Utilization of *Nitrosomonas* sp and *Nitrobacter* sp probiotic towards total suspended solid and ammonia level in nile tilapia culturing using aquaponic system

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Abstract. Nile tilapia (*Oreochromis niloticus*) is an aquaculture commodity which has high production and tendency to be cultured in intensive culturing system. However, the latter can accumulate high amount of organic waste (leftover feed and fish feces) which will cause poor water quality by increasing total suspended solid (TSS) level and ammonia level as well. The solution to the prior problem is by using the aquaponic system with the addition of *Nitrobacter* and *Nitrosomonas* bacteria in the probiotic application. Still, the effective probiotic dosage is yet to discover, so that this study aims to figure out the effective dosage of the probiotic to decrease TSS and ammonia level. This study used RAL as research design with 5 treatments repeated 4 times: control (no addition of probiotic), 0.5 mg/L, 1 mg/L, 1.5 mg/L, and 2 mg/L. The result shows that 1.5 mg/L is the most effective dosage which can decrease TSS and ammonia level into 16.18 ± 0.0571 mg/L and 0.178 ± 0.0673 mg/L respectively.

1. Introduction

Nile tilapia (*Oreochromis niloticus*) is an aquaculture commodity which has 1,084,281 ton production in 2015 and keeps increasing into 1,546,675 ton in 2018 [1]. A high number of Nile tilapia production is caused by the intensive culturing system, which leads to poor water quality. The latter is caused by accumulation of organic waste, which are leftover feed and fish feces [2]. Accumulation of organic waste can increase total suspended solid level (TSS) and ammonia level as well. The solution to the prior problem is by using aquaponic system. Aquaponics is a combination of aquaculture and hydroponic system, where leftover feed and feces in aquaponic system is used to fertilize the hydroponic component with closed recirculation system[3].

Besides closed recirculation system in aquaponic, the addition of *Nitrosomonas* and *Nitrobacter* in probiotic application is necessarily needed to minimalize organic waste accumulation by oxidizing ammonia compound into nitrate. The previous process is called as nitrification process [4]. Generated nitrate from nitrification process is absorbed by hydroponic component as a nutrient source [5]. Hence, the addition of *Nitrosomonas* and *Nitrobacter* probiotic in Nile tilapia culturing using aquaponic system hopefully can decrease total suspended solids and ammonia level by deciphering organic waste.

However, the effective dosage is yet to discover. The previous study stated that the addition of 1 mg/L *Nitrosomonas* and *Nitrobacter* probiotic does increase the biomass of 30 Nile tilapia fingerlings from 21 g into 25 g in 2 weeks [6]. The prior dosage is yet to know its effects on water quality parameter so that this study aims to figure out whether the addition of *Nitrosomonas* and *Nitrobacter* probiotic...
with the range ±1 mg/L dosage can decrease total suspended solids and ammonia level in Nile tilapia culturing using the aquaponic system.

2. Materials and Methods

2.1. Tools and Materials
This study is using 40 x 20 x 25 cm sized aquarium, 60 x12 x4,5 cm sized gutter as hydroponic system media, net pot, water pipe, water pump, aerator, rockwool, 3-5 cm sized Nile tilapia fingerlings, commercial fish feed, water spinach seed, *Nitrosomonas* and *Nitrobacter* probiotic, KMNO₄ 0.125 ppm, thermometer, DO meter, pH pen, and spectrophotometer.

2.2. Aquaponic system preparation
Aquaponic system preparation started off with sterilizing aquarium using KMNO₄, 0,125 ppm. Sterilized aquarium compiled with water pipe and gutter to form aquaponic system. Water spinach seed is sowed for 4 days, then planted on rockwool media inside the net pot. The gap for each net pot on the gutter is 10 cm. After the hydroponic system is completed, Nile tilapia fingerlings are added to the aquarium with the acclimatization process. The fish stocking density in each aquarium is 1 fish/L. Nile tilapia fingerlings are fed by commercial fish feed three times a day, with the feed amount of 5% fish biomass. *Nitrosomonas* and *Nitrobacter* probiotic with 5 different dosages is added as the main independent variable of the study. The dosage are 0.5 mg/L, 1 mg/L, 1.5 mg/L, 2 mg/L, and no addition of probiotic as control.

2.3. Data analyzing
Total Suspended Solid (TSS) and ammonia level in the aquaponic system are measured by spectrophotometry. Both TSS and ammonia value on spectrophotometry will be analyzed using Analysis of Variance (ANOVA). A significant result from the ANOVA test will be continued using Tukey HSD as post hoc test. The other supporting data such as water temperature, pH, Dissolved Oxygen (DO), Specific Growth Rate (SGR) of Nile tilapia, also stem length and total leaves of water spinach will be written descriptively.

3. Result and discussion
The result on table 1 shows that the addition of *Nitrosomonas* and *Nitrobacter* probiotic does decrease TSS level significantly (P < 0.05). The lowest TSS level occurred in P3 (16.18 ± 0.0571 mg/L) and P4 (16.68 ± 0.0557 mg/L). Tukey HSD post hoc test showed no significant difference between both P3 and P4 TSS level. Hence, the most effective probiotic dosage to decrease TSS level in an aquaponic system is 1,5 mg/L. The main source of TSS in the aquaponic system is anorganic substance (floated hydroponic roots, leftover feed, and fish feces) [7] and anorganic waste (rockwool)[8]. Decreased TSS level was occurred due to ammonia oxidation process by nitrification bacteria. Leftover feed and feces contained ammonia compound, which the latter will be deciphered by nitrification bacteria [9]. Reducing ammonia level from organic waste also reducing TSS level by degrading the organic waste itself. Bacteria do have a big role to control water quality by degrading organic substance [10].

