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# The Reuse of Shrimp Culture Wastewater Treated by Nitrification and Denitrification Processes

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*Abstract*—**In spite of wastewater from shrimp culture production contains high amount of ammonia nitrogen that is very toxic to shrimps, it will be available to reuse due to its good characteristic in terms of optimum salinity. To reuse this wastewater, ammonia nitrogen should be removed to the lower level than the toxic level. Also, the test for the effect of shrimp performance from wastewater reuse should be investigated. To succeed in this goal, a laboratory scale study was conducted to determine ammonia nitrogen removal by using aerobic and anoxic recirculation systems and the effects of recirculation water to growth and survival rate of white shrimp PL (*L. vannamei*). The experiments used to evaluate efficiency of aerobic and anoxic treatment were conducted at shrimp farm located at Chachoengsao Province, Thailand. Three treatment groups including fresh sea water (1), recycled without treatment (2) and recycled with treatment (3) were performed. The shrimp production wastewater with $NH_3$-N of 13.14±3.54 mg/L was passed through the designed reactors sizing of 6 L. The result showed that nitrifying bacteria converted approximately 98% $NH_3$-N to be $NO_3^-$-N. For group (1) and (2) operated without bacteria, it was found that only 5% and 25% was converted to $NO_3^-$-N. Also, $NO_3^-$-N was converted to be $N_2$ during denitrification while TN was approximately removed by 5% and 84% for group (2) and (3), respectively. The rate of $N_2$ production in group (3) was about 68 ml/m$^3$/day. The COD:N ratio for the anoxic period was 5.6 g of COD/g of $NO_3^-$-N. The wastewater recirculation affected the survival and growth rate of shrimp slightly. The growth and survival rate of shrimp in the group (2) showed a significant lower at the end of the 3rd cycle of recirculation water rearing than in the others ($p<0.05$). The growth and survival rates for group (1), (2) and (3) were 47% and 62%, 24% and 26% and 41% - 53%, respectively. It was indicated that treated wastewater by nitrification and denitrification recirculation systems had high potentially applied for shrimp aquaculture in closed system.**

*Index Terms*—**Shrimp Culture Wastewater, Nitrogen Removal, Nitrification, Denitrification.**

## I. Introduction

Water is the most important factor for the aquaculture process and the requirement continuously increases as shrimp farming is expanded. Over 350 shrimp nurseries exist in Thailand to produce [5] more than 50,000 million PL per year [7]. Lack of water resources leads to high costs of water and also in the current increase in environmental awareness, a high efficiency treatment system is needed to meet the consequent stringency in environmental legislation. A new approach to dealing with the ecological problems associated with aquaculture has been developed called recirculating aquaculture system (RAS). RAS confers ecological and economic advantages in that it facilities a reduction in the amounts of water and energy required [14].

However, water recirculation without ammonia removal will affect shrimp larvae performance. As aquatic animals exposed to ammonia with low concentration, it may cause osmoregulatory disturbances, blood acidosis and reduced respiratory efficiency. Furthermore, ammonia causes growth reduction and greater susceptibility to infectious diseases. A few studies that have been conducted on this problem have shown that there are a number of issues that should be addressed. Reference [17] reported that nitrification and denitrification biological process can remove almost 100% of ammonia. And others showed that it is possible to decrease the amount of water used in the system simply by providing the most basic biofilter. Reference [4] tested a commercially available nitrifying bacteria product to convert toxic ammonia and nitrite to less toxic nitrate and remain within safe limits for the entire Juvenile Pacific white shrimp (*L. vannamei*) culture period.

Although nitrification and denitrification are normally used for nitrogen removal in many fields as mention above, the literature review of ammonia removal in post larvae shrimp culture wastewater is rare. Also, rearing post larvae in recycled water is not well-known about how it affects the shrimp and whether it is worth the expense.

