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Investigation of bacteria composition in *Artemia* biomass culture under different conditions

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ABSTRACT

This study was conducted to determine the bacterial composition and its effect on *Artemia* performance in various *Artemia* biomass culture conditions. Newly hatched *Artemia franciscana* were cultured in 1.5L plastic bottles containing 1L of culture medium, with a density of 200 nauplii/L and salinity 30 ppt, and lasted for three weeks. In the first two days after stocking, *Artemia* was fed with *Chaetoceros* sp. Afterward, *Artemia*-formulated feed was used as daily food. In biofloc and bacteria treatments, the C/N ratio was regularly manipulated in the *Artemia* culture medium from day 5 and onwards. The statistical analysis results showed that culture conditions, as well as the bacterial development in those culture batches, affected *Artemia* performance. The best survival rate, individual length, and biomass were obtained in the seawater and biofloc treatment after 21 days of culturing. The total bacteria varied from 83–146 × 10^3 CFU/mL, with the highest value seen in the biofloc (BF) treatments. The biofloc treatments had a significantly higher bacterial density than the non-biofloc treatments. *Nitrosomonas* was better developed in seawater (SW) while the *Nitrobacter* was in sea salt (SS) medium.

INTRODUCTION

Artemia is a popular live feed used in aquaculture because of its high nutritional value and appropriate size (Sorgeloos *et al*., 1998). Adult *Artemia* has a high nutrient content, with high levels of protein (50 – 60%), amino acids, vitamins, and some digestive enzymes. In recent years, *Artemia* biomass culture has received more attention because of its wide use as a food source for fish, shrimp, and prawn (Nguyen *et al*., 2010; Nguyen, 2011). However, a large amount of biomass is only grown in outdoor systems, such as salt fields, which means that the biomass production is seasonal and is heavily influenced by the natural environment (Nguyen and Huynh, 2019). Therefore, indoor production of *Artemia* biomass is not only essential for the creation of an active biomass source, but also reduces the risk of pathogens and predators. However, indoor *Artemia* culture is quite costly because of the need for seawater transport, electricity power, feed, and labour (Nguyen and Huynh, 2019). To solve the cost problem, the replacement of the culture environment from seawater to sea salt has been

studied by Nguyen and Huynh (2019). The results of this experiment show that, when replacing seawater with sea salt at the rate of 50–70%, or even 100%, it is possible to apply *Artemia* biomass culture. However, there was an unknown amount of microflora in the sea salt medium, as it lacked many trace elements present in seawater (Kolev*et al*., 2013; Nguyen and Huynh, 2018). Additionally, biofloc technology, a method of water quality management based on heterotrophic bacteria growth that does not use water exchange or the addition of probiotic bacteria such as *Bacillus* into the culture medium to enhance the culture success (Nguyen *et al*., 2014; Nguyen and Huynh, 2019; Yao *et al*., 2018; Ngo *et al*., 2016),was recently applied in an *Artemia* biomass culture. According to Huynh (2013), heterotrophic bacteria in the biofloc system have a small size (3–5 µm), high nutrient value, and can be used as feed for *Artemia*. However, there is a lack of research on the development of bacteria in biofloc–incorporated *Artemia* culture systems, particularly regarding *Bacillus* spp., nitrogen-converted bacteria groups (namely *Nitrosomonas* spp. and *Nitrobacter* spp.) and pathogenic bacteria groups, like *Vibrio* spp., were previously believed to be strongly affected by culture conditions and to be related to the success of the culture batch. Our study stems from this context and aims to obtain results that can fill in knowledge gaps and further help advance for indoor *Artemia* biomass culture.

MATERIALS AND METHODS

Material

The seawater culture medium (SW) was prepared by diluting high saline water (80 ppt; collected and transported from Vinh Chau) in tap water to a salinity of 30 ppt, while the sea saltwater culture medium (SS) was prepared by dissolving 3 kg of sea salt in 100 L of tap water. Both mediums were then disinfected with 30 mg/L chlorine and strong aeration of both prepared mediums.

One gram of *Artemia franciscana* Vinh Chau strain was added into a 1.5 L conical plastic bottle containing 1 L of seawater at 30 ppt; the optimal conditions for hatching were maintained at a pH of about 7.5–8.6, with the temperature at 28°C, light 1000 lux and with continuous aeration (Sorgeloos *et al*., 1986). After 24h of incubation, newly hatched *Artemia* nauplii were harvested for stocking into the different culture medium (experimental treatments).

