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Research Article
Activities of NH_4^+ and NO_2^- Oxidizing Bacteria in a Recirculating System of Mud Crab (*Scylla serrata*) Culture with Different Number of Shelter

[1]Yuni Puji Hastuti, [2]Iman Rusmana, [3]Kukuh Nirmala and [4]Ridwan Affandi

[1]Study Program of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Indonesia
[3]Department of Aquiculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Indonesia
[4]Department of Management of Water Resources, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Indonesia

Abstract

Backgorund: The activities of NH_4^+ and NO_2^- oxidizing bacteria in aquaculture recirculating system can influence to environmental N-inorganic, physiologic response and growth performance of mud crab *Scylla serrata*. The culture of mud crab in a Recirculating Aquaculture System (RAS) involves the microbiological nitrogen removal including aerobic and anaerobic nitrogen conversion such as nitrification and denitrification. In nitrification process activities of ammonium (NH_4^+) and nitrite (NO_2^-) oxidizing bacteria can influence for a Dissolved Inorganic Nitrogen (DIN) like as ammonia (NH_3^-), nitrite (NO_2^-) and nitrate (NO_3^-) in the RAS environmental. **Objective:** The objective of this study aimed to isolate and determine the activities of ammonium (NH_4^+) and nitrite (NO_2^-) oxidizing bacteria in crab culture using a recirculating aquaculture system. **Materials and Methods:** With different quantities of shelters (4 and 6 shelters) in box cultivation of mud crab, the activity oxidizing bacteria be count by amount of NH_4^+, NO_2^- and NO_3^-. The concentrations of ammonia, nitrite and nitrate be measured with spectrophotometers in the Environmental and Microbiology Laboratory, Bogor Agricultural University. Ammonium (NH_4^+) and nitrite (NO_2^-) oxidizing activity tests were used to determine the enzyme activity and affinity to the culture substrate. **Results:** Based on the Michaelis-Menten kinetics analysis for ammonia oxidizer, it was found that treatment P4 showed a V_{max} value (Maximum speed) of 0.073 mM h^{-1} and a K_m (Michaelis-Menten) constant was 0.1909 mM. Whereas treatment P6 showed lower values, i.e., V_{max} of 0.0673 mM h^{-1} and K_m of 0.0788 mM, indicating that the affinity of enzymes related to ammonium oxidation by the bacteria in this treatment was higher. **Conclusion:** This study showed P6 give the best of NH_4^+ oxidizer activity and this was supported by the higher ammonium oxidation rate (12.72±5.89 μM h^{-1}).

Key words: Ammonium oxidizer, nitrite oxidizer, kinetics, mud crab (*Scylla serrata*), reticulation aquaculture system

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Corresponding Author: Iman Rusmana, Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The nutrient found in the highest concentrations in mud crab (*Scylla serrata*) culture waste is nitrogen which comes from feed waste, especially from fresh feed (trash fish). The culture system that could be applied to overcome this issue is the water recirculating system. Maintaining the quality of the crab culture medium should be taken seriously and pathogens must be avoided, therefore, an appropriate maintenance technique is needed to replicate the natural habitat[1]. The recirculating system is one of the systems and technology which is suitable for the effort to improve water quality, especially brackish water and sea water which could not be accessed by all areas. Recirculating with an oxidation reduction system is considered to be the appropriate system in protecting the aquaculture environment from nitrogen pollution[2]. Some compounds are produced by the degradation and decomposition of aquaculture waste both organic (lipid, carbohydrate and protein) and inorganic (NH_4 and NO_2 and NO_3). Direct negative effects could be caused by the toxicity of the inorganic nitrogen compounds themselves which consist of ammonia (NH_3^-) or ammonium (NH_4^+) and nitrite (NO_2^-)[3]. Certain concentrations of those compounds could cause death in aquatic animals. The compounds from nitrogen waste, in addition to causing death could also cause physiological, neurological and cytological disturbances which lead to stress and suppressed food consumption activities[4].