To solve some problems described above, a system consists of nitrification and denitrification reactor was designed. Ammonia is converted to nitrate by nitrification and nitrate is converted to nitrogen gas by denitrification [3]. The present study focuses on whether recirculation systems affecting nitrification and denitrification resulting in ammonia nitrogen removal from post larvae rearing water and how recirculation system affect the growth rate and survival rate of *L. vannamei* post larvae.

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## II. Materials and Methods

### A. Material Preparation

Preparation of shrimp: Nauplii Pacific white shrimp (Litopeneaus vannamei) were obtained from Charoen Pokphand Shrimp Hatchery (Chachoengsao, Thailand) and stocked in the recirculation system for seven days prior to experiments. Post larvae shrimps (PL1) were randomly distributed to the tanks in order to ensure a uniform size distribution across the treatment groups. Shrimp were kept under a 12 hour light : 12 hour dark photoperiod and fed with brine shrimp larvae and special shrimp powder feed (TNT 300).

Preparation of support media*:* Plastic tubes which was found to be the most effective media. The floating filter media was 0.5 cm diameter and 1.0 cm length. They contain a 750 m2/m3 specific area which calculated based on their external surface area. The packing density was 20.25 kg/m3 and 60 g. The characteristics of the floating filter media that provided by supplier were given in Table 9. These tubes made from high density polyethylene (HDPE) by KSP company that is incorporated with Charoen Phokphand Food Public Co.,Ltd.

Nitrifying biofilter*:* Synthetic wastewater prepared in 100 L tank from ammonium chloride (NH4Cl) at concentration of 100 mg-N/L was used in the experiment and sodium hydrogen carbonate (NaHCO3) was added in to increase the total alkalinity to 300 mg CaCO3/L. Then plastic media was added at a ratio of 1:3 (v/v) media to water. The optimum activity of microorganism fixed on the support media biofilter would be observed until the concentration of ammonia and nitrite were less than 1 mg-N/L and nitrate was not less than 10 mg-N/L.

Denitrifying biofilter: Synthetic wastewater prepared in 100 L tank from potassium nitrate (KNO3) at concentration of 100 mg-N/L was used in the experiment and 100 g sucrose was added to eliminate dissolved oxygen concentration to less than 0.5 mg O2/L. Then, plastic media were added in ratio of media to water 1:5 (v/v) and a complete mix inside the denitrification tank was achieved with a propeller at a speed of 100 rpm. The optimum activity of microorganism fixed on the biofilter would be observed until the concentration of nitrate was less than 1 mg-N/L.

Aerobic/Nirification Reactor: Laboratory scale up flow aerobic floating filter media reactor was used. The reactors were made of acrylic plastic with diameter and height of 0.10 m and 1 m, respectively. Total reactor volume was approximately 7.85 liters. The upper part of the reactor was filled with floating filter media of 0.30 m depth; Nitrifying biofilter for reactors used in treatment groups and sterilized filter media for positive and negative control group. Aquatic pumps were used to continuously feed wastewater from nursing tanks into all reactors and bypass valves were used to control their feeding rate. The influent was fed at the bottom and moved upward to the floating filter in reactors. The treated effluent was collected in nursing tank.

Anoxic/Denitrification Reactor: Laboratory scale up flow anoxic floating filter media reactor was used. The reactors were made of plastic boxes (25x55x75 cm). Total reactor volume was approximately 103.13 liters. The biofilter were placed in for 0.5 L; Denitrifying biofilter for reactors used in treatment groups and sterilized filter media for positive and negative control groups. Propellers were used to completely mix in the reactors by mixing rate at 100-150 rpm. The gas emission collector was connected at the top of the reactor.

Preparation of Gas Collector: Produced nitrogen gas was collected as soon as it released from the treated water. The gas collector consisted of chambers made of plastic. The gas chamber had a dimension of 7.0x7.4x7.4 cm. The chamber was installed inside a water bath which had a size of 18x18x18 cm. The gas collector worked on the water displacement principle.

