



# Water Quality Improvement by Wild Bacilli Isolated from Marine Environments

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**Abstract:** Huge feed volume and shrimp density loading in the intensive shrimp culture decreased water quality. Ammonia and nitrite, which are toxic substances, are occurred from feed and shrimp excretion. *Bacillus* and related genera (class Bacilli) are bacteria capable of transforming ammonia to nitrite and then nitrite to nitrate by nitrification. In this study, wild marine Bacilli were isolated from samples collected from seagrass beds Andaman Sea, Thailand. Bacilli strains were obtained by using the heat-shock method. Three bacilli bacteria were consisting of *Bacillus* sp. BC02 (DNA-based), *Bacillus* sp. BC05 (morphology-based) and *Virgibacillus* sp. BC06 (DNA-based) was obtained. This study aims to investigate these isolates in shrimp wastewater improvement compared with the commercial seed of *Bacillus* spp. product (PM-1). Seven wastewater treatments were separately tested by adding different formulas of bacteria. Each treatment was added for 1% (w/v) of  $10^7$  CFU/mL density and incubated for 7 days. Treatment of BC02+BC05 showed a significant TSS decrease (66.56%) and produced the highest nitrate concentration. The highest increase of OTP (84.97%) was found in the treatment of BC02+BC05+BC06. PM-1 product has presented the best BOD lessening (54.35%) and showed a non-significant reduction of ammonia (98.60%) with the highest nitrite ( $0.685 \pm 0.009$  mg/L) at the end of the experiment. *Virgibacillus* (BC06) has resulted in the highest significant reduction of COD (60.39%). Thus, it might be summarized that three marine isolates of *Bacillus*, *Virgibacillus*, and commercial PM-1 product have excellent potential to improve wastewater quality with no significant diferent. Marine *Bacilli* can be substitution used for commercial PM-1 products.

**Keywords:** *Bacillus*; *Virgibacillus*; Wastewater improvement, OTP, BOD, COD

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## 1. Introduction

Intensive shrimp culture has been popular in recent years. High-density rearing is necessary to use high protein feed for shrimp. This leads to a continuous increase of uneaten feed, feces, and wastes at the bottom of the pond according to

time and declines in water quality [1-2]. High ammonia and nitrite are some of the improper water qualities and are toxic to shrimp, which should be controlled [3]. In the huge pond, water exchangeable takes a lot of time and increase energy cost. Water discharging brings loads rich in nutrients and organic matter to surrounding environments [4]. And it creates a risk of the pathogen spreading to other farms and environments [5]. The microorganisms have been applied in shrimp culture as a biological process for managing these problems [1, 6]. Microorganisms should not be pathogenic bacteria, working as biodegradable organic matter, and removal of ammonia and nitrite in culture pond. Most microorganisms are *Bacillus* spp. as they role in nitrification and produce enzymes (i.e., amylase, lipase, and protease) that can decompose organic matter [1, 7-10]. Moreover, *Bacillus* spp. has been described as promoting the growth of green algae and diatoms in shrimp ponds. At the same time, they can control toxin-producing algae groups (blue-green algae and dinoflagellates) [11]. Furthermore, the advantage of the genus *Bacillus* is endospore formation which makes it comfortable to prolong shelf-life, storage, transport, and application in shrimp farms [12]. Although several commercial *Bacillus* products are currently used for water improvement in marine shrimp aquaculture, they are probably not marine species. This may result in being less effective in improving saline water quality. Therefore, the isolation of *Bacillus* from the marine origin is necessary because they may have more efficiency in enhancing water quality in marine shrimp aquaculture [9-10]. Thus, this study aimed to isolate wild marine bacteria, *Bacillus* spp. and *Virgibacillus* sp., apply them to improve water quality in shrimp ponds, and compare the efficiency with commercial *Bacillus* spp. (PM-1) product. Additionally, untapped, promising, and novel marine bacterial strains may be found for wastewater treatment.