Bacillus sp. was isolated from a shrimp grow-out pond, showing good results in regard to *Artemia* performance (Ngo *et al*., 2016) was mass cultured and used in this experiment.

Experimental design

A two factor experiment was set up, with two kinds of culture medium (sea salt/seawater) and three supplemental compounds producing six combination treatments as follows: (1) Seasalt-blank (nothing added, control; SS-B), (2) Seasalt-Bioflocs (added molasses; SS-BF), (3) Sea salt-*Bacillus* (added *Bacillus*; SS-*Bacillus*), (4) Seawater-Blank (nothing added, control; SW-B), (5) Seawater-Bioflocs (SW-BF), (6) Seawater-*Bacillus* (added *Bacillus*; SW-*Bacillus*). The experiment was conducted over the course of three weeks, each treatment undergoing three replicas.

Management

The system was designed in 1.5 L conical plastic bottles containing 1 L culture medium with a density of 200 nauplii/L, with light aeration. All treatments were kept in temperature-controlled room. *Artemia* were fed with *Chaetoceros* sp. for the first two days and then fed formulated feed twice a day (morning and afternoon) until the end of the experiment according to the feeding table of Nguyen (1993).

Biofloc (C:N = 5): according to Nguyen and Huynh (2018), using a formula of C:N = 5 produced *Artemia* with better length and biomass. Molasses (40% carbon) was added from day 5 onward and regulated in treatments every 3 days based on the TAN concentration measured in the culture medium (1 mg TAN was given 12.5 mL of molasses).

Bacillus sp., were added once a week to those bacteria (added *Bacillus)* treatments at 10^4 CFU/mL from day 5 onwards.

Water exchange was performed only for control treatments at 30% of culture volume on days 7 and 14, while biofloc and bacteria treatments did not take place for the entirethe experiment.

Data collection

Temperature and pH were measured at 8 AM and 2 PM by Hanna pens. TAN was measured every three days and NO_2^- was recorded weekly using a Sera test (Germany).

Survival at days 7 and 14 after hatching was counted individually and calculated according to the formula:

$$\text{SR (\%)} = \text{final number of } \textit{Artemia} \text{ at day 7 or 14/ Initial number of } \textit{Artemia} \text{ at stock x } 100$$

Individual lengths of *Artemia* on days 7and 14 were recorded by randomly measuring the individual length of 30 *Artemia* in each treatment. *Artemia* was measured from the head to the telson of the organismunder a specific microscope (Olympus SZ51, Japan) and calculated with the formula:

$$L\ (mm) = A/10 \times 1\gamma$$

where L is the length of *Artemia* (mm), A is the number of bars in the microsope ruler andγ is binocular magnification.

Final *Artemia* biomass production is a lump sum of biomass harvested in wet weight and was collected at the end of the experiment.

Bacterial samples were collected from day 14 of the culture and periodically every three days after that until end of experiment by using sterilized Falcon tubes and TCBS, NA, MSM, nitrite/nitrate-calcium-carbonate mediums for analysis and density determination. The total bacteria, *Bacillus* and *Vibrio*, were determined with the agar-plating method while the nitrogen-converted bacteria group was determined with the MPN (Most Probable Number) system (Ehrlich, 1975). For the MPN method, ammonium-calcium-carbonate medium was prepared for ammonia oxidizing bacteria (AOB) and nitrite-calcium-carbonate medium for nitrite oxidizing bacteria (NOB).

Bacterial composition determination

Vibrio spp. and total bacteria was cultured on agar plates using TCBS and NA media, respectively (HiMedia Laboratories, India). *Bacillus* spp. was cultured in a *Bacillus*-selective medium (Harwood and Archibald, 1990; Ngan, 2012). Only plates showing CFU between 30 and 300 colonies were counted and reported as estimated CFU/mL or CFU/g (APHA, 2017). The number of bacteria was calculated with the formula:

Bacteria density (CFU/mL) = number of colonies× dilution

N-converted group (AOB and NOB) was estimated by looking up the value in the most probable number technique –MPN (dos Reis Souza *et al*., 2020).

Data analysis

The data were calculated using mean and standard deviation on Excel software; the statistical program Statistica version 7.0 was used to compare the mean between the treatments using two way-ANOVA and Tukey-HSD analysis at the significance level ($p < 0.05$). For bacteria density, the data was log transformed before statistical analysis was run.

RESULTS AND DISCUSSION

1 Environmental factors

During experiment, the average temperature in the morning ranged from 22.6–22.7^{o}C and the afternoon fluctuated from 25.5–25.6^{o}C. The average pH values in the morning and the afternoon ranged from 8.27–8.28 and 8.43–8.47, respectively. Abiotic factors during the experiment were not significantly different between treatments ($p > 0.05$), and these factors were in the appropriate range for *Artemia* development (Nguyen *et al*., 2007).