### B. Experimental System Operation

#### 1) Nitrification and Denitrification Efficiency Test

The experimental set-up included three groups of three replicates each. They are groups of new sea water used in rearing, rearing without and with nitrification and denitrification treatment which were assigned as positive control (Group 1), negative control (Group 2) and treatment (Group 3), respectively. They were placed an aquaculture greenhouse at Chachoengsao nursery of Charoen Pokphand Food public company limited, Thailand.

Each system comprised of an aquaculture tank and a water treatment facility system. A small aquarium pump fed the water from the aquaculture tank to the nitrification reactor, and the outlet water flowed to the culture tank. Each aquaculture tank was filled with 90 L of sea water (25-30 ppt) and was maintained at 30±2 ºC. The aquaculture tank were stocked with *L. vannamei* post larvae (PL1) at a density of 150 pcs/L. Dry and living feed was supplied 6 times a day at a rate of approximately 3-6 g/million PL per day. All groups operated without water exchange except group 1 in which 50% of the water was exchanged every day. Air was supplied by perforate pipes at a flow rate of 2.5 L/min to sustain the dissolved oxygen value in optimum range (5.5-6.5 mg $O_2$/L). Shrimp were harvested at day 14 and wastewater was transferred to the denitrification reactor and treated for 14 days.

#### 2) Performance of Shrimp Rearing in Recycled Water Test

The experimental set-up included three groups of three replicates each. They are groups of new sea water used in rearing, 100% recycled untreated and 100% recycled treated water with nitrifying and denitrifying bacteria treatment which were assigned as positive control (Group 1), negative control (Group 2) and treatment (Group 3), respectively. They were placed an aquaculture greenhouse at Chachoengsao nursery of Charoen Pokphand Food public company limited, Thailand.

Each system comprised of an aquaculture tank and a water treatment facility system. A small aquarium pump fed the water from the aquaculture tank to the nitrification reactor, and the outlet water flowed to the culture tank. Each aquaculture tank was filled with 90 L of sea water (25-30 ppt) and was maintained at 30±2 ºC. The aquaculture tank were stocked with *L. vannamei* post larvae (PL1) at a density of 150 pcs/L. Dry and living feed was supplied six times a day at a rate of approximately 3-6 g/million PL per day. Air was supplied by perforate pipes at flow rate of 2.5 L/min to sustain the dissolved oxygen value in optimum range (5.5-6.5

mg $O_2$/L). Shrimp were harvested in day 14 and wastewater were transferred to the denitrification reactor and treated for 14 days. Then treated water were filtered using 1 micron filter bags before used for post larvae stocking and water exchange in the next cycle. The treated water was used for three times and compared with group 1 which used new water to stock post larvae and carry the 50% water exchange rate every day.

### C. Sampling and Laboratory Analysis

The monitoring of system performance started from the beginning of the experiment and sampling twice a week. Most of the analyses in this study were carried out according to the methods described in Standard Method for Examination of Water and Wastewater 20th Edition [2].

Samples were filtered through 0.45 µm glass-fibre filter (GF/C) for the determination of $NO_2^-$ and $NO_3^-$. Alkalinity was measured by the titrimetric method as per Standard Methods [2]. $NO_2^-$-N was measured by the colorimetric method as per Standard Methods [2] as well as was measured by the G10VIS spectrophotometer at 543 nm. $NO_3^-$-N was measured by the colorimetric method as per Standard Methods [2] as well as was measured by the G10VIS spectrophotometer at 410 nm.

In addition, shrimp were sampled to monitor the total length (TL), % total length (≥ 8.00 mm), %CV and survival rate every three days until harvest (14 days of experiment). Total length of shrimp was the average length of 50 post larvae. Survival rates were checked by sampling shrimp in the tank with a 500 ml plastic beaker at three points and calculating the total amount of shrimp compared with the initial amount.