## 2. Materials and Methods

### 2.1 Isolation of marine Bacilli

The samples, including soil and seawater, were collected from seagrass beds at Libong Island, Trang province, Andaman Sea, Thailand. Targeted marine *Bacilli* were isolated by the heat-shock method [13]. One gram of soil and 1 mL of water samples were taken to each tube. The tubes were heated at 80 °C for 20 minutes and immediately shocked in ambient water. The soil sample was diluted with 10 mL of sterilized seawater. Then all sample solutions were diluted by 10 folds serial dilution. Each 0.1 mL of dilution was spread out on Nutrient Agar (NA) plates with an additional 3.2% sea salt, and plates were incubated at 37 °C for 24 hours. Single colony on plates was picked up by loop and streaked on fresh NA plates (3.2% sea salt) 2-3 times until pure isolates were obtained. *Bacilli* cells were observed by the unique characteristics of gram-negative, rod shape, endospore-forming, and positive catalase testing [10]. Then, DNA extraction of each strain was done using a commercial DNA extraction kit (Genomic DNA mini kit, Geneaid). Their 16S rRNA genes were amplified by PCR using a set of universal primers 27F (5'-AGAGTTTGATCATGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). After PCR product purification by GF-1 AmbiClean Kit (PCR/Gel) (Vivantis), DNA sequencing was determined with the same primers by Macrogen manufacturing [9]. DNA sequences were compared within GenBank/EMBL/DDBJ database using the Blast program. A phylogenetic tree of marine isolates and related species was constructed by the MEGA 11 program [14].

### 2.2 Optimal salt requirement of marine Bacilli

Each strain of candidate *Bacilli* was cultured in Nutrient Broth (NB) with 3.2% sea salt by inoculating a 1-loop of bacteria into a 10 mL tube containing NB (3.2% sea salt) shaking at 110 rpm for 24 hours. 1 mL of each isolate was transferred into a tube containing 9 mL of NB at different salinity levels of 0, 5, 10, 15, 20, 25, 30, 35, and 40 ppt. All tubes were shaken at 110 rpm for 24 hours, and measured cell density was using a spectrophotometer at 600 nm wavelength.

### 2.3 Water improvement efficiency

The water treatment efficiency of isolated marine *Bacilli* was compared with PM-1. A commercial bacterial product consisted of 3 *Bacillus* species (*B. subtilis*, *B. licheniformis*, and *B. megaterium*). One gram of PM-1 seed product was mixed with 2.5 L water, 5 g shrimp feed, and 50 mL molasses, then closed the cover. Air has been blowing continuously for 36 hours. Seed starters of isolated marine *Bacilli* and PM-1 were carried in a 250 mL flask with NB (3.2% sea salt), shaking at 110 rpm for 24 hours. After that, each isolated *Bacilli*'s cell suspension was started at a concentration of  $10^7$  CFU/ml. Then, 70 mL (1%) of each single strain suspension (PM-1, BC02, BC05, and BC06) was transferred into a 10-L glass jar containing 7 L of water from the shrimp

pond. Treatment BC02+BC05 was prepared at a 1:1 ratio (35:35 mL). Likewise, treatment BC02+BC05+BC06 was mixed in a 1:1:1 ratio (23.3 mL each). These mixture treatments were carried on under the same condition as a single treatment in a 10-L glass jar. The experiment was conducted for 7 days by air supplying for 24 hours in every treatment and water quality parameters were measured daily, including dissolved oxygen, salinity, temperature, ammonia, nitrite, and nitrate [15]. The total suspended solid was measured on days 1, 3, 5 and 7 of the following experiment [16]. Additionally, orthophosphate [15], BOD<sub>5</sub> [17], and COD [18] were analyzed at the initial and the end of the experiment.

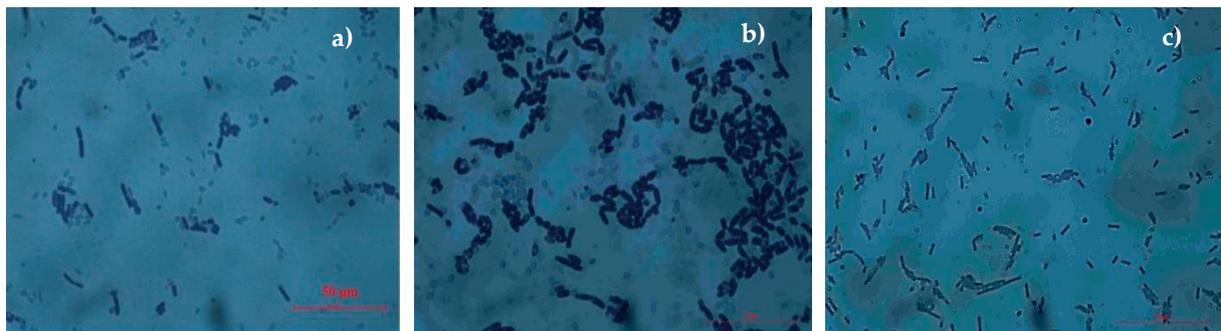
#### 2.4 Statistical Analysis

Variations in water quality parameters were investigated between treatments and control using Analysis of Variance followed Completely Randomized Design. Duncan's New Multiple Range Test and T-Test at 95% confidence levels were used to determine the mean difference among the treatments. These analyses were performed using the R program.