The aquaculture system for mud crabs (*Scylla serrata*) is inseparable from the nitrogen cycle that occurs in it. The recirculating system involves microbiological processes: Aerobic/anaerobic binding of nitrogen gas (N_2), autotroph/heterotroph nitrification processes and the aerobic/anaerobic oxidation processes of ammonium (NH_4^+) and the mineralization process[5]. Nitrogen waste removal is an important issue in culture environment. The recirculating system is used to avoid the toxic effects of N-inorganic produced by the culture. In aerobic conditions to eliminate ammonium, the ammonia (NH_3^-) oxidation process can be done by increasing the nitrification process which is by utilizing nitrate forming (ammonium oxidizers) and nitrite forming (nitrite oxidizer) bacteria so that ammonia is changed into nitrite then nitrate (nitrification).

The unstable condition between nitrification and denitrification in the environment strongly affects the organic and inorganic chemical condition in the environment and would affect the growth stability of aquaculture biota. On the 30th day of the vannamei prawn culture system in fish ponds, nitrite-producing bacteria could reach numbers[6] of up to 7.03 log CFU L^{-1} and the number continued to increase as the cultivated biota aged. Some other environmental factors which are related to the condition of nitrification bacteria are temperature, oxygen and pH[7]. The culture of mud crabs (*Scylla serrata*) has an effect on the ammonia, nitrite and nitrate contents which increase as the biota aged and this was related to the survival of the biota[8]. The nitrite produced on day 45 was 1.02 mg L^{-1} or equivalent to 22.173 μM and after day 100 the nitrite produced was 37.391±11.304 μM. Nitrite could inhibit the ability of haemoglobin in blood in binding oxygen, leading to the formation of methemoglobulin. The presence of nitrite in culture environments is extremely toxic to *Pacifastacus leniusculus* Dana (Crustacea: Decapoda) in the 48th h (LC_{50}- 0.7 mM NO_2^-). Continuous exposure to NO_2^- in culture media and active accumulation of nitrite in the hemolymph could cause inhibition of Cl^- absorption[9]. Crabs are able to absorb and distribute a variety of Na ions, Potassium and detect K-ATPase activity through their antennal glands[10].

The stability of the nitrification and denitrification process strongly determines the dynamics of ammonia, nitrite and nitrate in the environment. There needs to be an analysis of the activity of ammonia oxidizing bacteria, nitrite oxidizing bacteria and the amount of nitrate produced as they are important in the success of the recirculating system. This study was aimed to isolate and test the activity of ammonium oxidizing bacteria and nitrite oxidizing bacteria and to observe the growth dynamics of inorganic nitrogen which is produced in relation to the physiological response, growth and Survival Rate (SR) in the recirculating system culture of mud crab (*Scylla serrata*) in different shelters.

MATERIALS AND METHODS

Mud crab recirculating culture system (*Scylla serrata*). Ten mud crabs (*Scylla serrata*) weighing between 50 and 100 g were kept in culture tanks sized 60×80 cm with a capacity of 60 L of sea water which used the recirculating system. Data of environmental parameters included physical and chemical parameters of the water which were measured continuously and periodically so that the data could represent various supporting factors using Water Quality Checker (WQC) equipment with the APHA[11]. The test biota (mud crabs) were kept parallelly with three treatments triplicate. There were two sets of filtering tanks used as filters for each treatment consisting of two sets of closed system tanks and open system tanks each with a capacity of 220 L and had two water sluicegates, one inlet and one outlet. Water from the culture tanks which had a capacity of 60 L was then channelled to the FA filtering tank (closed system) which consisted of sand,

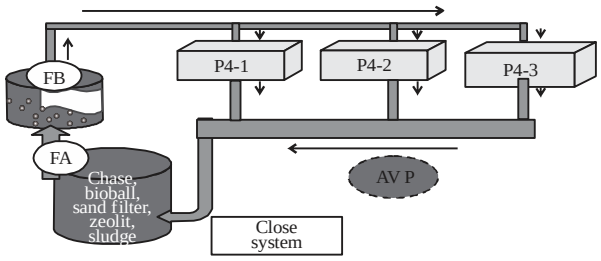

Fig. 1: Mud crab recirculating culture system (*Scylla serrata*)

activated charcoal, zeolith, bioball, gauze and cotton fiber. Then the water was channelled to the FB filtering tank (open system) without any addition of UGF and fiber glass then was channelled back to the culture tank and so forth (Fig. 1).