| Treatment | TSS (mg/L) ±SD | Ammonia (mg/L) ±SD |
|-----------|----------------|--------------------|
| P0        | 21.93a ± 0.0644 | 0.264a ± 0.0046    |
| P1        | 19.91ab ± 0.0671 | 0.2185b ± 0.0156   |
| P2        | 19.28b ± 0.0586  | 0.214b ± 0.0048    |
| P3        | 16.18c ± 0.0571  | 0.178c ± 0.0673    |
| P4        | 16.68c ± 0.0557  | 0.172c ± 0.0673    |

Overall TSS average level ranged between 16.18 – 21.93 mg/L, where those TSS level considered safe as tolerated by cultured fish. Indonesia government regulation number 82 in 2001 [11] stated that
the maximum TSS level for aquaculture is 50 mg/L. High TSS level can cause hypoxia in fish due to obstructed suspended particles filtered in fish gill.

The result in table 1 also showed the addition of *Nitrosomonas* and *Nitrobacter* does decrease the ammonia level significantly (P<0.05). Lowest ammonia level occurred in P3 (0.178 ± 0.0673 mg/L) and P4 (0.172 ± 0.0673 mg/L). Tukey HSD post hoc test showed no significant difference between both P3 and P4 ammonia level. Hence, the most effective probiotic dosage to decrease the ammonia level in the aquaponic system is 1.5 mg/L. Decreasing ammonia level is occurred due to the nitrification process by *Nitrosomonas* and *Nitrobacter* bacteria. The main source of ammonia in the aquaponic system is leftover feed and fish feces. Leftover feed contained protein, then protein degradation into amino acid. Protein degradation process explained as a process where protease enzyme hydrolyzes protein into polypeptide compound, oligopeptides, and amino acids [12]. Amino acid then modified into ammonia by deamination process [1]. Ammonia (NH₃) hydrolyzed and become ammonium (NH₄⁺). Ammonium oxidized into nitrite (NO₂⁻) by *Nitrosomonas* sp. as Ammonia-Oxidizing Bacteria. The process continued by oxidizing nitrite into nitrate (NO₃⁻) by *Nitrobacter* sp. as nitrite-oxidizing Bacteria. Prior process is known as nitrification process [13]. Highest ammonia level occurred in P0 or known as an aquaponic system without any addition of probiotic. The highest ammonia level is 0.264 ± 0.0046 mg/L. High ammonia level in the aquaponic system without any addition of probiotic is occurred due to limited nitrification bacteria which generates naturally.

| Table 2. Water temperature, dissolved oxygen, and pH average level |
|---------------------------------------------------------------|
| **Treatment** | **Measurement day** | **Water temperature (°C)** | **DO (mg/L)** | **pH** |
| P0 | Day 0 | 28.58 | 4.708 | 7.825 |
|   | Day 28 | 28.43 | 4.573 | 8.037 |
| P1 | Day 0 | 28.82 | 4.802 | 7.912 |
|   | Day 28 | 28.85 | 3.337 | 7.225 |
| P2 | Day 0 | 28.87 | 4.665 | 7.9 |
|   | Day 28 | 28.26 | 3.24 | 7.25 |
| P3 | Day 0 | 28.97 | 4.58 | 7.85 |
|   | Day 28 | 28.93 | 3.012 | 7.175 |
| P4 | Day 0 | 28.76 | 4.61 | 7.875 |
|   | Day 28 | 28.76 | 3.142 | 7.225 |

Please write the result measuring of water temperature during experiment.

Overall ammonia average level ranged between 0.172 – 0.264 mg/L. Those range of ammonia level considered safe for fish culture. Maximum tolerated ammonia level by Nile tilapia is 2 mg/L. Ammonia level higher than 2 mg/L can cause damage in fish gill, liver, and kidney tissue [14].

Overall water temperature from each aquarium ranged between 28.26 – 28.97°C. Those range of water temperature considered optimal for Nile tilapia growth and well-tolerated by nitrification bacteria. Optimal water temperature range for Nile tilapia growth is 25 – 30°C [15], while tolerated water temperature for nitrification bacteria ranged between 5-30°C [1].

Dissolved Oxygen (DO) level in P0 described as relatively stable with DO level 4.573 mg/L on day 28, whereas DO level on the other treatment (besides P0) decreasing with DO level ranged between 3.012 – 3.337 mg/L on day 28 (table 2.). Decreasing DO level in the aquaponic system with the addition of *Nitrosomonas* and *Nitrobacter* probiotic is caused by the nitrification process by *Nitrosomonas* and *Nitrobacter*, where both of them are aerobic bacteria. The latter explained as bacteria which uses oxygen in a certain amount to oxidized organic substance [16]. Overall DO level categorized as well tolerated by both Nile tilapia and nitrification process wherein tolerated DO for Nile tilapia ranged between 2 – 5 mg/L and tolerated DO for nitrification bacteria is 2 mg/L and above [16].

pH level in P0 described as relatively stable with pH level 8.037 on day 28, while pH level on the other treatment considered decreasing with pH level ranged between 7.175 – 7.25. Decreasing pH level is due to the oxidation process by nitrification bacteria [17]. Those pH range categorized as well
tolerated by Nile tilapia and nitrification bacteria, where Nile tilapia can tolerate pH level 6 – 8.5 [18] and nitrification bacteria can tolerate pH level 5.8 – 8.5 [1].

4. Conclusion
The addition of *Nitrosomonas* and *Nitrobacter* probiotic in Nile tilapia culturing using aquaponic system decreased TSS and ammonia level significantly, with the most effective dosage at 1.5 mg/L.

5. References
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