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ACTIVITY OF NITRIFYING BACTERIA (AMMONIA OXIDIZER AND NITRITE OXIDIZER) IN BRACKISHWATER PONDS (TAMBAK) IN BENGKALIS ISLAND, RIAU PROVINCE

By: Feliatra

Lab. Marine Microbiology, Fisheries and Marine Science Faculty,
University of Riau Campus of Bina Widya km 12,5 SP Panam Pekanbaru 28293 Indonesia.
Fatra@lovemail.com

ABSTRACT

A research study was carried out from April to July 1999, on brackishwater ponds in Bengkalis Island, Riau Province, Indonesia. Observations and samplings were taken from three stations. Six nitrifying bacteria were isolated from ammonium oxidizer and four from nitrite oxidizer. The nitrifying bacteria activity varied from 0,024 to 0,092 ppm/day for ammonia oxidizer, and from 0,032 to 0,052 ppm/day for nitrite oxidizer. These nitrifying bacteria can reduce the ammonia concentration; in the brackish water ponds. In the absence of nitrifying bacteria, the ammonia concentration was so toxic, it was killed the shrimp at 31 days after sampling.

Keywords : nitrifying bacteria, brackishwater ponds, ammonia, nitrite, nitrate.

I. INTRODUCTION

Nitrification is a decomposing process of ammonia to nitrite and nitrate. In an aquatic ecosystem, the process acts important role in the nitrogen cycle. Nitrifying bacteria and phytoplankton as primary producers of inorganic matters, have a unique relationship to the need of ammonia and nitrate. In one aspect, the nitrifying bacteria produces nitrate which is consumed by phytoplankton, and in another aspect, there is a competition to consume the available ammonia (Feliatra and Bianchi, 1993).

In an aquatic ecosystem, only nitrifying bacteria produces nitrate which will be consumed by primary products of inorganic matters. Meanwhile, in a brackishwater ponds ecosystem, the heterotrophic organism or bacteria will mineralize organic substance of unconsumed food from over feeding rate, and of organism excretion, hence increasing ammonia concentration. This product could kill fish and shrimp cultures (Goldman et al, 1985).

Shrimp is a primary export product of Indonesia fishery. The commodity has significantly increased economic activity all over Indonesian, such as in the island of Java, Sumatra, Kalimantan, Sulawesi and Irian Jaya. The more developed technology in the aquaculture industry; in this case the brackish water pond culture; the more problems are encountered. The development of brackishwater pond culture was started in the 1980's, and it contributes significantly to the national income. However, the recent harvest failure was caused by the worsening of water quality.

Brackishwater pond, which is rich in organic substances; fertilizer and nutrient; is an ideal place to grow the

heterotrophic bacteria. Its decomposition products; ammonia; at certain limit can be harmful to fish and shrimp, hence causing failure of harvesting. Moreover, ammonia produced by organism and macro zooplankton excretion yields to 75% of the excretion (Goeyens, et al, 1991). Ammonia is toxic in a low dissolved oxygen concentration (Boyd, 1990). One organism which utilizing ammonia, is nitrifying bacteria.

The role of nitrifying bacteria in an aquatic ecosystem is in nitrogen cycle. As a primary producer of inorganic matters, the bacteria can change productivity of the ecosystem from regenerative productivity to a new productivity. Due to existing issues, it is important to understand the activity of nitrifying bacteria in a brackish waterpond ecosystem.

II. METHODOLOGY

The research was carried out from April to July 1999, in brackishwater ponds, Bengkalis Island, Riau Province. Observations and samplings were taken at three stations. Pond station I was located at Kelapa Pati, pond station II was at Pangkalan Batang, and pond station III was at Sei Alam. Analyses of nitrifying bacteria were conducted at Microbiology Laboratory at marine science and fisheries faculty, riau University Indonesia.