## III. Results and Discusstion

### A. Nitrogen Removal

#### 1) Nitrifying Biofilter

The nitrifying biofilter was conducted by adding 100 mg-N/L $NH_4Cl$ into 100 L of 25-30 ppt sea water and the alkalinity value was adjusted to be 300 mg $CaCO_3$/L. Plastic media were added to the reactor at ratio of 1:3 (v/v) and the air was continuously added with the rate of 2.5 L/min. The result showed that total ammonia nitrogen (TAN) gradually reduced after five days of the experiment until it was nearly zero after 15 days. In addition, alkalinity rapidly reduced because of ammonia oxidation produced hydrogen ions that destroyed buffer capacity at the rate of 100 g $CaCO_3$ per 14 g-N or 7.14 mg$CaCO_3$/L per 1 mg-N/L [16]. Nitrite nitrogen continuously increased in the first period until day 10 of the experiment it decreased to nearly zero after 20 days. This was in agreement with the report by Reference [11] who explained that biomass production in the nitritification process was higher than nitratification therefore in the first period of nitrification system nitrite accumulates at a high level until nitrobacter performed adequate amount the nitrite concentration would be eliminated and became to equilibrium.

After 10 days the nitrate nitrogen was gradually produced and continuously increased through the end of the preparation period. In contrast, nitrite nitrogen was rarely found after 20 days. This is due to the nitratification rate was higher than nitritification rate therefore the concentration of nitrite nitrogen in the aeration tank left was found less than 0.1 mg-N/L [15].

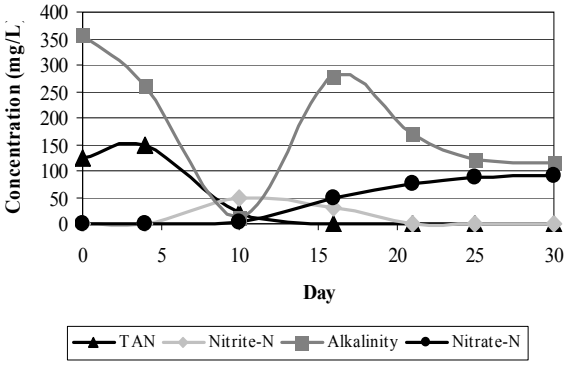


Figure 1. TAN, Nitrite nitrogen, Nitrate nitrogen and alkalinity concentration for the entire of the nitrification starter period.

The pH on day 10 (6.57) was lower than typically optimum values (7.0-8.4) therefore it might cause nitrification reduction of approximately 50% [15]. As in the first period ammonia oxidation generated high amounts of hydrogen ions that rapidly neutralized basic condition in water therefore sodium hydrogen carbonate and lime were used to rise up buffer capacity and pH values in the late period of preparation.

Although sodium chloride affected nitrifying bacteria it could moderately acclimatize and grow in high salinity water. Reference [11] and [12] reported that total nitrogen removal efficiency of nitrifying bacteria reduced just 20% after adjusting the sodium chloride concentration from 0 to 30,000 mg/L. This group of bacteria could perform well if it was acclimatized in saline water for a while. In the next section of the experiment the nitrification processed in 25-30 ppt wastewater from shrimp culture therefore the salinity of the water in the preparation tank was 24-27 ppt to induce the efficiency of bacteria.

It indicated that nitrifying bacteria preparation was conducted by adding 100 mg-N/L $NH_4Cl$ into 100 L of 25-30 ppt sea water and maintained the alkalinity value at 300 mg $CaCO_3$/L. Plastic media were added at ratio of 1:3 (v/v) and controlled the other parameters in optimum range; pH (7.0-8.4), temperature (30-32 ºC) and dissolved oxygen (>2 mg$O_2$/L). The nitrifying biofilter media was ready to use within 30 days.

#### 2) Denitrifying Biofilter

The preparation of the denitrifying biofilter was conducted by adding 100 mg-N/L $KNO_3$ into 100 L of 25-30 ppt sea water. Sucrose was added for 100 g and plastic media were added at ratio of 1:5 (v/v). The result showed that nitrate nitrogen gradually reduced until it was nearly zero after 30 days. Afterwards, nitrogen gas gradually occurred and alkalinity continuously increased at the rate of 4.28 g $CaCO_3$/g $NO_3$-N. It supported the theory explained that typically increasing of alkalinity in the denitrification system was approximately 3.57g $CaCO_3$/g$NO_3$-N that was reduced [10].