### 3. Results and Discussion

#### 3.1. Wild marine Bacilli

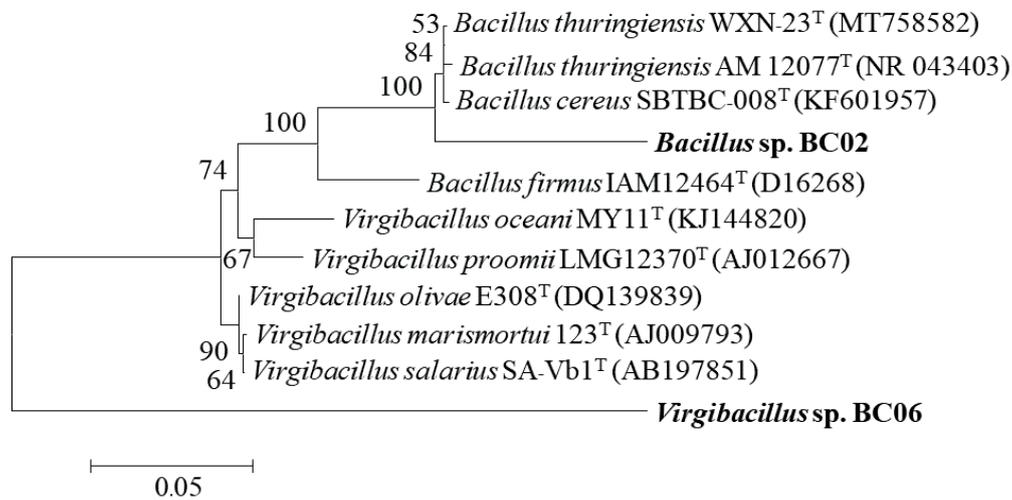
Bacilli bacteria were isolated from water and soil samples collected on seagrass beds at Libong Island, Trang province, which was manipulated by the heat-shock method. Although fourteen isolates were gram-positive and showed rod shape, only 3 isolates found endospore forming, consisting of BC02, BC05, and BC06 (Figure 1). Endospore formation is a unique characteristic of *Bacillus* when environmental conditions are far from optimal, e.g., high temperature and dry. Utilizing this capability will therefore make the isolation process easier. In addition, endospore-forming *Bacillus* is proficient for further use in shrimp ponds. The three Bacilli isolates were selected to study the water improvement efficiency further.



**Figure 1.** Characteristic of three marine endospore-forming Bacilli; a) BC02, b) BC05, and c) BC06.

As a result of partial 16S rDNA sequencing, *Bacillus* sp. BC02 showed a similarity of about 95% to *Bacillus thuringiensis* (Figure 2). The bacteria *B. thuringiensis* was widely known that they inhabited soil and produced specific proteins to kill insects. Therefore, *B. thuringiensis* has been widely used as a biocontrol of insect pests in agriculture [19]. However, [20] reported the isolation of marine *B. thuringiensis* from marine sediment. Then several publications of marine *B. thuringiensis* from marine sources were reported [21, 22]. In aquaculture, [23] reported the potential of marine *B. thuringiensis*, isolated from shrimp intestines, as a probiotic against a pathogenic bacterium in marine shrimp. Another research [24] published the isolation of *B. thuringiensis* from the goldfish intestine and screening it for use as a probiotic. In addition, there are some studies on the application of *B. thuringiensis* to inhibit parasitic nematodes in fish [25] or to control insects (mosquitoes) that as carriers of disease in water and wastewater [26]. However, no publication of *B. thuringiensis* directly used for water treatment. This research may first study *B. thuringiensis* for water treatment. Another isolate, BC06, has low similarity, about 83% related to the genus *Virgibacillus* (Class Bacilli). Genus *Virgibacillus* was reclassified, separating from the genus *Bacillus* in 1998 [27]. Although *Virgibacillus* can be isolated from marine and non-marine sources, they were reported as a halophilic bacterium [28-31]. The percentage of similarity result (83%) of partial 16S rRNA was deficient. Therefore, strain BC06 may require rearrangement when the 16S

rRNA gene full length is studied. In the case of strain BC05, the DNA extraction had a problem of low quantity and quality. Hence this isolate was still unidentified.