The nitrifying bacteria activity and water quality parameters of brackish water ponds were studied. The activity of nitrifying bacteria was measured by using specific inhibitors. Two inhibitors were used to inhibit the bacteria activity, they were Allylthiourea as the ammonia oxidizer inhibitor (Hauck, 1980; Hall, 1984), and Chlorate as the nitrite oxidizer inhibitor (Belser and Mays, 1980; Hynes and Knowsles, 1983).

Water quality parameters measured were salinity, temperature, dissolved oxygen, transparency concentration of ammonia (Solorzano, 1969), nitrite (Bendschreider and Robinson, 1952), and nitrate (Anonymous, 1990). The composition of culture medium for ammonia oxidizer and nitrite oxidizer was based on Feliatra and Bianchi's (1993), which consisted of $(NH_4)_2SO_4/NaNO_2$ $NaHCO_3$, K_2HPO_4, multivitamins and minor element.

Sample Isolation to identify the bacteria was logically used the method according to Bergey's Determinative Bacteriology (Holt et al, 1994). Conducting series of physical and biochemical tests, included gram-staining, motility, shape of cell, cell-joined type, aerobic and aerobic test, growing temperatures, colony colour, pigment formation, colony size, colony shape observed from inside, beside and upside, and production of catalyses and oxidation.

III. RESULT AND DISCUSSION

3.1. Brackish Water Pond Condition

In general, the geometry conditions for the three brackish water ponds are the same. The pond sizes were 3000 m^2. These ponds used the same fertilizer, feed type and density. The shrimp was harvested at the age of 100 to 120 days. Pond management, such as liming, drainage, and fertilization was using urea, PSP and organic fertilizer, in order to grow natural food plankton. Feeding of artificial food was by distributing evenly all around the pond. The food was always being adapted to the age, size and weight of shrimp, which was around 2 to 3% of shrimp body weight. The food was produced by PT. Charoen Pokphan, with composition of 36 to 41% protein, 6 to 8% lipids, 3% fiber, 12% water contain and 1% ash contain. Feeding frequency was also adapted to the shrimp

age, which was 4 to 5 times daily. The stocking density for each pond varied from 25 to 30 pieces/m^2 size of post larve (PL) 11 to PL 15. During the research, the age of the shrimp was 53 days (station I), 30 days (station II), and 42 days (station III).

Table 1. The Brackish water pond condition in Bengkalis Island

No	Parameter	Station I	Station II	Station III
1.	Size of ponds (M^2)	3000	3000	3000
2.	Age of Shrimp (days)	53	30	42
3.	Fertilizer type	Urea, TSP & PK	Urea, TSP & PK	Urea, TSP & PK
4.	Food type	Pellet	Pellet	Pellet
5.	Feeding frequency (per day)	5	4	5
6.	Density (piece/ m2)	25	30	30

3.2. Water Quality Parameters of Pond

In shrimp culture, water quality is an important factor, because if it is not observed well, the shrimp culture could fail. As the intense and feeding frequency influences the water quality. The average temperature of two water samples were not highly fluctuated, ranging from 30 to 31^{O}C, This range is normal for tropical waters. Buwono (1993) reported that the best temperature for growing shrimp is varied from 25 to 30^{O}C, the acceptance temperature for shrimp life varies from 18 to 35^{O}C. Meanwhile, the optimal temperature for nitrifying bacteria was 20 to 30^{O}C (Fock and Verstate, 1977). Watson and Watenbury (1971) reported that Nitrospina and Nitrosococcus grew optimum at temperature ranging from 25 to 35^{O}C. The growth would be disturbed below 14^{O}C.

Salinity was one of the factors influencing the shrimp growth. The pond salinity varied from 19 to 22 ppt. Sumartini and Aspiriyanto (1990) indicated that shrimp grew well at salinity of 30 ppt, and rapidly grew at salinity of 10 ppm. Salinity range for shrimp to grow was 10 to 35 ppt. Research in the Rhone Estuarine of France indicated that the increase of salinity, decreases the nitrifying bacteria activity (Feliatra and Bianchi, 1993). They also described that high salinity fluctuation in water would also cause high tolerance of salinity.