Samples were collected from a number of different locations in the culture system:

AV P4 : Culture tank with 4 shelters
AV P6 : Culture tank with 6 shelters
Control (FC) : Culture tank without any shelters
FA : Closed system filter tank
FB : Open system filter tank

The assessment of the activity of nitrification bacteria (ammonia/NH_3 oxidizing bacteria and nitrite/NO_2 oxidizing bacteria) were done on samples collected from locations P4, P6 and control using a composit method, each sample was assessed twice.

Nitrification bacteria isolation and morphology: Three of the last dilutions from the water samples (10^{-4}, 10^{-5} and 10^{-6}) from the the recirculating system were isolated in nitrification media which consisted of heterotrophic medium (using glucose as the source of carbon) and ammonia as the sole source of nitrogen and were incubated for 7 days. The activity assessment was conducted on 1 mL samples of water from various collection locations throughout the recirculating system and was then diluted using physiological NaCl solution (0.85%) through serial dilution. Then 1 mL of the results of the last three dilutions was inoculated to 9 mL liquid medium (growth medium) which had a composition (per liter) of 0.9 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.005 g $FeCl_3$, 0.1 g $MgSO_4.7H_2O$, 0.0184 g $CaCl_2$, 0.25 g yeast extract and 5 g Na_2CO_3 was added as a source of carbon. Samples were then incubated for 7 days on a shaker incubator at 80 rpm, room temperature (28-31°C). The cell growth and ammonium oxidation activity were observed on 7 and 8 days, then were observed for growth morphology. Ammonium oxidizing activity could be measured by analyzing the ammonium, nitrite and nitrate content. In addition to the amount of Ammonium Oxidized (AO), nitrite and other compounds that were produced, the microbe enzyme affinity was also calculated based on kinetic results (Michaelis-Menten/(K_m)) and the V_{max} produced[12,13].

Ammonium and nitrite oxidizing activity assessment: The percentage of Oxidized Ammonium (AO) and nitrate or nitrite compounds formed was calculated using the following equation:

$$AO = \frac{AK - AP}{AK} \times 100$$

Where:
AO = Percentage of oxidized ammonium
AK = The concentration of ammonium in the control medium
AP = The concentration of ammonium in the bacteria-inoculated medium

The percentage of the amount of nitrate produced (PNA) or the percentage of the amount of nitrite produced (PNI) was calculated using the following equation:

$$PNA = \frac{NT - NK}{AK - AP} \times 100$$

Where:
PNA = The percentage of amount of nitrate produced
NT = The nitrate content in the treatment suspension (inoculated with the bacterial isolate)
NK = The nitrate content in the control (not inoculated with bacteria)
AK = The ammonium content in the control
AP = The ammonium content in the treatment suspension

Nitrification bacteria abundance in the integrated recirculating system in the mud crab (*Scylla serrata*) culture: One milliliter of water sample from various collection

locations in the recirculating system was diluted using physiological NaCl solution (0.85%) through serial dilution. Then 1 mL of the results of the last three dilutions was inoculated to 9 mL growth medium and 5 g Na_2CO_3 which functioned as a source of C was added. Ammonia oxidizing bacteria were detected by adding 1 g NH_4Cl, while nitrite oxidizing bacteria were detected by adding 1 g $NaNO_2$. The samples were incubated for 7 days at room temperature. The ammonia oxidizer test was declared positive if the the bacterial culture produced was pink to purplish after the addition of the reagents sulfanilamide 1% and Naphthalene Ethylene Diamine (NED) 0.1%, whereas the nitrite oxidizer test was declared positive if the bacterial culture produced was yellow after the addition of the reagents brucine and 90% concentrated sulfuric acid (H_2SO_4)[13].