**Figure 2.** The phylogenetic tree of two marine Bacilli, BC02 and BC06, is based on partial 16S rDNA (Bar = 0.05  $\mu$ m).

### 3.2. Salt tolerance for growth of marine Bacilli

Salt tolerance was conducted in 9 levels, including 0, 5, 10, 15, 20, 25, 30, 35, and 40 ppt. The result showed that strain BC02 has a wide range of optimal growth at 0-35 ppt, like strain BC05 has optimal growth at 0-30 ppt. In comparison, strain BC06 has a narrower range of optimal growth at 20 to 40 ppt. Three marine isolates in this study were obtained from marine sources. Therefore, they showed the character of a wide range of salt-tolerant, according to other studies [9-10]. These might result from the various physiological preferences of each strain.

### 3.3. Water quality improvement of marine Bacilli and PM-1 bacterial product

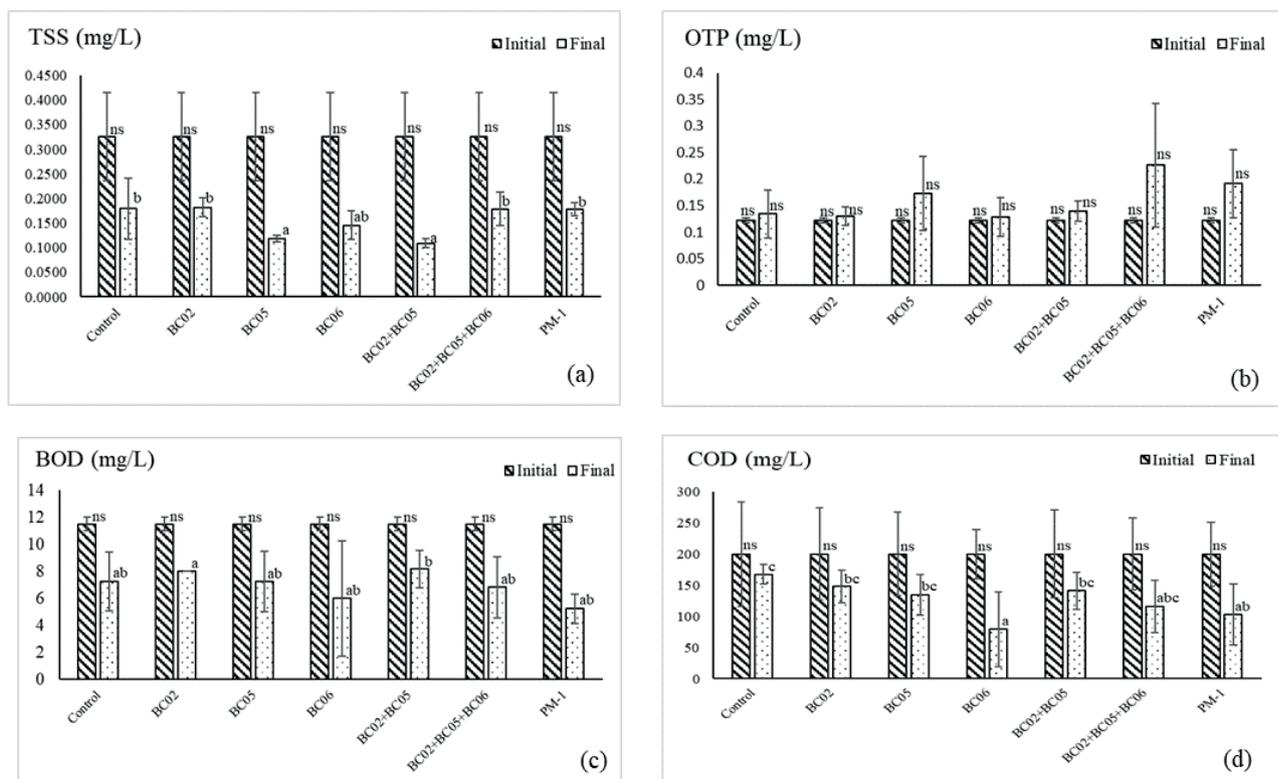
#### 3.3.1. Total suspended solid (TSS)