Dissolved oxygen concentration in pond water; besides being used for shrimp respiration; is also used for organic substance oxidation carried out by heterotrophic bacteria. The dissolved oxygen concentration in ponds observed varied from 6,2 to 6,6 ppm. Difference in the value was caused by different numbers of aerators used. Buwono (1993) found the best dissolved oxygen concentration for shrimp culture was 5 to 10 ppm. Nitrification process of an ecosystem is better if dissolved oxygen is always available. According to Gunderson and Mountain (1973), nitrification process decreased at dissolve oxygen less than 0,3 mg/l. Aleem, Hoch and Varner (1965) described that at dissolved oxygen circulation in nitrite oxidation, electron acceptor of nitrate was obtained from water, and it was not from dissolved oxygen

The pH of pond water samples were varied from 7,0 to 7,7. These values were appropriate for shrimp culture. Sumartini and Aspriyanto (1996) agreed that this is the normal pH for shrimp, and that pH of less than 5 would inhibit the growth. Wong Chong (1975) reported the optimal pH for productive growth of nitrifying bacteria was at pH of 7 to 8. He also mentioned that PH would influence on the activity of ammonia oxidizer, of which the activity of nitrosomonas would decrease as pH increases.

Pond water transparency directly correlates to the sunlight penetration, hence

photosynthetic process. The transparency of pond water varied from 25 to 30 cm. This range is relatively well. Based on Suyanto and Mujiman's (1995) that the best water transparency for shrimp growth varied from 25 to 24 cm. Distribution and existence of nitrifying bacteria are also influenced by water transparency. Ammonia and nitrite oxidation could be inhibited by sunlight. Olson (1981) reported that 50% of ammonia and nitrite oxidation was inhibited by light intensity of 4 x 10^{14} to 1,1 x 10^{15} quanta cm^{-2} S^{-1}

Depth of pond water varied from 75 to 96,5 cm (Table 2). These values were ideal for the growth of shrimp cultured. Suyanto and Mujiman (1995) described that the depth of pond water for larvae cultivating were from 75 to 100 cm, which functions as a temperature stabilizer. Water depth is an important parameter, because the activity distribution of ammonia oxidizer takes place at three depth layers. At water surface nitrification activity was minimum, because ammonia oxidizer competed with phytoplankton to consume ammonia. Mesophotic area was the highest ammonia oxidation area, that could reach 60% of nitrite concentration available in the water. The third layer was aphotic area, where nitrification process would decrease as depth increase (Olson, 1981).

Table 2. The average of water quality parameters of two water samples

No	Station	Temperature (^{0}C)	Salinity (ppm)	Dissolved oxygen (ppm)	PH	Transparency (cm)	Depth of Pond (cm)
1.	Station I	30	19	6,2	7,9	25	75,0
2.	Station II	30	22	6,5	7,5	25	87,5
3.	Station III	31	20	6,6	8,0	30	96,5

3.3. Concentration Variation of Inorganic Nitrogen

Inorganic nitrogen is always a limiting factor in an ecosystem of land fishery. Three dominate inorganic nitrogenous; ammonia, nitrite, nitrate; were analyzed from surface and bottom layers of the brackishwater ponds. The ammonia concentration varied from 0,271 to 0,443 ppm (Table 3). Over all, the ammonia concentration was higher at the bottom than at the surface, as shrimp activity was higher at the bottom. In addition to that, the food residue resulting from over feeding activity, would sink to the bottom. The organic substance would than be decomposed by heterotrophic bacteria resulting in the increase of ammonia.