The concentrations of TAN (NH_3/NH_4^+) and NO_2^- in the experiment tended to be low at the beginning and increase gradually over time due to the accumulation of organic matter, such as feces, peeled shells, leftovers, etc. The creation of biofloc b adding mollasses, as well as of *Bacillus*, had relatively low NO_2^- and TAN content due to the active activity of nitrogen-metabolizing bacteria, especially *Nitrosomonas* and *Nitrobacter* (Nguyen *et al*., 2021; Ngo *et al*., 2015).

The TAN and NO_2^- concentration during the experiment are shown in Table 1. Throughout the 21-day experimental period, TAN and NO_2^- concentrations tended to increase gradually throughout, ranging from 0.5–2.0 mg/L, respectively. The biofloc treatments had lower concentrations than the non–biofloc treatments, which is consistent with Nguyen and Huynh (2019). However, compared with previous studies, the concentrations of TAN and NO_2^- were higher, but still within the appropriate range for *Artemia* to grow and develop, as it is a species that can adapt to environments with high levels of TAN and NO_2^- (Dhont and Lavens,1996).

Table 1. Average TAN and NO_2^- concentrations during the experiment.CM: culture medium; SC: supplemental compound; SS-SW: sea salt–seawater; BF: biofloc; blank: control treatment. The standard deviation was added (mean ± SD) for each value. Values in a single column for each part showing different superscript letters are significantly different ($p<0.05$)

Factor	Treatment code	TAN (g/L)	NO_2^-(g/L)
Culture medium (CM)	SS	1.02 ± 0.17^a	0.96 ± 0.25^a
	SW	1.00 ± 0.19^a	0.93 ± 0.24^a
Supplemental compound (SC)	Blank (control: B)	1.18 ± 0.12^a	1.17 ± 0.00^a
	Biofloc (BF)	0.82 ± 0.06^b	0.75 ± 0.09^b
	Bacillus	1.04 ± 0.12^a	0.92 ± 0.27^{ab}
CM + SC	SS-B	1.19 ± 0.15^a	1.17 ± 0.00^a
	SS-BF	0.83 ± 0.04^b	0.72 ± 0.10^b
	SS-*Bacillus*	1.05 ± 0.04^{ab}	1.00 ± 0.29^{ab}
	SW-B	1.17 ± 0.11^a	1.17 ± 0.00^a
	SW-BF	0.81 ± 0.08^b	0.78 ± 0.10^b
	SW-*Bacillus*	1.02 ± 0.16^{ab}	0.83 ± 0.29^a

Statistical results showed that the addition of different supplemental compounds to the *Artemia* culture medium had an impact on the concentration of TAN and NO_2^- in the experiment ($p < 0.01$; Table 1). However, the use of different types of culture medium and the interaction between the two factors did not affect the concentration of TAN and NO_2^- in water (p-value ranging from 0.54–1.00). The data in Table 1 indicates that biofloc medium was always the best option in term of removing nitrogen products, and that TAN and NO_2^- was lower ($p < 0.05$) compared to the control and *Bacillus*, although he *Bacillus* also enhanced the water quality. Many previous studies have confirmed that adding *Bacillus* or molasses to *Artemia* culture not only led to better *Artemia* performance but

also improved water quality criteria by the activation of bacteria in a nitrogen cycle (Huynh *et al*., 2013; Ngo *et al*., 2014; 2016). *Bacillus* act as probiotic bacteria, while molasses provides a substrate (carbon) for the growth of heterotrophic bacteria.

2 Effect of culture conditions on *Artemia* performance

2.1 Survival rate

The survival rate of *Artemia* is shown in Table 2. In the first week, the survival rate of *Artemia* fluctuated from 86.5–91.2% and then decreased to 63.8–78.3% by the following week. Statistical analysis on survival at D7 (day 7; Table 2) indicated that neither culture medium nor the supplemental compound (BF and *Bacillus*) played a role in survival of *Artemia* ($p > 0.05$), although there was an interaction between them ($p = 0.02$). The highest survival was recorded in the SW-BF treatment, at 91.2%, and was statistically different ($p < 0.05$) from SS and SW-*Bacillus* treatment (87%). There was no statistical difference ($p > 0.05$) with with the remaining treatments.