All treatments showed no significant difference between each other on days 1 and 3, but they presented the same trends as TSS gradually decreased. While the end of the experiment, BC02+BC05 treatment was the lowest TSS ( $0.109 \pm 0.009$  mg/L) which has no significance ( $P > 0.05$ ) with treatments of BC05 and BC06 ( $0.1177 \pm 0.0067$  and  $0.145 \pm 0.029$  mg/L, respectively). The TSS of control, BC02, BC06, BC02+BC05+BC06 and PM-1 were  $0.182 \pm 0.019$ ,  $0.145 \pm 0.029$ ,  $0.1787 \pm 0.034$ ,  $0.178 \pm 0.0139$  and  $0.179 \pm 0.062$ , respectively with no significance ( $P > 0.05$ ) as shown in Figure 3a. Conversely, they were significantly different ( $P < 0.05$ ) with BC02+BC05. Adding all 3 marine Bacilli decreased TSS from  $0.326 \pm 0.000$  mg/L to the range of  $0.109 \pm 0.009$  to  $0.182 \pm 0.019$  mg/L (~ 44–66%) at the end of the study. Exceptionally, BC02+BC05 showed the lowest TSS. It might be because they have co-metabolism to degrade or trap the particle suspension in the water sample. The result was according to the study of *B. velezensis* that showed TSS removal of brewery wastewater for 55% [32]. In an aquaculture pond, the solid suspension will be overshadowed by light through water which affects the primary production process of water. The high suspension will directly harm aquatic animals by clogging their gills, leading to hard breathing, slow growth, late hatching, and growth of larvae. Therefore, using a couple of wild marine Bacilli (BC02+BC05) could reduce TSS and indirectly help promote the well-being of aquatic animals.

#### 3.3.2. Orthophosphate concentration ( $PO_4^{3-}$ )

Orthophosphate has been monitored in all treatments. The initial concentration of orthophosphate was  $0.1224 \pm 0.000$  mg/L and continuously increased until the last day of the experiment with

the range of  $0.1287 \pm 0.0361$  to  $0.2264 \pm 0.1165$  mg/L. Treatment of BC02+BC05+BC06 was showed the highest quantity of orthophosphate ( $0.2264 \pm 0.1165$  mg/L), but has no significance ( $P > 0.05$ ) with treatments of control, BC02, BC05, BC06, BC02+BC05 and PM-1, which their orthophosphate volume were  $0.1300 \pm 0.0168$ ,  $0.1731 \pm 0.0697$ ,  $0.1287 \pm 0.0361$ ,  $0.1394 \pm 0.0196$ ,  $0.1914 \pm 0.0638$  and  $0.1345 \pm 0.0452$  mg/L, respectively (Figure 3b). Although the treatment BC02+BC05+BC06 was the highest  $PO_4^{3-}$ , major nutrients needed by most phytoplankton consist of nitrogen in the forms of nitrite ( $NO_3^-$ ), ammonia ( $NH_4^+$ ), and urea ( $CO(NH_2)_2$ ). As well as phosphorus in orthophosphate ( $PO_4^{3-}$ ) or soluble reactive phosphorus that are good soluble for instant utilization on phytoplankton and aquatic plants growth [1]. In the part of insoluble phosphorus will be slowly decomposed by bacteria and released into a soluble form. Several *Bacillus* species were mentioned about their ability in phosphate elimination, such as cellulolytic *Bacillus* species can reduce phosphate by 81% at the cell concentration of  $10^9$  CFU/g [33]. *B. subtilis*, *B. cereus*, and *B. licheniformis* showed phosphate reduction [34], and *B. velezensis* reduced total phosphorus in channel catfish pond by 19% [35]. Moreover, the mixture of *B. megaterium* and *B. subtilis* can remove total phosphorus by about 80.3% [36].



**Figure 3.** Water parameter variations during the experiment; (a) total suspended solid: TSS, (b) orthophosphate ( $PO_4^{3-}$ ) concentration: OTP, (c) biological oxygen demand: BOD, (d) chemical oxygen demand: COD.