Total ammonia nitrogen and nitrite nitrogen were constant at nearly zero during the entire experiment. It showed that the

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reaction was denitrification dissimilation as nitrate nitrogen totally converted to nitrogen gas and total ammonia nitrogen and nitrite nitrogen did not generate [11].

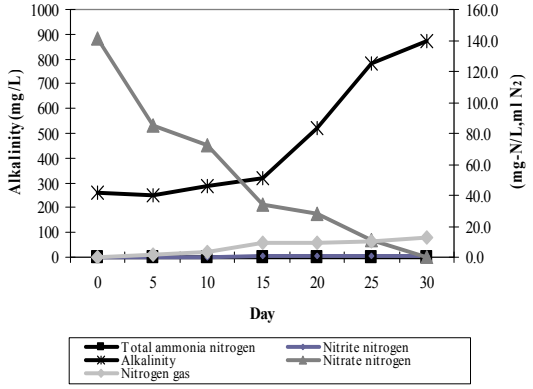


Figure 2. Nitrogen compounds and alkalinity concentration for the entire of the denitrification starter period.

In this study pH was controlled in a range of 7.03-8.40 agreement with typically values [8]. Optimum pH value for denitrification process was 7-9. On the first day of preparation, dissolved oxygen in water was 5.03 mg $O_2$/L above the optimum value (0.3-0.8 mg$O_2$/L). However, sucrose added to increase BOD and COD concentration could eliminate oxygen in the experimental tank to nearly zero.

Denitrifying bacteria could moderately acclimatize and grow in high salinity water. Reference [12] and [13] reported that denitrification efficiency of dnitrifying bacteria rapidly reduced when sodium chloride concentrations were changed. Thus, it could offer good performance if it was acclimatized in saline water for a while. In the next section of the experiment the denitrification processed in 25-30 ppt wastewater from shrimp culture therefore the salinity of water in preparation tank was 24-27 ppt to induce the efficiency of bacteria.

Temperatures in this study was controlled in range of 28-30 ºC for high denitrification efficiency. According to optimum temperature and dissolved oxygen for denitrification should be more than 20 ºC [15].

Denitrification processes in which heterotrophic bacteria needed a carbon source to generate new cells and use as energy source could be completed by adequate amounts of carbon source added into the reactor [9]. In this study, sucrose was used as a carbon source and the C:N ratio was approximately 7.6 that relate to the theory that COD per Nitrate nitrogen which should be at least 3-7 [11].

It indicated that denitrifying bacteria preparation was conducted by adding 100 mg-N/L $KNO_3$ into 100 L of 25-30 ppt sea water and added 100 g of sucrose. Plastic media were added at ratio of 1:3 (v/v) and controlled the other parameters in optimum range; pH (7.0-9.0), temperature (30-32 ºC) and dissolved oxygen (<0.5 mg$O_2$/L). The denitrifying biofilter media was ready to use within 30 days.

*3) Efficiency of nitrification in shrimp wastewater treatment*

Ammonia nitrogen in shrimp culture water at density of 150 pcs/L was 13.14±3.54 mg-N/L and aerated by supercharge at the rate of 2.5 L/min. The experiment was divided into three groups; group 1: new sea water used and did the water exchanged 50% daily, group 2: without nitrifying bacteria and group 3: with nitrifying bacteria. Group 2 and 3 kept the same water for the entire the experiment. The control conditions in this experiment were pH (8.0-8.3), dissolved oxygen (≥ 5 mg$O_2$/L), temperature (28-30 ºC) and alkalinity (>200 mg$CaCO_3$/L).