Table 2. Survival, individual length, and biomass of *Artemia* in different treatments. CM: culture medium; SC: supplemental compound: SS-SW: sea salt–seawater; BF: bioflocs; blank: control treatment. The standard deviation is added (mean ± SD) for each value. Values in a single column for each part showing different superscript letter are significantly different ($p<0.05$)

Factor	Treatment code	Survival rate (%)		Individual length (mm)		Biomass (g/L)
		Day 7	Day 14	Day 7	Day 14	
CM	**SS**	88.8 ± 1.9^{a}	68.0 ± 4.2^{b}	3.7 ± 0.7^{b}	5.7 ± 1.2^{b}	3.7 ± 0.4^{b}
	SW	88.6 ± 2.7^{a}	73.4 ± 4.3^{a}	4.3 ± 0.8^{a}	6.7 ± 1.3^{a}	4.2 ± 0.7^{a}
SC	**Blank**	88.0 ± 2.3^{a}	66.4 ± 3.1^{c}	4.3 ± 1.2^{a}	5.0 ± 0.7^{c}	3.6 ± 0.7^{b}
	BF	90.3 ± 1.5^{a}	75.7 ± 3.4^{a}	4.1 ± 0.4^{b}	7.5 ± 0.8^{a}	4.3 ± 0.6^{a}
	Bacillus	86.5 ± 2.5^{a}	70.1 ± 3.4^{b}	3.7 ± 0.4^{c}	6.1 ± 0.8^{b}	4.0 ± 0.2^{ab}
CM + SC	**SS-B**	86.5 ± 0.9^{b}	63.8 ± 0.7^{d}	3.5 ± 0.5^{c}	4.5 ± 0.5^{d}	3.3 ± 0.5^{b}
	SS - BF	89.3 ± 0.4^{ab}	73.0 ± 0.5^{b}	4.3 ± 0.3^{b}	6.9 ± 0.5^{b}	3.8 ± 0.3^{ab}
	SS - *Bacillus*	89.8 ± 0.3^{a}	67.2 ± 0.4^{cd}	3.4 ± 0.3^{c}	5.6 ± 0.4^{c}	3.8 ± 0.1^{ab}
	SW-B	89.5 ± 0.7^{ab}	69.0 ± 0.4^{bc}	4.1 ± 0.4^{b}	5.4 ± 0.4^{c}	3.8 ± 0.8^{ab}
	SW - BF	91.2 ± 0.4^{a}	78.3 ± 0.7^{a}	5.5 ± 0.4^{a}	8.1 ± 0.5^{a}	4.9 ± 0.3^{a}
	SW - *Bacillus*	86.7 ± 0.4^{b}	73.0 ± 0.7^{b}	3.9 ± 0.3^{b}	6.6 ± 0.7^{b}	4.1 ± 0.1^{ab}

On D14 (day 14) there was a reversible trend with D7 (day 7), with the results showing that both culture medium and the supplemental compound affected *Artemia* survival ($p<0.05$), but no interaction between them was found ($p>0.05$). The seawater was associated with a better survival than sea salt (73.4% versus 68%; $p<0.05$) and the survival rate of supplement compound wasnot significantly different when compared among BF, *Bacillus* and the control ($p<0.05$). As a sequence of those differences, highest

survival rate was also found in the SW-BF treatment, at 78.3%, and was significantly different from the other treatments ($p<0.05$; Table 2).

The different effects of experimental factors (culture medium and supplemental compound) at D7 and D14 were probably due to the presence of heterotrophic bacteria in the culture. According to Nguyen and Huynh (2019), when molasses is added at a ratio of C:N = 5, it promotes the growth of heterotrophic bacteria in the environment increases survival rate of *Artemia*. Pham (2014) also reported that the addition of a mixture of *Bacillus* bacteria could contribute to the increased survival rate of *Artemia* compared with no supplementation. Because the molasses and *Bacillus* were added on day 5, its effect on D7 was still not clear. However, at D14, the bacteria in the culture medium had already developed and proliferated such that its effect became strong. The SS medium, despite having similar macronutrients as SW but lesser amounts of many trace elements found in natural seawater (Kolev*et al*., 2013), may cause a lack of these traces when *Artemia* becomes bigger, resulting in lower survival.

2.2 Length

Individual length of *Artemia*is presented in Table 2. At day 7, the length of *Artemia* was in the range of 3.4 – 5.5 mm and 4.5 – 8.1 mm after 14 days of rearing. The statistical analysis showed that both culture medium and supplemental compounds played a significant role to the individual length of *Artemia* on day 7 ($p < 0.05$), and that the interaction of these factors also affected *Artemia* length ($p < 0.05$). Similarly, the length of *Artemia* on day 14 was also affected by culture medium and supplemental compound but there was no interaction between them ($p > 0.05$). The SW-BF treatment yielded the highest length on both days 7 and 14, at 5.5 and 8.1mm, respectively, and was significantly different ($p < 0.05$) from the other treatments.