### 3.3.3. Biochemical oxygen demand (BOD<sub>5</sub>)

The initial BOD<sub>0</sub> of all treatments was  $11.50 \pm 0.000$  mg/L and tended to decrease until the 5-day incubation ended. The lowest BOD<sub>5</sub> was PM-1 treatment ( $5.25 \pm 1.0897$  mg/L), which showed 54.35% of BOD<sub>5</sub> decreasing. There was not significantly different ( $P > 0.05$ ) with treatments of BC02, BC05, BC06, BC02+BC05+BC06 and control ( $8.00 \pm 0.0000$ ,  $7.25 \pm 2.2500$ ,  $6.00 \pm 4.2525$ ,  $6.83 \pm 2.2684$  and  $7.25 \pm 2.1794$  mg/L, respectively), but was significance ( $P < 0.05$ ) with a treatment of BC02+BC05 ( $8.17 \pm 1.3769$  mg/L) (Figure 3c). Since the first day to the end of the 5-day experiment, Bacilli from both marine isolates and PM-1 can decrease BOD by approximately 36-54%, except BC02 and BC02+BC05 have 28-30% of BOD removal. BOD is the oxygen bacteria and other microorganisms require for organic degradation in water, with an optimal range of  $\leq 6$  mg/L. Only 5.25 (PM-1) and 6.00 (BC06) mg/L were found in this study, which was less than the standard BOD of

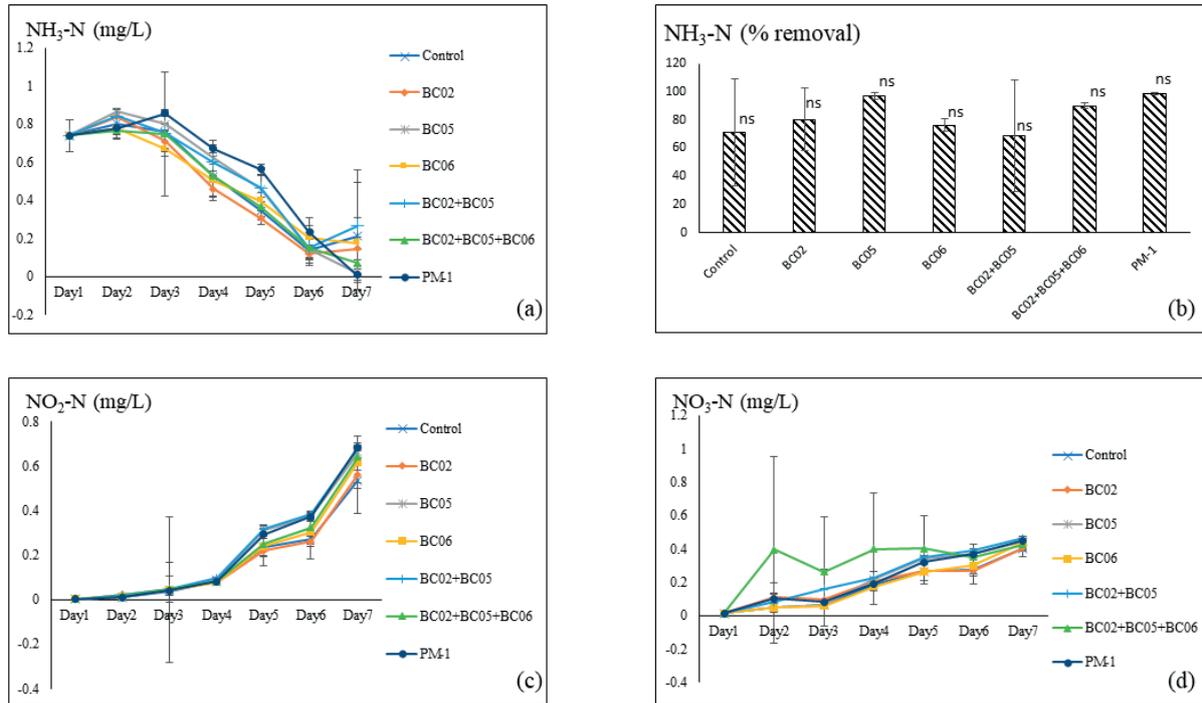
aquaculture effluent ( $\leq 20$  mg/L). The result of Bacilli species was similar to the result of *B. velezensis*, which showed 46% of BOD removal [32]. While isolated cellulolytic *Bacillus* had reduced BOD by 90% [33], another *B. subtilis* was 93% of BOD removal [37].