Analysis of ammonium, nitrite and nitrate: The chemical analysis of the quality of water as the culture medium was performed based on Greenberg *et al.*[13]. The total ammonia was measured using a spectrophotometer at a wavelength of 640 nm. Five milliliters of sample were filtered using 0.22 µL Whatman's filter paper and 0.2 mL of 10% phenol alcohol, 0.5% nitroprussid and 0.5 mL of a mixture of hypochlorite and citric acid 20% (1:4) were added. After being set aside for 1 h, reagent was added until the color changed to blue and was measured using a spectrophotometer.

The nitrite parameter was measured by taking 5 mL of filtered water sample and by adding 0.1 mL sulfanilamide 1% and 0.1 mL NED 0.1%, then setting it aside for 10 min. The addition of the reagent would produce a pink to purplish coloration and the color absorption was measured using a spectrophotometer. The nitrate content was measured by taking 5 mL of the water sample from the sytem and filtering it. Then 0.2 mL brucine 0.5% and 4 mL concentrated sulfuric acid were added then set aside for 0.5 h. After that, a reagent was added which would then result in a yellow color. The resulting absorption of the color was measured at a wavelength of 420 nm[11].

Data analysis: The data which were collected were tabulated and analyzed using Microsoft Excel 2007 and Minitab 2015. The analysis of variance (ANOVA) was conducted with the t-test (Tukey's test) at a confidence interval of 95%.

RESULTS

Morphologically, the most of ammonium oxidizing bacteria which grew in the system were translucent, slightly convex in elevation and spheroid. A small number of the others were flat in the elevation and were irregular in shape. The nitrite oxidizing bacteria were also dominated by these characteristics: translucent, flat elevation on the isolate and spheroid in shape (Table 1).

Based on the isolation results of the ammonia oxidizing bacteria on 7 and 8 days, it was discovered that the NH_4^+ oxidizing bacteria could oxidize an amount of 5.7126±5.47 µM or 33% and could form NO_2^- at an amount of 16.9748±0.05 µM or 56.916%. In P6, the ammonia oxidizing bacteria had an activity of 12.7273±5.89 µM or 74.0074% and could form NO_2^- at an amount of 16.7608±0.78 µM or 43.061% (Table 2).

The analysis of the Michaelis and Menten[12] kinetics for ammonium oxidizers revealed that P4 had a quantitative V_{max} (maximum velocity) of 0.0731 mM h^{-1} and a K_m

Table 1: Morphology of ammonium/NH_4^+ oxidizing bacteria and nitrite/NO_2^- oxidizing bacteria

Codes	Isolate code	Colony morphology	Cell colony
NH_4^+ oxidizing bacteria	P6.2	Medium, convex, translucent, mucous	100
	P4.1	Medium, translucent, circular, undulate, raised, gripped the medium, anchor-like	7
	P4.2	Medium, white, convex	7
	P4.3	Medium, translucent, circular, entire, raised	14
	P6.1	Medium, translucent, flat	7
Nitrite oxidizing bacteria	P4.1	Medium, flat, white	76
	P4.2	Medium, yellow, circular, undulate, raised	5
	P6.1	Large, white, circular, lobate, flat	20
	P6.2	Medium, undulate, convex, translucent	44
	P6.3	Medium, translucent, convex	120

Table 2: Activity of bacteria oxidizing ammonium into nitrite (ammonium oxidizer)