The length of *Artemia* tended to be higher in the seawater treatment and lower in the sea salt treatment, while the supplemented treatments, the biofloc, and *Bacillus* treatments yielded higher *Artemia* length than the control treatments only in day 14, clearly showing their effects (the addition of *Bacillus* and molasses was only done from day 5). These results were consistent with former studies from Huynh *et al*.(2013), as the results showed that more heterotrophic bacteria were grown in bioflocs. In addition, some beneficial bacteria in *Bacillus* treatment can be a direct food source for *Artemia*, improving length (Nguyen *et al*., 2007).

The difference in the effect of the culture medium and supplemental compound on the length of bacteria in this experiment can be attributed to the addition of biofloc to the culture system, as biofloc promoted the growth of heterotrophic bacteria in the culture medium and increases *Artemia* length (Nguyen and Huynh, 2019). Moreover, *Artemia* length was greater in seawater than in sea salt because of the higher trace mineral content in seawater (Kolev*et al*., 2013). The interaction of two factors only affected *Artemia* length on day 7, but did not affect it on day 14, which can be explained by *Artemia*

having reached the adult stage by day 14 and was able to adapt to the environment better. Moreover, as explained above, molasses and *Bacillus* were added on day 5, meaning that the bacteria likely had not occupied the entire culture medium by day 7 and therefore expressed different conditions on day 14.

2.3 Biomass at harvest

After 21 days, biomass of *Artemia* fluctuated from 3.3–4.9 g/L (Table 2). The highest biomass in the SW–BF treatment (5.9 g/L) was significantly different ($p < 0.05$) from the lowest in the SS treatment (3.3 g/L). The other treatments were in the intermediate range between the lowest and the highest values. Although there were differences between treatments, this difference was not statistically significant ($p > 0.05$). When using seawater to culture *Artemia*, the biomass at harvest was 1.1 times higher than in salt water and the difference was statically significant ($p < 0.05$). However, there was not much difference between the treatments. The results indicate that the supplemental compound also affected biomass of *Artemia*, and that biofloc and *Bacillus* treatments were associated with a higher biomass than the control treatment and were statistically significant ($p < 0.05$). However, the interaction between two factors produced a non-statistically significant effect on the biomass of *Artemia* ($p > 0.05$).

Nguyen and Huynh (2019) also reported that using biofloc as a supplemental compound can increase the development of heterotrophic bacteria and the biomass at harvest compared to culture under normal conditions. Here, the non–biofloc treatments did not produce the conversion of nitrogen from waste to bacterial cell establishment as the biofloc treatments. Like the survival rate and length of *Artemia*, the biomass of *Artemia* was higher when cultured in seawater than in saltwater because of the higher trace mineral content (Kolev*et al*., 2013).

3 Effect of culture conditions on the growth of bacteria

In aquatic environment, expecially culture conditions where feed with high protein was used, it will quickly stimulate the presence of microbial nitrogen cycling in which amonium derived from extra feed, animal wastes... is oxidized to nitrite and further to nitrate (NO_3^- is the plant accessible form) by two functional groups kown as nitrifying bacteria including ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), sometimes referred as *Nitrosomonas* and *Nitrobacter* as major genus, therefore these bacteria are play a vital role in nitrogen cycle of aquaponics well as terrestial environment (Gee *et al*., 1990; Stief, 2013). In aquaculture, maitaining of these bacteria at a heathy level concerns to a good water quality of the culture system, resulting in a culture suscess.

Whenever bacteria are introduced into a new environment, they need time to develop their population via growth and competition with opportunistic bacteria. Emerenciano *et al*. (2017) confirmed that in a bioflocs system, heterotrophic bacteria

colonize the feces, molts, dead organisms and unconsumed food to produce bacterial biomass and then the nitrifying bacteria are naturally promoted by the presence of ammonia and the accumulation of flocculated matter (used as a substrate). In former studies on this issue have also shown that, a week after inoculation of probiotics in shrimp culture and nursery, bacteria densities, especially *Nitrosomonas* and *Nitrobacter* were still low (Pham *et al*., 2010; 2021). So in the present study, the sampling for bacteria density determination was begin at day 14 in order to let bacteria growing.