Ammonia is important for nitrifying bacteria activity, because the ammonia is the only energy source for the bacteria. Olson (1981) reported that the activity of ammonia oxidizer was hyperbolically at substrate concentration of 0,048 to 0,164 ppm. Carlucci and Strickland (1968) described that the activity of ammonia oxidizer increased as ammonia concentration increased. In pure culture condition, activity of ammonia oxidizer occurred at 0,24 to 40,6 ppm ammonia concentration.

The level of nitrite concentration varied from 0.011 to 0,049 ppm (Table 3). Generally, the nitrite concentration was higher at bottom than at surface. Nitrite is a product of ammonia oxidation by ammonia oxidizer. Study conducted in the Rhone Estuarine by Feliatra (1994), found that the nitrite concentration varied from 0,096 to 163,6 ppm and it was also higher at bottom than at surface. Olson (1981) found that the required nitrite concentration of nitrite oxidizer was minimum at 0,048 ppm.

Nitrate is the final product of the bacteria nitrification. The nitrate concentration measured from the ponds varied from 0,195 to 0,354 ppm (Table 3). The concentration was higher at bottom than at surface. Nitrate is always the

limiting factor in an aquatic ecosystem because the primary source of nitrate in aquatic was from the nitrification process (Beronsky and Nixon, 1990). Carlucci and Strickland (1968) mentioned that in a water culture, an increase in oxidation value was proportional to the increase in ammonia uptake by nitrifying bacteria.

Table 3. Average concentration of inorganic nitrogen in brackish water pond ecosystem

No	Station	Ammonia (ppm)	Nitrite (ppm)	Nitrate (ppm)
1	Station I Surface	0,271	0,011	0,223
2	Station I Bottom	0,382	0,043	0,354
3	Station II Surface	0,246	0,011	0,195
4	Station II Bottom	0,317	0,035	0,284
5	Station III Surface	0,362	0,012	0,216
6	Station III Bottom	0,443	0,049	0,276

3.4. Number of Nitrifying Bacteria in Pond Water

Ammonia oxidizer and nitrite oxidizer are the only bacteria involved in the nitrification process. The number of ammonia oxidizer found from the observed ponds varied from 3,1 x 10^2 to 1,65 x 10^3 cell/ml (Table 4). The count was higher at bottom than at surface. Similar variation was also observed from the nitrite oxidizer count, which varied from 2,9 x 10^2 to 1,44 x 10^3 cell/ml (Table 4). Number of the bacteria correlated to the substrate concentration; ammonia and nitrite. However this number was lower than it was found by Perfettini and Bianchi (1990) at a closed culture system, which was 5 to 6 x 10^6 cell/ml. The lower count could be a result of lower energy substrate concentration; ammonia and nitrite. Feliatra and Bianchi (1993) found in the Rhone Estuarine that the number of nitrifying bacteria decreased from lower salinity of 3,7 to 2,17 x 10^6 cell/ml to higher salinity of 0,88 x 10^6 cell/ml.

Table 4. Average count of nitrifying bacteria from the brackish water ponds observed

No	Station	Ammonia Oxidizer (cell/ml)	Nitrite Oxidizer (cell/ml)
1	Station I Surface	4 x 10^2	2,9 x 10^2
2	Station I Bottom	6,6 x 10^2	5,7 x 10^2
3	Station II Surface	3,6 x 10^2	3,1 x 10^2
4	Station II Bottom	1,65 x 10^2	1,44 x 10^3
5	Station III Surface	3,1 x 10^2	2,7 x 10^2
6	Station III Surface	1,15 x 10^3	1,12 x 10^3

