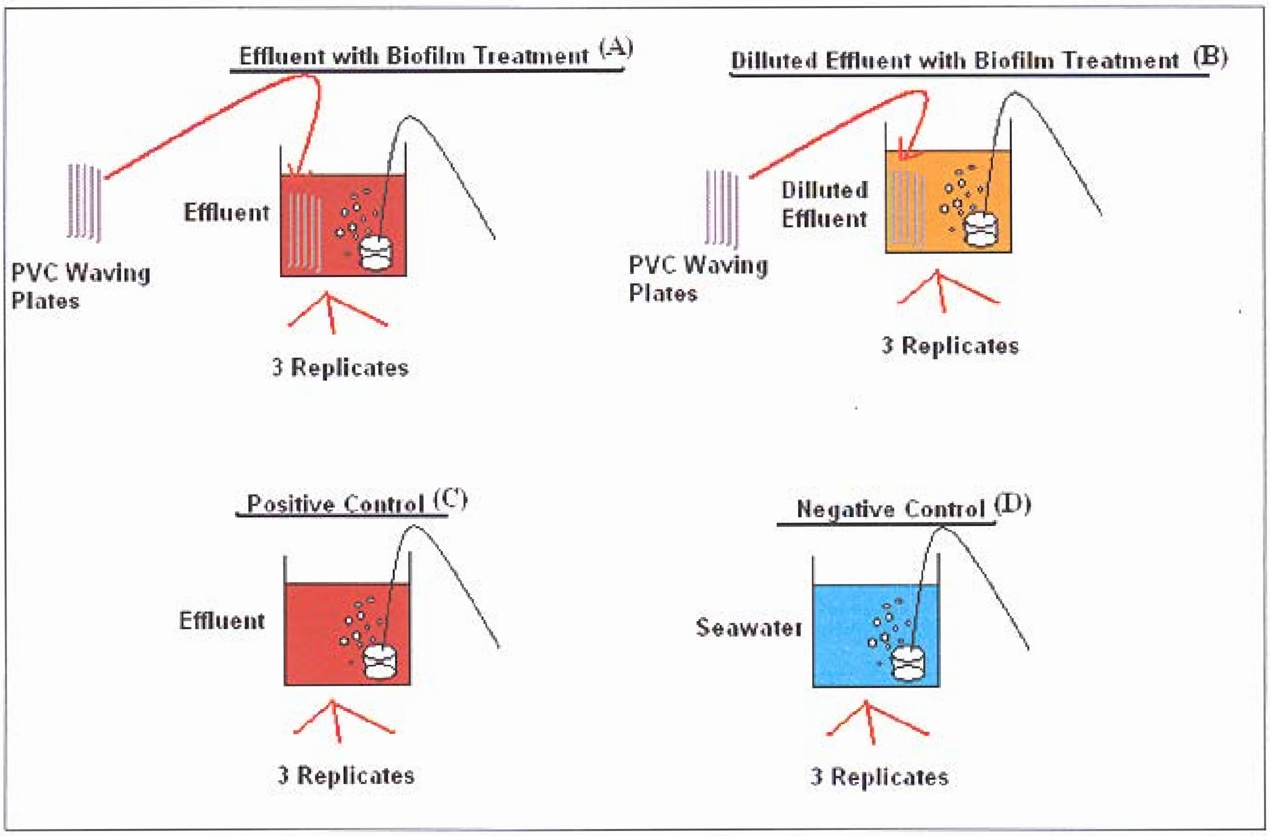

Figure 4: Waste Water Treatment by Biofilm in the Laboratory.

3.4 Nutrient Analysis

The content of nutrients was analyzed using HACH Kit (DR 2010) and 3 readings were taken for each sample. Ammonia nitrogen (NH_3-N) was analyzed following the Standard Method 8038 based on Nessler method. Nitrate (NO_3^-) was carried out using Standard Method 8192 based on cadmium reduction method, while nitrite (NO_2^-) using Standard Method 8507 based on diazotizaleon. The determination of reactive phosphorus (PO_4^{3-}) was carried out using Standard Method 8048 based on Powder Pillow Method and the silicate (SiO_2) was analyzed following the Standard Method 8186 based on heteropoly blue method.

The over range readings from the spectrometer HACH kit were overcome with the dilution of the water sample. The dilution ratio ranged from 1: 10 to 1: 10,000 according to the degree of over range reading in analysis of ammonia-nitrogen, nitrite, nitrate, orthophosphate and silicate. The formula used to calculate the actual value is:

Data after dilution / (dilution ratio)

The mean of the replicates for each of the 4 treatments (A: effluent stock with biofilm, B: diluted effluent stock with biofilm, C: effluent without biofilm and D: influent without biofilm) is calculated and recorded.