Statistical analysis on the effect of culture conditions on bacteria is shown in Table 3. The results indicate that total bacteria and *Bacillus* were affected by culture conditions involving culture medium, as well as the addition of probiotics or molasses, more than by nitrogen-converted bacteria (known as nitrifying bacteria, such as *Nitrosomonas*, *Nitrobacter*, and other heterotrophic bacteria). The nitrogen converted bacteria was affected by culture medium only.

Table 3. p-value of experimental factors on bacterial density

	ANOVA VALUE P							
Treatment	**Total bacteria**		***Bacillus***		**AOB (*Nitrosomonas*)**		**NOB (*Nitrobacter*)**	
	Day 14	**Day 21**	**Day 14**	**Day 21**	**Day 14**	**Day 21**	**Day 14**	**Day 21**
CM	<0.01	<0.01	<0.01	0.849	0.284	<0.01	<0.01	<0.01
SC	<0.01	<0.01	<0.01	<0.01	0.394	0.776	0.556	0.441
CM*SC	<0.01	<0.01	<0.01	0.119	0.01	0.01	0.129	0.073

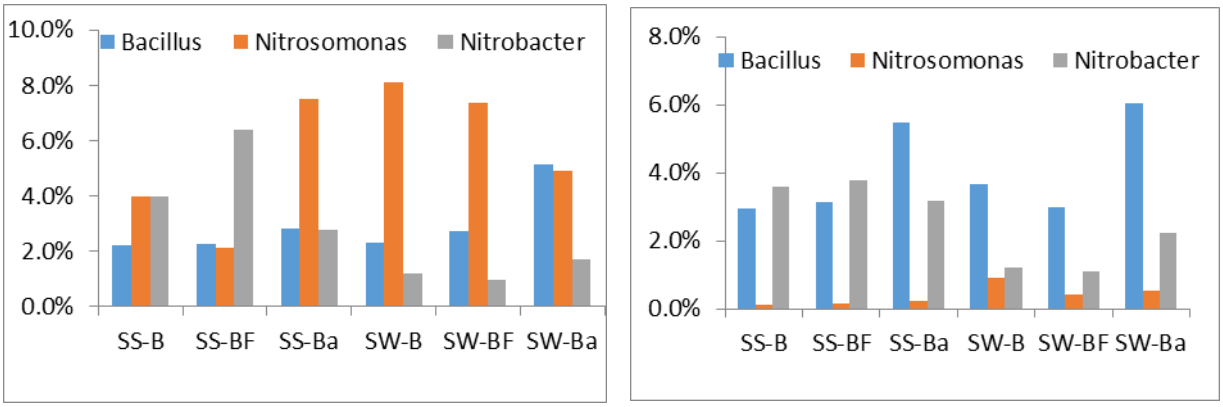

Figure 1. Percentage of *Bacillus*, *Nitrosomonas* and *Nitrobacter* in relation to total bacteria at day 14 (left) and day 21 (right) of culture.

Results from Fig.1 show that *Bacillus* accounted for 2.2–6%, *Nitrosomonas* was at 0.13–8.1%, and *Nitrobacter* was 1–6.2% of the total bacteria during experiment. The *Bacillus* increased in density, were 3–6% of total bacteria at day 21, when longer culture was run. The nitrogen converted bacteria reached peaks at day 14 and slowed down at day 21, especially *Nitrosomonas* presented with less than 1%.

3.1 Total bacteria

The variation of total bacterial density at day 14 and 21 is shown in Fig.2. The results indicate that the bacterial density tended to reach peak at day 14 (101×10^3–146×10^3 CFU/mL) and decrease at the end of the experiment (day 21), with a density of 58×10^3– 83×10^3 CFU/mL. The SW–BF treatment and SS–BF treatments reached the peak density in day 14 and 21, with densities of 146×10^3 CFU/mL and 83×10^3 CFU/mL, respectively. On both days 14 and 21, the two treatments were not statistically different ($p > 0.05$) from each other and significantly higher ($p < 0.05$) than the other treatments.