3.5. Isolation and Identification of Nitrifying Bacteria

a. Ammonia Oxidizer Bacteria

Ammonia oxidizer is nitrifying bacteria, which is able to oxidize ammonia to become nitrite. From the three ponds observed, six ammonia oxidizers were isolated. All isolates were gram-negative which required oxygen for their life. They produced catalyses, oxidized cytochrome, and did not produce H_2S. The cell shape of strains Tam 1 and Tam 5 was coccus; while strains Tam 2, Tam 3, Tam 4 and Tam 6 was rod. Except the isolate 1, all other isolates were motile. The colony size of isolate Tam 1, Tam2, Tam 3 and Tam 5 was 1 to 2 mm, isolate Tam 4 was larger than 1 to 3 mm, and isolate Tam 6 was less than 1 mm. The optimal growth temperature was from 30 to 35^O C, the well growth was from 20 to 30^O C, and they grew minimum at 5^O C (Table 5).

b. Nitrite Oxidizer Bacteria

Nitrite oxidizer is a bacteria which is able to oxidize nitrite to nitrate. There were four strains obtained from the brackishwater ponds. These four strains were gram-negative, aerobic, catalyses, oxidize negative, oxidize cytochrome, and no H_2S production. The bacteria grew optimum from 30 to 35^{O}C, grew well from 20 to 30^{O}C and grew minimum at 5^{O}C. Three of the isolates shape were coccus, these were TNO2, TNO3 and TNO4, while TNO1 isolates was rods. The colony size of isolate TNO1 and TNO3 was 1 to 2 mm, isolate TNO2 and TNO4 was less than 1mm. Isolates TNO1, TNO3 and TNO4 were motile and TNO2 was not (Table 5).

Table 5. Morphology, Physical and Chemical Characteristics of Nitrifying Bacteria Observed from the Research Locations

Morphology	Tam 1	Tam2	Tam3	Tam 4	Tam 5	Tam6	TNO1	TNO2	TNO3	TNO4
Shape of cell										
- Coccus	+			+	+			+	+	+
- Rods		+	+			+	+			
Gram Staining	-	-	-	-	-	-	-	-	-	-
Motility	-	+	+	+	+	+	+	-	+	+
O_2 Requirement	+	+	+	+	+	+	+	+	+	+
Oxidize	-	-	-	-	-	-	-	-	-	-
Catalyses	+	+	+	+	+	+	+	+	+	+
Oxidized Cytochrom	+	+	+	+	+	+	-	-	-	-
Gas H_2S forma-tion	-	-	-	-	-	-	-	-	-	-
Colony Colour										
- Transparent										
- Turbid	+		+			+				+
- Yellow		+			+		+	+	+	
- Crème				+						
Colony Size										
- Larger than 1 mm						+		+		+
- 1 to 2 mm	+	+	+		+		+		+	
- 2 to 3 mm				+						
Shape colony										
- Upside	B	B	BB	T	BO	BO	B	BO	BO	BO
- Below	U	U	BB	BC	BO	BO	BO	BO	BO	BO
- Beside	C	M	K	M	D	D	M	K	K	D
Cell Joint-Type										
- Separate	+	+	+	+	+	+	+	+	+	+
- Clump										
Temperature growth										
- 5^{O}C	TL	TL	TL	TL	TL	TL	TL	TL	TL	TL
- 20 –to 30^{O}C	TB	TB	TB	TB	TB	TB	TB	TB	TB	TB
- 30 –to 35^{O}C	TO	TO	TB	TO	TO	TO	TO	TO	TO	TO
Halophilic										
- NaCl 0%	TL	TL	TL	TL	TL	TL	TL	TL	TL	TL
- NaCl 0,7%	TB	TB	TB	TB	TB	TB	TB	TB	TB	TB
- NaCl 2%	TB	TB	TB	TB	TB	TB	TB	TB	TB	TB
- NaCl 3%	TO	TO	TO	TO	TO	TO	TO	TO	TO	TO

3.6. Activity of nitrifying bacteria

Oxidation of ammonia to nitrite by ammonium oxidizer bacteria is the primary stage of nitrification process. In the

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observed brackishwater pond ecosystems, The level of ammonia oxidizer varied from 0,024 to 0,092 ppm/day (Figure 1). Oxidation of nitrite to nitrate is the second stage of nitrification process. The bacterial activity of nitrite oxidizer at the second stage of nitrification, was higher at bottom than at surface of the ponds. This condition was due to a higher concentration of energy substrate sources of the two bacteria. This phenomenon was reported by Ward (1986) that there was a correlation between ammonia concentration and the activity ammonia oxidizer; the higher ammonia concentration, the higher ammonia oxidizer activity would be.