Isolate in treatment	Initial NH_4^+±SD	Oxidized NH_4^+±SD (µM)	Percentage of NH_4^+ oxidized	Average NO_2^- formed (µM)	Percentage of NO_2^- formed
P4	17.0068±0.87[a]	5.7126±5.47[a]	33.5904[a]	16.9748±0.05[b]	56.916
P6	17.1973±1.32[a]	12.7273±5.89[a]	74.0074[b]	16.7608±0.78[a]	43.061

Similar letters in one column demonstrates that there was no significant difference in a 5 % scale, $p<0.05$ a significant difference between treatments

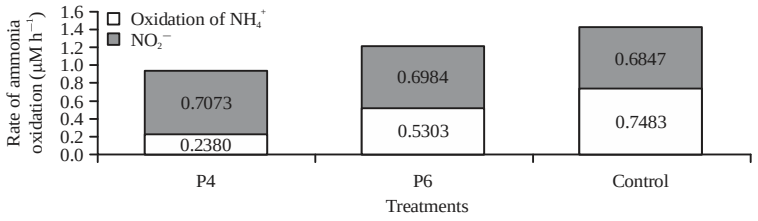

Fig. 2: Ammonium (NH_4^+) which was oxidized and nitrite accumulated by the mud crab Recirculating Aquaculture System (RAS)

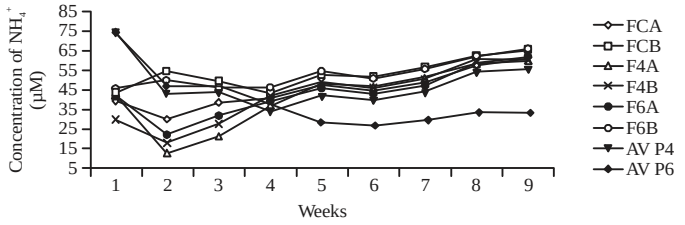

Fig. 3: NH_4^+ dynamics in the mud crab (*Scylla serrata*) Recirculating Aquaculture System (RAS)

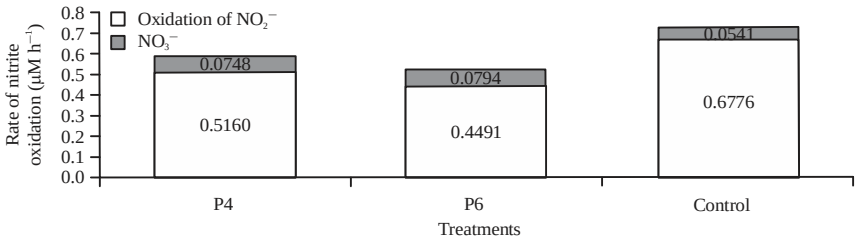

Fig. 4: Oxidized nitrite and nitrate accumulation in the mud crab Recirculating Aquaculture System (RAS)

Table 3: K_m and V_{max} values of ammonium oxidizers

Codes	K_m (mM)	V_{max} (mM h^{-1})
P4	0.1909	0.0731
P6	0.0788	0.0673
Control	0.1322	0.0704

(Michaelis-Menten constant) of 0.1909 mM, whereas P6 had a V_{max} of 0.0673 mM h^{-1} and a K_m of 0.0788 mM. The P6 was discovered to have a K_m value that was relatively lower, demonstrating that P6 had a higher enzyme affinity to the substrate than P4 (Table 3).

Treatment P4 had an NH_4^+ oxidation rate of 0.2380 μM h^{-1} and a nitrite accumulation of 0.7073 μM h^{-1}. The P6 had an NH_4^+ oxidation rate of 0.5303 μM h^{-1} and a nitrite accumulation of 0.6984 μM h^{-1} (Fig. 2).

The activity of the oxidizing bacteria and oxidation rate could be measured by observing the dynamics of NH_4^+ compound content which functions as the substrate for ammonium oxidizing nitrification bacteria in the Recirculating Aquaculture System (RAS). The dynamics of the total ammonia movement in the system had an increasing trend as time lapsed (from 1-9 weeks) except in tank P6 which had a decreasing trend after 4 weeks. The lowest total ammonia was observed in culture tank 6 where there was a decrease in the amount of amonium from 74.244 μM in 1 and continued to fall, reaching 27.33 μM in 6 weeks, then increased slightly in the system as the culture time lapsed (Fig. 3).