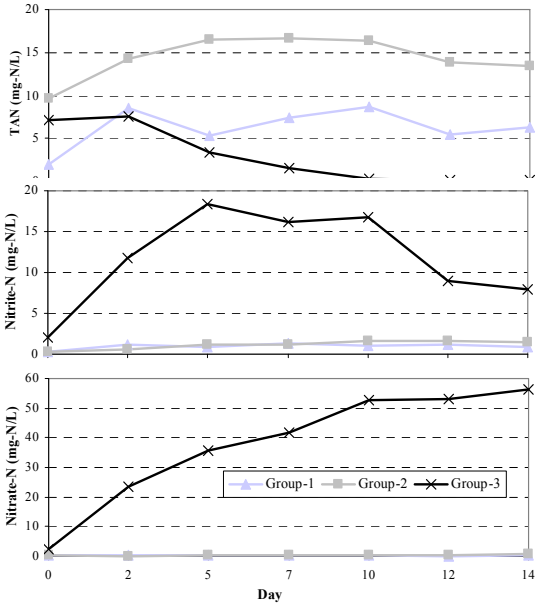


Figure 3. Nitrogen compounds changing during shrimp culture with nitrification reactor.

The results in Figure 3 showed that total ammonia concentration in group 3 was the lowest compared with group 1 and 2. It indicated that nitrifying bacteria is highly effective in nitrogen removing bacteria. It differed from group 2 in ammonia nitrogen accumulated at significantly higher amounts than group 1 and 2. It could be explained that high amounts of ammonia came from shrimp feed which contained high amount of protein (40-60% protein) and was complementary with no water exchange. In addition, ammonia nitrogen in group 1 did not accumulate during culture as daily exchange of water lowered the ammonia concentration.

Nitrite nitrogen and nitrate nitrogen in group 3 were the highest compared with group 1 and 2 where nitrite and nitrate were rarely generated. Although group 1 and 2 operated under the same conditions as group 3 they did not perform ammonia oxidation well. As describe in nitrifying preparation that it took at least 30 days to generate this group of bacteria therefore group 1 and 2 which started without nitrifying bacteria could not build up biomass of this species in the period of 14 days especially for group 1 where 50% of the water was changed daily.

Efficiency of total ammonia nitrogen removal in culture tank after 14 days of experiment by nitrification process is shown in Table 13. Removal percentage in group 3 (98%) was significant higher than group 1 (50%) and 2 (7%). Thus, the nitrifying biofilter had higher performance than water exchange for nitrogen removal in shrimp culture water.

The relationship between total ammonia nitrogen and

nitrate nitrogen in the nitrification process should occur as it showed the nitrification efficiency of the system [16]. $R^2$ for group 1, 2 and 3 were 0.1556, 0.0011 and 0.8705, respectively.

*4) Efficiency of denitrification in shrimp wastewater treatment*

Shrimp culture wastewater was transferred to denitrification reactors. The experiment divided into two groups. These were group 2 without denitrifying bacteria and group 3 with denitrifying bacteria. The control conditions in this experiment were pH pH (7.0-9.0), temperature (30-32 ºC) and dissolved oxygen (<0.5 $mgO_2$/L).

The results in Figure 18 show that ammonia, nitrite and nitrate nitrogen concentrations in group 3 were lower than group 2 after 12 days. Nitrogen gas was generated in group 1 more than group 2. It indicated that denitrifying bacteria performed effective high nitrate removal. It differed from group 2 that ammonia nitrogen accumulated at significant higher amounts and nitrogen not presented entire the experiment.

Although group 2 operated in the same conditions as group 3 they did not perform nitrate conversion to nitrogen gas well. As described in denitrifying preparation it took at least 30 days to generate this group of bacteria and nitrate nitrogen was rare at the initial start of denitrification.