### 3.3.4. Chemical oxygen demand (COD)

Initial COD in all treatments was  $200.2933 \pm 26.0721$  mg/L and gently declined until the last day of the experiment. The treatment of BC06 was the lowest COD ( $79.3333 \pm 45.2409$  mg/L), and there was no significance ( $P > 0.05$ ) with BC02+BC05+BC06 ( $115.8267 \pm 31.6936$  mg/L) and PM-1 treatment ( $103.1333 \pm 33.7703$  mg/L), but significantly different ( $P < 0.05$ ) with BC02, BC05, BC02+BC05 and control treatments (Figure 3d). Although there is no COD regulation in aquaculture effluent, COD levels below 120 mg/L are recommended for standard industrial effluent. According to this study, all treatments had lower COD than the standard industrial effluent. Only BC06 can reduce COD reached to about 60% as the best biodegradation in this study, while others diminished COD within 26-48%, and control without bacteria added resulted in only 16%. This might be because of the different species of *Bacillus*. For example, BC06 showed a high capability for organic degradation in high salinity conditions. [38] reported the isolation of *Bacillus* sp. LY from a membrane bioreactor (MBR), by testing water treatment efficiency for 24 days, can reduce COD up to 71.7%. Another study reported the COD removal efficiency of *B. velezensis* in a very high load of COD concentration (4,000 mg/L). Authors suggested that *B. velezensis* can remove COD volume by about 55% [32]. In addition, a COD reduction above 90% of another *Bacillus* was described by [33] and mentioned *B. subtilis* had an 80% COD decrease [37]. Furthermore, *B. flexus* and *B. licheniformis* were suggested as effective species for COD, removing about 70-80% [39]. *Bacillus* spp. has a good ability for COD removal because it can produce several digestive enzymes, surfactants, hydrocarbons, phenols, fatty acids, and ketones to decompose organic matter as nutrients for growth [39].

### 3.3.5. Concentrations of Ammonia ( $NH_4^+$ ), Nitrite ( $NO_2^-$ ) and Nitrate ( $NO_3^-$ )

On the first day, ammonia concentration was  $0.7389 \pm 0.0840$  mg/L and showed diminished trends in all treatments. Despite the increased concentration of all treatments on day 2, it lightly decreased from day 3 to the end of the experiment (day 7). The treatment of PM-1 has presented the lowest ammonia removal percentage (98.60%) and has no significance ( $P > 0.05$ ) with treatments of control, BC02, BC05, BC06, BC02+BC05, and BC02+BC05+BC06, which their ammonia removal was 71.13, 80.26, 97.30, 76.16, 64.14 and 90.13%, respectively (Figure 4a, 4b). Reduction trends resulted in nitrite concentration of every treatment on day 1 with the initial value of  $0.0030 \pm 0.0002$  mg/L. From day 2 until the last day, nitrite slowly rose. PM-1 has produced the highest nitrite ( $0.6851 \pm 0.0087$  mg/L) and is not significantly different ( $P > 0.05$ ) from others (Figure 4c). In the case of nitrate, initial nitrate was  $0.0143 \pm 0.0034$  mg/L and gently raised since day 1, except for BC02, BC02+BC05+BC06, and PM-1, have slightly decreased on day 3. Then again, they all increased on days 4 to 6. Only BC02+BC05+BC06 has resulted in a slight decline. At the end of the experiment, all treatments had higher nitrate than the initial as BC02+BC05 was the highest ( $0.4629 \pm 0.0012$  mg/L) and had significance ( $P < 0.05$ ) with control only (Figure 4d). All three strains of Bacilli showed 64.14–98.60% of ammonia removal ability while increasing nitrite from 0.0029 mg/L up to about 0.5623–0.6830 mg/L. The result suggested that the nitrification process was driven in this study. According to another study, a consortium of *B. cereus*, *B. amyloliquefaciens*, and *Pseudomonas stutzeri* can remove ammonia for 84.89%, whereas nitrite and nitrite nitrate concentrations were increased. The authors suggested that bacterial consortium contained both ammonia-oxidizing and nitrite-oxidizing bacteria [6]. *Bacillus* species were the miracle bacteria that can eliminate high strength of ammonium concentration. [9] reported that *Bacillus* sp. had an ammonium removal efficiency of 42.28% at a very high concentration (815.86 mg-N/L). However, ammonium removal efficiency will be decreased when the lack of carbon sources.