Based on the results of the nitrite oxidizing bacteria isolation results, on 7 and 8 days of incubation, the NO_2^- oxidizing bacteria were able to oxidize an amount of 12.3834±5.85 or 90.6581% and could form NO_3^- at an amount of 1.7955±0.17 μM or 12.8118%. The ammonia oxidizing bacteria from P6 had an activity of 10.7772±5.58 μM or 80.8669% and could form NO_2^- at an amount of 1.9046±0.68 μM or 11.0499% (Table 4).

Based on the K_m and V_{max} values, P4 had a higher K_m than P6, 0.0385 mM. The P6 had a lower Km value, only 0.0962 mM (Table 5).

Isolates grown from the mud crab Recirculating Aquaculture System (RAS) had very good nitrite oxidation and nitrate accumulation rates. Accumulation rate of nitrate formed in P4 was an average of 0.4491 μM h^{-1}, whereas in P6 it was 0.0794 μM h^{-1} (Fig. 4).

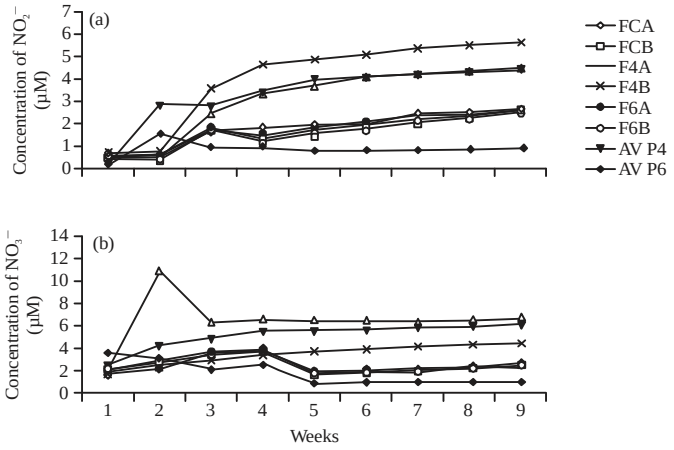

Fig. 5(a-b): Dynamics of (a) NO_2^- and (b) NO_3^- in the mud crab Recirculating Aquaculture System (RAS)

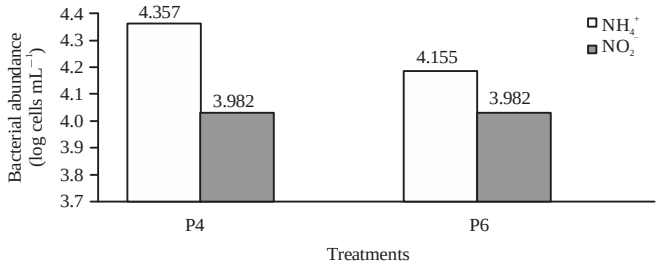

Fig. 6: Ammonium (NH_4^+) oxidizing bacteria and nitrite (NO_2^-) oxidizing bacterial abundance in the mud crab Recirculating Aquaculture System (RAS)

Table 4: Activity of bacteria in oxidizing nitrite into nitrate (nitrite oxidizer)

Isolate in treatment	Initial $NH_4^+ \pm SD$	Oxidized $NH_4^+ \pm SD$ (µM)	Percentage of NO_2^- oxidized	Average NO_2^- formed (µM)	Percentage of NO_3^- formed
P4	13.7795 ± 0.46^a	12.3834 ± 5.85^a	90.6581^b	1.7955 ± 0.17^c	12.8118^a
P6	13.3345 ± 0.68^a	10.7772 ± 5.58^a	80.8669^b	1.9046 ± 0.68^c	11.0499^a