3.5 Statistical Analysis

The content of nutrient in the different concentration treated effluent on the particular week was analyzed. The data was statistically analyzed by using one-way analysis of variance (one-way ANOVA) at a level of significance of 0.05 with the SPSS software. The one-way ANOVA was used because the samples that compared under the hypothesis have all come from the same population. The underlying theory of one-way ANOVA is to compare the variation between the treatments (the samples) with the variation within the treatments. If the differences observed are significant, means were compared by the multiple ranges Tukey's test.

4. Results

4.1 Water Quality

The water quality parameter in shrimp pond and lab was shown in Table 1. The lab have a higher parameter reading in pH, salinity and dissolved oxygen and the pond have a higher parameter reading only in temperature.

Table 1: Water Quality in Shrimp Pond and Lab.

Site	Parameter			
	pH	Salinity (‰)	Dissolved Oxygen (mg/l)	Temperature(°C)
Pond	7.07	18	3.07	28.53
Lab	8.7	20	7	22

4.2 Initial (Day 0) Concentration of Influent and Effluent

The mean concentration of nutrients in influent and effluent prior to the experiment (Day 0) was shown in Table 2. The nutrients that were monotored in this study were ammonia-nitrogen (NH_3-N), nitrite (NO_2^-), nitrate (NO_3^-), orthophosphate (PO_4^{3-}) and silicate (SiO_2). In general, nutrient concentration is higher in effluent compare to the influent. In effluent, nitrite showed the highest concentration among the nutrients before the experiment.

Table 2: Mean Reading of Nutrients Concentration Data on the Initial Day (Day 0).

Treatment	Nutrient(mg/l)				
	Ammonia-nitrogen (NH_3-N)	Nitrite (NO_2^-)	Nitrate (NO_3^-)	Orthophosphate (PO_4^{3-})	Silicate (SiO_2)
Influent	0.5 ± 0.05	1.01 ± 0.01	1.3 ± 0.05	0.4 ± 0.05	0.7 ± 0.05
Effluent	1.5 ± 0.05	13.5 ± 0.05	1.5 ± 0.05	1.4 ± 0.05	0.6 ± 0.05

4.3 Nutrient Concentration After Treatment

The main comparison in this experiment was between treatment A (Effluent stock with biofilm plates) and C (Effluent stock without biofilm plates). The effect of dilution (Treatment B) was also compared with undiluted effluent (Treatment A). The overall observation shows that the biofilm treatment had reduced constantly the nutrient of orthophosphate and silicate. On the final day of the experiment (Day 21), the biofilm had reduced the orthophosphate from 1.8 mg/l in Aquarium C (untreated effluent) to 0.8 mg/l in Aquarium A (biofilm treated effluent) and silicate from 496 mg/l in Aquarium C to 425 mg/l in Aquarium A.

Ammonia Nitrogen

Figure 5 showed the mean concentration of ammonia nitrogen for 3 weeks for each treatment respectively. The initial day (Day 0) concentration of ammonia-nitrogen in influent was represented by dotted lines while effluent by straight lines. The concentrations of ammonia nitrogen were reducing constantly in all of the experiment. After one week the concentration of ammonia-nitrogen was reduced in all the treatments with a lower concentration than the effluent. In the first week, the concentration was higher in the Treatment C (untreated effluent) at 1.0 mg/l compared to Treatment A (treated effluent) at 0.8 mg/l. But the nutrient concentration was increased on the second week in Treatment A at 1.2 mg/l compared to Treatment C at 1.0 mg/l. On the third week, Treatment A and C was equal and lower than Day 0 influent. The ammonia nitrogen in treated effluent of Treatment A was reduced from 1.5 mg/l to 0.8 mg/l during the first week but increased to 1.2 mg/l for the second week before it decreased again to 0.2 mg/l in the third week. Treatment C (untreated effluent) shows that ammonia nitrogen was reduced from 1.5 mg/l to 1.0 mg/l during the first week but remain unchanged until the second week before it decreased rapidly to 0.2 mg/l in the third week. The effect of dilution show that the concentration was lower in the first week but no differences after 2 weeks before become higher in the third week. Treatment D which contained influent showed that concentration of ammonia-nitrogen was stagnant to 0.2 mg/l after the first week until the end of the experiment. From the ANOVA, there was a significance different for the ammonia-nitrogen treatment (P = 0.0001). The Tukey Test showed that the effluent initial day had a significant different with all the treatment. The concentration between Treatment A and Treatment C was reduced nearly the same in Day 21.