**Figure 4.** Water parameter variations along the experiment; (a) ammonia, (b) ammonium removal (%), (c) nitrite, (d) nitrate.

Nitrification of bacteria can transform ammonia to nitrite but cannot convert nitrite to nitrate as a full nitrification process. Ammonium oxidizing bacteria (AOB) will be altered ammonia to nitrite and continuously transform to nitrate by nitrite-oxidizing bacteria (NOB). Thus, three isolated marine Bacilli might be instead in the group of AOB, which can only converse ammonia to nitrite, agreed with [9] suggested that isolated *Bacillus* species could be ammonium oxidizing bacteria due to ammonium conversion characteristics. Additional research of *Bacillus* species for nitrogenous effluent treatment [7] reported *B. subtilis* A1 for water treatment capability for 120 hours with 33.51% ammonia removal and 20.4% of decreased nitrite. [40] also studied using *B. subtilis* ( $10^8$  and  $10^5$  CFU/mL) in a shrimp pond. They described that *B. subtilis* with high concentration ( $10^8$  CFU/mL) could control ammonia and nitrite better than with low concentration ( $10^5$  CFU/mL). While [10] reported that the starter volume of *Bacillus* with 1% and 5% was no significance for ammonium removal efficiency. Besides the single culture of *Bacillus*, a mixture or consortiums of them were used for water treatment. For example, mixed *Bacillus* (*B. megaterium* and *B. subtilis*) reported efficiency of nitrogenous waste (ammonia, nitrite, and nitrate) reduction by approximately 35-76%. This result had good efficiency rather than compared mixture (*B. megaterium* and *B. coagulans*) [36]. Moreover, a consortium of *B. cereus*, *B. amyloliquefaciens*, and *Pseudomonas stutzeri* was studied with 84.89% of ammonium removal [6]. The consortium of microorganisms can be an advantage over single culture because they have cooperative interactions between the co-cultivated microorganisms that enhance the nutrient removal rate [6]. This study compares the water treatment efficiency of three Bacilli isolates, consisting of BC02, BC05, and BC06, with microbial seeds (PM-1), resulting in a decrease of total organic matter and an increase of orthophosphate in all isolates with no significance ( $P > 0.05$ ) compared with PM-1 product. PM-1 has shown high efficiency in decline BOD<sub>5</sub> with 54.35% while presenting only 48.51% COD removal, which was lower than BC06 (60.39%). Furthermore, PM-1 product has the utmost ability to diminish ammonia (98.60%) and raise nitrite (from 0.0029 to 0.6851 mg/L) since the initial day, but there was no significant difference ( $P > 0.05$ ) with 3 marine Bacilli isolates. Augmentation of nitrate has resulted in a mixed culture of BC02+BC05 that highest produced about 0.4629 mg/L from 0.01428 mg/L on the first day, which showed significantly ( $P < 0.05$ ) with control only. *Bacillus* spp. can improve water because it produces several enzymes to lyse nutrients and organic matter, which have been involved in nitrification/denitrification reactions. Furthermore, *Bacillus* species have suggested that they are proficient in maintaining water quality in aquaculture, which is simple

and cost-effective [1]. Other advantages: *Bacillus* species could reduce pathogenic microorganisms in aquaculture and control toxin-producing phytoplankton (blue-green algae and dinoflagellates) in shrimp farms [11].

#### 4. Conclusions

Three marine *Bacilli* presented endospore-forming were isolated from soil and water samples on seagrass beds. Strain BC02 was identified as *Bacillus thuringiensis*, and BC06 was genus *Virgibacillus*. Unfortunately, strain BC05 could not be identified. These isolates have been utilized for shrimp water improvement at a density of  $10^7$  CFU/ml for 7 days. *Bacilli* utilization in water treatment caused better water quality as about a 50% decrease of TSS, COD, BOD, and  $\text{NH}_3$ , and only  $\text{NO}_3^-$  has significantly increased. Marine *Bacilli* isolates have no significant capabilities compared to commercial PM-1 product. Therefore, these *Bacilli* isolates can develop into an alternative microorganism product with efficiency equal to commercial PM-1. Therefore, these *Bacilli* isolates have the potential to become a substitute microbial product with a level of efficacy comparable to that of commercial PM-1. Further research should be done to determine how marine *Bacilli* and PM-1 can enhance the water quality in shrimp ponds.

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