Similar letters in one column demonstrates that there was no significant difference in a 5 % scale, $p<0.05$ a significant difference between treatments

Table 5: Nitrite oxidizer K_m and V_{max} values

Codes	K_m (mM)	V_{max} (mM h^{-1})
P4	0.0385	0.0749
P6	0.0962	0.0825
Control	0.0625	0.0160

The dynamics of nitrite compoud content in the mud crab Recirculating Aquaculture System (RAS) increased as time lapsed except in P6 which had a decreasing trend until 8 weeks, reaching 0.9933 µM, whereas in treatment P4, the environment had an constantly increasing amount of nitrite, from 0.251-5.054 µM in 8 weeks of the culture (Fig. 5a).

In contrast with the nitrite dynamics within the system, the nitrate content dynamics presented a decreasing trend as time lapsed (1-4 weeks). Starting 5 weeks, the decrease in nitrate content started to stabilize. However, the decrease in nitrate content reached a figure below 2 µM (Fig. 5b).

The inorganic compound oxidation rate is affected by the bacterial abundance/number of oxidizing bacteria within. In P4, using the MPN (Most probable number), the ammonia oxidizing bacterial abundance reached 4.357 log cells mL^{-1}, whereas the nitrite oxidizing bacteria had a lower abundance, 3.982 log cells mL^{-1}. The P6 had ammonia oxidizing bacterial abundance of 4.155 log cells mL^{-1} and a nitrite oxidizing bacterial abundance of 3.982 log cells mL^{-1}. This demonstrates that the ammonia oxidizing bacterial abundance of P6 was relatively lower than P4 (Fig. 6).

DISCUSSION

Cell domination in an environment demonstrates the presence of a group of cells based on the total bacterial colonies which grow the most based on the available substrat.

Nitrification bacteria in the mud crab (*Scylla serrata*) recirculating culture system with a culture medium of sea water with a salinity of 25 ppt demonstrated the dominance of bacterial colonies whose morphology was medium, convex, translucent and mucous for ammonium oxidizing bacteria using 6 shelters. On the other hand, the nitrite oxidizing bacteria were dominated by bacteria whose morphology was medium, translucent and concave using 6 shelters. Production of organic nitrogen from decomposition that would be affected inorganic materials and the activity of bacteria involved in the ammonification process in stable salinity conditions[14].

The activity of ammonium oxidizer is strongly affected by existing substrate and environmental conditions. If adjusted to the amount of substrate in the system, it seen that P6 culture had an average amount of ammonium concentration lower than P4 culture. The ammonium oxidizer activity rate was strongly affected and influenced by the condition and amount of available substrate. The P6 had a relatively higher ammonium oxidation activity and percentage than that of P4, these affect the amount or total content of ammonia in the environment, demonstrated by $p<0.05$ in the ANOVA analysis and Tukey's test. Potential ammonium oxidizer has a positive correlation with the enzymes involved in ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA)[15].

An enzyme's affinity affects its activity in oxidizing ammonia during the nitrification process. The gene ammonia monooxygenase enzyme (amoA) which is involved in the ammonia oxidizing process in the nitrification process[16]. Based on the kinetic results, P6's was lower than that of P4, demonstrating that P6's enzyme affinity towards the substrate was higher that of P4 ($p<0.05$).