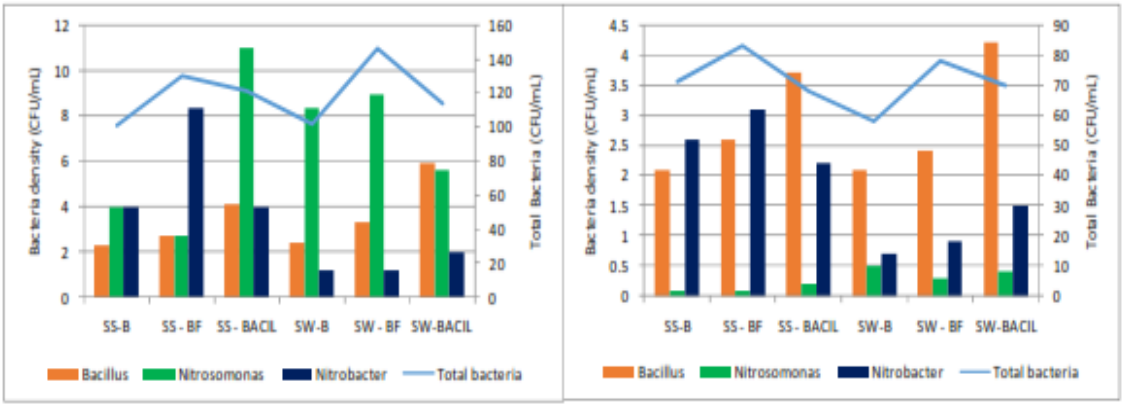

Figure 2. Bacterial density in the experiment on days 14 (left) and 21 (right).

According to the statistical results (Table 3), both the culture medium and supplemental compounds had an impact on total bacterial density ($p < 0.01$). Additionally, the interaction of these two factors also created an effect on total bacterial density throughout the experiment ($p < 0.01$). This can be explained by the fact that, since most heterotrophic bacteria need a substrate to grow on, the addition biofloc not only provided the substrate but also nutrients for them (Nguyen *et al*., 2014). Therefore, the treatments with added biofloc had a higher total bacterial density than the non–biofloc treatments. Beside, the culture mediumm (seasalt and seawater) eventhough have similar macronutrients but seasalt contains lesser amounts of many trace elements found in natural seawater (Kolev *et al*., 2013) and this might effect to the growth and diversity of bacteria community as Shadia *et al*., (2016) assumed that there was a relationship between limiting nutrients and microbial growth, development and diversity.

3.2 Bacillus

The results of *Bacillus* density variation are shown in Fig. 1. *Bacillus* density reached 2.3×10^3–5.9×10^3 CFU/mL at day 14 and remained stable towards the end of the experiment, with density varying from 2.1×10^3–5.9×10^3 CFU/mL at day 21. On day 14, the highest density of *Bacillus* was recorded in the SW–*Bacillus* group; both SW and SS–*Bacillus* treatments were significantly higher than all other treatments ($p <$

0.05). Statistical results showed that, at day 14, both culture medium, the supplemental compound, and their interactions had an impact on bacterial density ($p < 0.01$)

At day 21, SS – *Bacillus* and SW – *Bacillus* treatments reached densities of 3.7×10^3 and 4.2×10^3 CFU/mL, respectively, which was significantly higher than the other treatments ($p < 0.05$). When the *Bacillus* population was more stable, at the end of the experiment, only the supplemental compound affected the bacterial density, and the use of sea salt or seawater for culture medium no longer had any effection this bacterial population. This may be because the periodic addition of *Bacillus* mixture kept the *Bacillus* population in the additional treatments relatively stable (Pham and Tran, 2014). Moreover, the salinity was 30 ppt, at which the adaptation time of bacteria to the environment is shorter and the filter feeding activity of *Artemia* is lower than at high salinity (Ngo *et al*., 2015). *Bacillus* species are widerly used in many probiotic products that take advantage of their wide range of physiologic characteristics and their ability to produce a host of enzymes, antibiotics, and other metabolites... (Olmos *et al*., 2020), promoting growth, heath and biomass of host animals. The high densities of *Balcillus* in those *Bacillus* added treatments gave better *Artemia* performance and biomass in this experiment compared to the blank treatments (Table 2) might benefit from this species.

3.3 Ammonium oxidizing bacteria (Nitrosomonas)

In aquatic environment, expecially culture conditions where feed with high protein was used, it will quickly stimulate the presence of microbial nitrogen cycling in which amonium derived from extra feed, animal wastes... is oxidized to nitrite and further to nitrate (NO_3 is the plant accessible form) by two functional groups kown as nitrifying bacteria including ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), sometimes referred as *Nitrosomonas* and *Nitrobacter* as major genus, therefore these bacteria are play a vital role in nitrogen cycle of aquaponics well as terrestial environment (Gee *et al*., 1990; Stief, 2013). In aquaculture, maitaining of these bacteria at a heathy level concerns to a good water quality of the culture system, resulting in a culture suscess.