Activity of nitrifying bacteria in brackish water pond ecosystem was relatively higher than the activity in the Rhone Estuarine observed by Feliatra and Bianchi (1993), which was 0,029 ppm/day at salinity of 15 ppm.

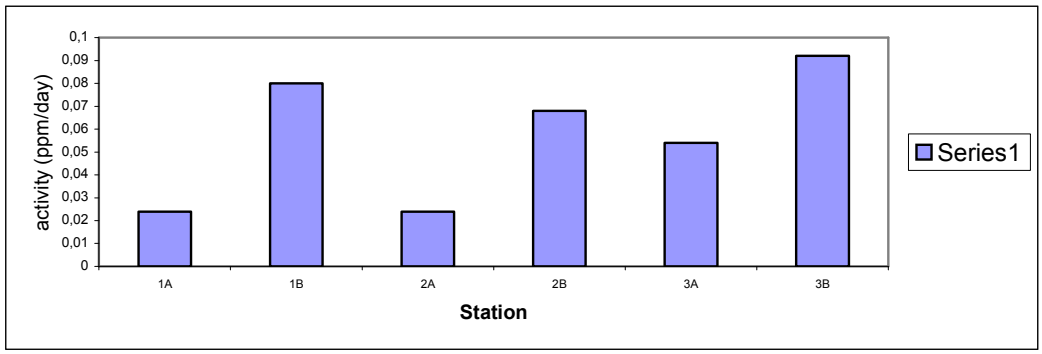

Figure 1 - Activity of ammonia oxidizer bacteria
Note : A= Surface B = Bottom

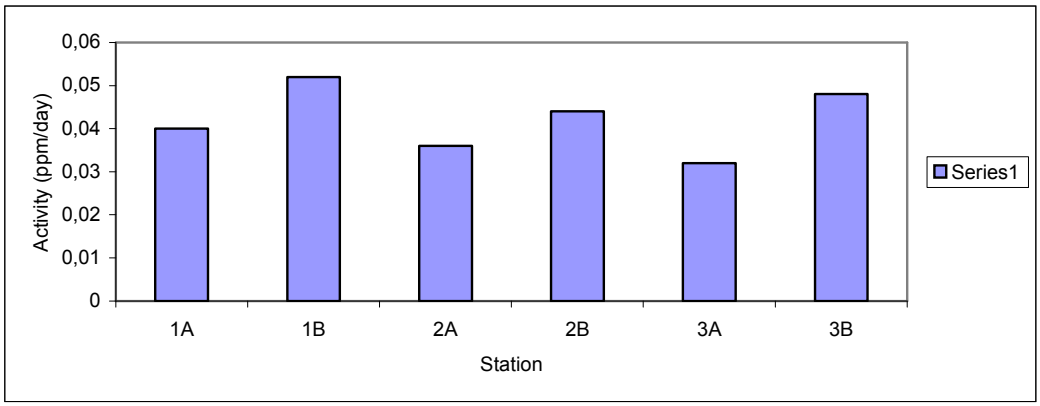

Figure 2 - Activity of nitrite oxidizer bacteria
Note : A = Surface B = Bottom

3.7. Role of Nitrifying Bacteria in the Brackish Water Pond Environment

The two stages of nitrification is a complete process in which ammonia oxidizer oxidizes ammonia to nitrite, which then is being oxidized to become nitrate by