The oxidation rate of the bacteria can be described by the ammonium oxidation activity in the determined time unit. On 7 and 8 days, P6 had a higher oxidation rate than that of P4. The bacteria's high ammonium oxidation rate in addition to the aforementioned high enzyme affinity resulted in a higher oxidized NH_4^+ percentage, leading to a lower NH_4^+ content in the environment compared to the other treatments. Camargo and Alonso[3] stated that when the water temperature and pH tend to increase, the concentration of NH_3^- would also increase but the concentration of NH_4^+would decrease and vice versa. As the concentration and exposure time increased, the ammonia and nitrite reduced the growth and survival rate of tiger crabs[17]. The dynamics of ammonium in the recirculating medium did not demonstrate a significant difference between P4 and P6, it was seen from the Tukey's test ANOVA result ($p>0.05$). Oxidation of ammonia into nitrite involves two processes, ammonia (NH_3^-) is converted into hydroxylamine (NH_2OH) by the ammonia oxidizing enzyme, ammonia monooxygenase (AMO) and the second step hydroxylamine (NH_2OH) is converted into nitrite (NO_2^-) by hydroxylamine oxidoreductase[18]. The enzyme AMO is coded by the a*moA* gene and enzyme HAO is coded by the *hao* gene[19]. Nitrite as the most unstable compound could be quickly oxidized into nitrate by the nitrite oxidoreductase (NOR) enzyme[20]. *Nitrospira* and *Nitrospina* spp., populate fresh water and sea water environments, especially in low NO_2 conditions[21]. Percentage results of the value of nitrite could be oxidized, P4 and P6 were not significantly different, it was seen from ANOVA test ($p>0.05$). The low percentage of nitrite oxidation by the bacteria did not describe a lower nitrite oxidizing bacteria activity in P6 compared to P4.

Nitrite oxidation condition can be affected to enzyme affinity by each treatment. Based on Michaelis and Menten[12] kinetic analysis results, nitrite oxidizer for P4 was that it had enzyme affinity higher than that of P6. In crustaceans, nitrite can bind with the hemocyanin in the blood, reducing the blood/haemolymph's ability to bind oxygen[22]. Russo *et al.*[23] stated that the presence of nitrite in aquatic environments could be in the form of nitrite ions (NO_2^-) and in the form of ionized nitrous acid (HNO_2). Based on the nitrite content, the p-value of P4 and P6 was less than 0.05, demonstrating that there was a significant difference between the nitrite in the recirculating system of 4 and 6 shelters. The amount of nitrite in aquatic environment is strongly affected by microbe diversity, ammonium oxidizing bacterial activity and enzyme affinity of the indigenous microbes.

The dynamic of inorganic nitrogen can be seen in the each filter (F4 and F6) of treatments, a significant effect on the condition of the crab culture environment (AV P4 and AV P6). Culture tank P6 had a higher ammonium oxidizer activity and a lower K_m (Michaelis-Menten), demonstrating a higher enzyme affinity. High enzyme affinity signifies the role of enzymes in microbial activity on the existing substrate and would have a positive effect on the amount of ammonium and nitrite in the aquatic environment. Production of organic nitrogen through the decomposition process will have a direct impact on organic matter and the bacteria involved in the ammonification process in stable salinity is high ammonium is accumulated at certain depths and would easily form organic materials in anoxic conditions[14]. However, for nitrate compounds, based on the covariant analysis performed, the nitrate values of P4 and P6 were significantly different ($p<0.05$). Nitrite produced through ammonia oxidation is converted to nitrate (NO_3^-) by the key enzyme nitrite oxidoreductase (NXR). Nitrification process in the environment

is usually performed by Chemolithoautothrophic bacteria, for example in waste-water treatment to reduce the ammonia burden[24].

Bacterial abundance demonstrates the density of bacterial cells in an ecosystem. In this recirculating system, the abundance of ammonia oxidizing bacteria had a tendency to be higher than the abundance of nitrite oxidizing bacteria. Bacterial abundance was supported by the results of the ammonia oxidizing activity which was higher than that of the nitrite oxidizing bacteria.

CONCLUSION

Activity assessment of ammonium oxidizer in the the recirculating culture system resulted in a relatively higher activity in P6 than in P4 and this was supported by the results of the kinetic test based on Michaelis-Menten quantitative test, which demonstrated lower results for P6 than that of P4, which could be interpreted as the enzyme affinity to substrate P6 was higher than that of P4. The total amount of ammonium and nitrite in the aquatic culture environment of P6 had tendency to be lower than those of P4 and the control.

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