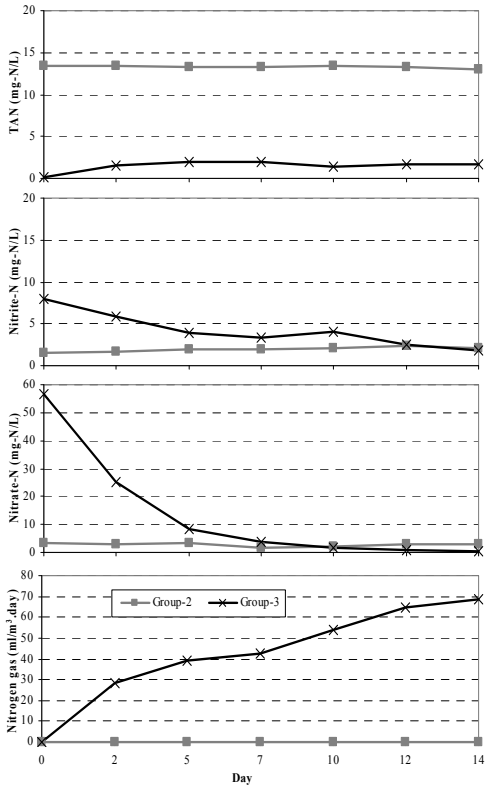


Figure 4. Nitrogen compounds changing during denitrification system.

The efficiency of the denitrification process showed in Table 13. Removal percentage of BOD and COD in group 2 and 3 were 69%-12% and 76%-43%, respectively. The reduction of COD in group 3 was explained that denitrification process which heterotrophic bacteria needed a carbon source to generate new cells and use as energy source could be completed by adequate amounts of a carbon source added into the reactor [9].

TABLE I: BOD, COD, TAN AND TOTAL NITROGEN REMOVAL

| Parameters | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| BOD removal (%) | - | 69% | 76% |
| COD removal (%) | - | 12% | 43% |
| TN removal (%) | - | 5% | 84% |
| TAN removal (%) | 50% | 7% | 98% |

Total nitrogen related with amounts of organic nitrogen, ammonia, nitrite and nitrate therefore ammonia removal with nitrification and nitrate removal with denitrification reduced the total nitrogen value [16]. In group 3 the total nitrogen removal showed high efficiency (84%) it indicated that nitrifying and denitrifying biofilter performed well and is suitable to use for nitrogen compounds eliminated in wastewater.

*B. Effects of recycled water on shrimp performance*

*1) Water quality of Recycled water*

After a nitrification and denitrification process the treated water was used three times to culture post larvae shrimp and the water quality after each cycle was determined and compared with shrimp culturing standard. The results showed that water quality after the 1st and the 2nd cycle of recirculation were under standard limits but the water quality after the 3rd cycle showed the concentration of nitrite nitrogen to be higher than standard thus it indicated that water treated by using a nitrification and denitrification system could be reused for three cycles. Otherwise nitrite nitrogen would be toxic to post larvae if treated water was used for the fourth cycle [6].

During shrimp culture in each cycle, the nitrification reactor was connected to the culture tank and after the shrimp were harvested wastewater was treated in the denitrification reactor. The results in Table 14 show that the efficiency of TAN, TN, BOD and COD removal in group 3 were higher than group 1 and 2

TABLE II: BOD, COD, TAN AND TOTAL NITROGEN REMOVAL IN EACH CYCLE OF RECIRCULATION

| | Treatment | %Removal | | | |
|---|---|---|---|---|---|
| | | TAN | TN | BOD | COD |
| 1st cycle reused | Group 1 | 14% | - | - | - |
| | Group 2 | 16% | 2% | 65% | 8% |
| | Group 3 | 93% | 81% | 67% | 54% |
| 2nd cycle reused | Group 1 | 12% | - | - | - |
| | Group 2 | 15% | 1% | 65% | 9% |
| | Group 3 | 92% | 82% | 66% | 52% |
| 3rd cycle reused | Group 1 | 13% | - | - | - |
| | Group 2 | 14% | -2% | 62% | 5% |
| | Group 3 | 94% | 80% | 63% | 50% |

*2) Shrimp Growth Rate*

Growth rate of shrimp was not significantly different between group 1 and group 3 for 1st, 2nd and 3rd cycle of recirculation ($p<0.05$). In contrast, growth rates of shrimp in

group 2 were opposite to number of recirculation cycle increased. From this results it showed that ammonia accumulated in culture water affected osmoregulation and blood acidity in shrimp and caused high mortality of post larvae shrimp (Boyd *et al.*, 1998 and G. Alcaraz *et al.*, 2003). (Figure 5)

After 14 days of the experiment, although shrimp in group 3 were shorter than group 1, both of them still passed the standard level (TL $\geq$ 8.00 mm, %TL $\geq$ 70% and %CV $\leq$ 12.00%) but group 2 did not pass the standard at all cycles of recirculation.