nitrite oxidizer. Time required by ammonia oxidizer to consume ammonia in situ varied from 4,72 to 12,92 days (Table 6). At the water surface, the oxidation time was longer than at the bottom, even though the ammonia concentration at the bottom was higher than at the water surface. Primarily because the number of bacteria at the bottom was higher than at the surface, and only ammonia oxidizer consumed ammonia which was produced by heterotrophic bacteria and cultured organism excretion. At the water surface, there was competition to consume ammonia between ammonia oxidizer and primary producers. Ammonia oxidizer could decrease the accumulation of ammonia concentration resulting from heterotrophic decomposition and culture organism excretion, which could produce 0,024 to 0,092 ppm/day of ammonia. In the absence of nitrifying bacteria intervention to oxidize ammonia; shrimp larvae died 11 days, and adult shrimp died 31 days after taking sample. Buwono (1993) found that the best ammonia concentration for the shrimp growth was less than 3 ppm, and for shrimp larvae was less than 1 ppm.

Time required for nitrite oxidizer to produce nitrate did not vary very much. From the three stations and the two different depths, they varied from 4,29 to 8,85 day (Table 7). In general, the time required by nitrite oxidizer consuming ammonia was longer at the bottom than the water surface. The longer time required to produce nitrate at the bottom was primarily caused by nitrate directly consumed by the primary producers at the water surface; while at the bottom, nitrate ion should undergone turbulence before reaching the water surface, and then being consumed by primarily producers. Bianchi et al (1993) reported that the primary producer required nitrate of 0,062 ppm/day. Therefore, from this research, nitrate produced by nitrite oxidizer of 0,032 to 0,052 ppm/day could provide nitrate requirement of primary producer as a nitrogen source at level of 5,16 to 8,29%.

Table 6. Concentration of Ammonia, Activity of Ammonia Oxidizer and Time Required Consuming Ammonia in-situ

No.	Sampling Station	Ammonia Concentration (ppm)	Activity of ammonia oxidizer bacteria (ppm)	Time require to consume ammonia (days)
1	Station 1 Surface	0,271	0,024	11,29
2	Station 1 Bottom	0,382	0,080	4,72
3	Station 2 Surface	0,246	0,024	10,25
4	Station 2 Bottom	0,317	0,068	4,66
5	Station 3 Surface	0,362	0,028	12,92
6	Station 3 Surface	0,443	0,092	4,82

Table 7. Concentration of Nitrate, Activity of Nitrite Oxidizer and Required to Produce

No.	Sampling Station	Concentration of nitrate (ppm)	Activity of nitrite oxidizer bacteria (ppm/day)	Time require to produce nitrate (days)

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1	Station 1 Surface	0,223	0,052	4,29
2	Station 1 Bottom	0,354	0,040	8,85
3	Station 2 Surface	0,195	0,044	4,43
4	Station 2 Bottom	0,284	0,036	7,88
5	Station 3 Surface	0,216	0,048	4,5
6	Station 3 Surface	0,276	0,032	8,62

IV. CONCLUSION

From the study, it was found that the quality of brackishwater pond correlates well to its cultured production. There were no accumulation of ammonia concentration from mineralization product of organic substances, such as food and fertilizer. Ammonia concentration at the observed pond ecosystem was relatively low and was not harmful to the shrimp life, as it was consumed by enough nitrifying bacteria.

Ten isolates of nitrifying bacteria was obtained from the ecosystem. Six were ammonia oxidizer isolates (Tam1, Tam2, Tam3, Tam4, Tam5 and Tam6) and four were nitrite oxidize isolates (TNO1, TNO2, TNO3, TNO4)

The role of nitrifying bacteria in an aquatic ecosystem is to reduce accumulation of ammonia concentration. If there is no nitrifying bacteria in the brackishwater pond, the growth of shrimp larvae at the age of 11 day would be inhibited. Moreover, the adult shrimp would be inhibited at the age of 31 days. The nitrification process could provide 5,16 to 8,29 % of nitrate that was required by primary producer at the pond ecosystem.

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