Nitrosomonas was considered to be the most effective bacteria in conversion of ammonium waste into nitrite, a process that requires several days. The density of *Nitrosomonas* bacteria during 21 days of culture period is shown in Fig.1. Between two sampling times, on day 14 and 21, the density of *Nitrosomas* group decreased from 2.4×10^3–11×10^3 CFU/mL to 0.1×10^3–0.5×10^3 CFU/mL. On day 14, the SS-*Bacillus* treatment reached its highest density of *Nitrosomonas*, which was significantly higher ($p < 0.05$) than the SS-BF treatment and not statistically significant compared to the other treatments ($p > 0.05$). According to the results, only the interaction between the two factors (culture medium and supplemental compound) affected the density of *Nitrosomonas* ($p < 0.05$).

At day 21, the density of *Nitrosomonas* reached its highest point in the SW treatment, but the difference was not statistically significant ($p > 0.05$) with the two treatments SW-BF and SW-*Bacillus*. However, if compared to the sea salt group, it was significantly higher ($p < 0.05$). According to the statistics results (Table 3), the interaction of the two factors also affected to the density ($p < 0.05$). In addition, the density of *Nitrosomonas* was also affected by the use of different types of culture medium in the experiment ($p < 0.01$). This is probably due to biomass production in SW being better (4.2 g/L vs. 3.7 g/L; Table 2), resulting in more waste and therefore more substrates for bacteria. Moreover, the SW medium had better trace elements relative to SS (Kolev *et al*., 2013), resulting in a more diverse heterotrophic bacteria community. Furtheremore, according to Pham (2010), the *Nitrosomonas* strain is less affected by light than *Nitrobacter*, meaning that the *Nitrosomonas* strain can still grow under nonlight conditions that sometime happened during this experiment.

3.4. Nitrite oxidizing bacteria (Nitrobacter)

Like *Nitrosomonas*, *Nitrobacter* was the most effective during denitrification. They converted harmful nitrite to nitrate, which can be used by phytoplankton. Therefore, this species together with Nitrosomonas is very important in wastewater treatment, as well as in aquaculture recycling systems.

Statistical results (Table 3) showed that using different types of culture medium (SS vs. SW) had an effect ($p<0.05$) on the density of *Nitrobacter*. The density variation of the *Nitrobacter* group is shown in Fig. 2. On day 14, the bacterial density increased, ranging from 1.2×10^3–8.3×10^3 CFU/mL, and then decreased to 0.7×10^3–3.1×10^3 CFU/mL at the end of experiment.

At day 14, the SS-BF treatment reached the highest density (8.3×10^3 CFU/mL), but the difference was not statistically significant compared to the SS and SS-Bacillus treatments (4.0×10^3 CFU/mL, $p>0.05$), although it was statistically significant compared to the seawater treatment ($1.2–2.0\times10^3$ CFU/mL, $p<0.05$).

Like day 14, at day 21, *Nitrobacter* densities were only affected by the culture medium ($p<0.05$) and not by their complementary composition and interactions ($p>0.05$; Table 4). The SS-BF reached the highest density on day 21 (2.5×10^3 CFU/mL). However, its differences from the treatments SS, SS-*Bacillus* and SW-*Bacillus* were not statistically significant ($p>0.05$) and were statistically significant ($p<0.05$) with the remaining treatments, which used the SW medium ($0.7–1.5\times10^3$ CFU/mL)

In general, with both SS and SW media, the biofloc creation (adding molasses) gave the best results for development of bacteria, with a total count consistently higher than the others. *Bacillus* was similar across all treatments, except those which were supplemented with this species. The difference was notable with nitrogen-converted bacteria; *Nitrosomonas* was better developed in SW while Nitrobacter was better in SS

medium. However, this issue needs more study to confirm and understand it, as our study lasted only for three weeks.

3.5 Vibrio

There was no *Vibrio* found in water samples at day 14 and 21, and the last check on *Artemia* biomass also did not reveal them. This could be because the time for experiment was too short, resulting in no infection.

CONCLUSION

Artemia performance was better in seawater than in seasalt culture medium, highest survival, length and biomass were obtained when they was cultured in seawater combined with bioflocs, but it was not significantly different from the sea salt medium.

Total bacteria achieved higher densities with biofloc and *Bacillus* addition treatments compared to the blank treatment in both SW and SS media.

Bacillus grow better in seawater and the addition of *Bacillus* to the *Artemia* culture also improved survival, length and biomass. Addition of this species to the culture will enhance the density and their occupation in the culture medium.

Nitrosomonas was better developed in SW, while *Nitrobacter* was better developed in SS medium.

There was no *Vibrio* found in this study.

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