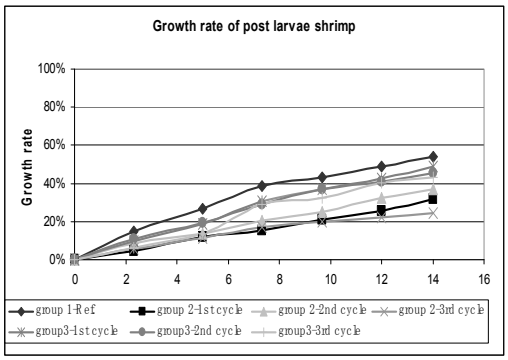


Figure 5. Growth rate of shrimp rearing in treated water.

*3) Shrimp Survival Rate*

As shown in Figure 6, shrimp survival rate in group 3 which used the aerobic and anoxic recycled water for culturing showed a significantly higher survival rate than group 2 in all three occurrences of reusing the water. For the 1st, 2nd and 3rd cycles of recycled water the survival rate of shrimp in group 2 and 3 were 26% and 46%, 27% and 48% and 26% and 53%, respectively ($p<0.05$). When in group 1, new water was used, the survival rate of shrimp was significant higher than group 2 but it was not significant different between groups 1 and 3. This result showed the effect of ammonia accumulated in the culturing water on post larvae shrimp. Ammonia concentrations in group 2 were higher than the toxic level for post larvae shrimp. Thus, it affected osmoregulation and blood acidity in the shrimp and caused high mortality of post larvae shrimp [3].

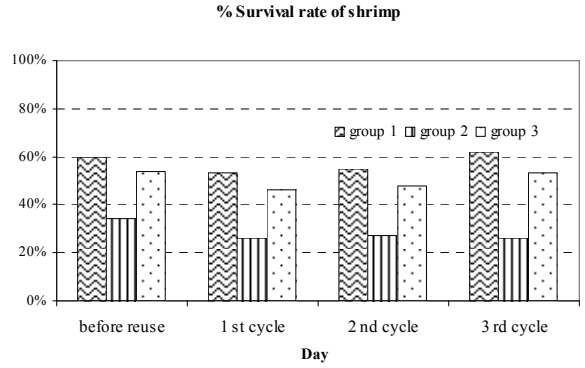


Figure 6. Survival rate of shrimp reared in treated water.

The results as mentioned above indicated that wastewater treated by aerobic and anoxic system could be recycled three times to use in shrimp culture process at the rate of 100% recycle. Shrimp culture together with the nitrification and denitrification took around 28 days for each cycle. Furthermore, it reduced water preparation and treatment costs by approximately 70%.

## IV. Conclusion

Main conclusions derived from this study are summarized as follows:

The results of the three laboratory systems, which proved to be almost identical in most of the monitored parameters, showed that effective operational capability of the nitrification and denitrification biofilter could be easily evaluated. This recirculation process was shown to produce consistently good results over a long period with hardly any need to compensate for the loss of system water (except for evaporation losses), and with effluents having low nitrate levels (below 1 mg-N/l).

The 3rd cycle recirculation water after treatment with the aerobic and anoxic systems slightly affected the shrimp survival and growth rate *($p<0.05$)*. The water quality of wastewater from the 3rd cycle recirculation was over the standard limit thus it should not be used for a further cycle.

Recirculation of aerobic and anoxic treated water in shrimp culturing process reduced at least 50% of water cost in shrimp production. In addition, the environmental awareness and cooperate social responsibility are the main points of future trade. Thus, it is excellent supporting for a sustainable aquaculture business.

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