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Investigation of Ability to Remove NH_3 and NO_2 By Combination of *Yucca Schidigera* Extract and *Bacillus* Strains

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Abstract:
Background*: Aquaculture have developed more and more strongly in many countries around the world. However, a intensive shrimp or fish farming model always have issues in pond water pollution. NH_3 and NO_2 toxic gases are two direct threats to survival rate and growth of aquatic animals. It is necessary to find out a economical and environmentally friendly solution in order to control NH_3 and NO_2 in aquaculture ponds. Therefore, this study was carried out to get an effective approach based on the combination of Bacillus sp. and Yucca schidigera extract in NH_3 and NO_2 control.*
Materials and Methods*: Bacillus sp. is isolated on NA medium from sludge samples collected in Can Gio Mangrove Forest. Screening Bacillus strains and dilution of Yucca schidigera extract which have the most effective ability to remove NH_3 and NO_2 through experiments with initial concentration of 10, 20, 40, 80 and 160 ppm. Investigating the combination of Yucca schidigera extract and screened Bacillus sp. in NH_3 and NO_2 removal. This study used sodium nitroprusside which acts as a catalyst for the formation of indophenol derivatives in order to determine NH_3; sulphanilic acid and N-(1-naphthyl) ethylenediamine were used for formation of an azo dye to determine NO_2 by spectrophotometry with absorbance at 640 nm (NH_3) and 540 nm (NO_2).*
Results*: Three strains of Bacillus sp. BIO_2, BAL_3, AQ_1 were isolated from Can Gio Mangrove Forest which have ability to remove approximately 55 ppm ammonia and 59 ppm nitrite within 72 hours. Yucca schidigera extract with 1/2 dilution had the best result among treatments with removing 24.314 ppm of ammonia and 12.081 ppm of nitrite within 72 hours. The combination of Bacillus sp. BIO_2, BAL_3, AQ_1 and Yucca schidigera extract can remove approximately 90 ppm ammonia and 94 ppm nitrite within 72 hours.*
Conclusion: *The combination of Yucca schidigera extract and Bacillus sp. BIO_2, BAL_3, AQ_1 enhance removal ability of ammonia and nitrite.*
Key Word*: Bacillus; Yucca schidigera; Ammonia Nitrite Removal; Yucca Extract.*

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I. Introduction

Aquaculture have developed more and more strongly in many countries around the world. Intensive shrimp or fish farming model has been applied widely and brought high economical efficiency for farmers, but this led to pond water pollution by sedimentation of feed and organic residues. Especially, nitrogen compounds such as ammonia and nitrite, which increase rapidly in the pond, are toxic to fish and shrimp even at very low concentration. Periodic water change or changing water when ammonia and nitrite levels are too high that are often used to maintain a safe level of toxic gases. However, this water exchange will occur frequently and consume a large amount of water that increases costs and is able to contaminate pathogens from a polluted river or sea (Mohapatra et al., 2012). Therefore, it is imperative to create a environmentally friendly bioremediation which sustainably controls water quality and effectively reduces costs in aquaculture.

The use of microorganisms to control water quality in aquaculture has been widely applied in recent years (Ninawe and Selvin 2009; Verschuere et al. 2000). *Bacillus* sp. is a major component of many probiotics that are widely used in aquaculture for the purpose of improving pond quality. They possess many beneficial biological properties, including being able to form spores for easy long-term storage, and are resistant to pathogenic bacteria in seafood, which can reduce residual organic content and remove toxic gases NH_3 and NO_2 in the pond. *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus cereus*, *Bacillus pumilus* of the *Bacillus* species have been evaluated as potential microorganisms for biological control and water quality improvement. Additionally, in recent decades, many researchers have shown the efficacy of the extract of a desert plant - *Yucca schidigera* in ammonia (Santacruz-Reyes and Chien, 2010a; Santacruz-Reyes and Chien, 2010b; Tidwell

et al., 1992) and nitrite removal (Calisto-Vargasmachuca et al., 2015) in aquaculture. Although *Yucca schidigera* has been shown to control ammonia and nitrite in livestock and aquaculture, there has been no research that shows the combination of *Yucca schidigera* and *Bacillus* sp. in reducing ammonia and nitrite in the pond. Therefore, the objective of this study was to isolate *Bacillus* strains which have high ability of ammonia and nitrite removal, determine the effect of combination of *Yucca schidigera* and screened *Bacillus* strains in ammonia and nitrite removal.

II. Material And Methods

2.1. Collection of samples and Isolation of *Bacillus*

The sludge samples were collected from at least 10 cm of depth in sterile vials from Can Gio Mangrove Forest, located in southeast of Ho Chi Minh city, Vietnam. The samples were diluted with saline and plated onto isolation media (Nutrient Agar) by spread plate method and incubated at 37^{o}C for 24 hours. Taking the individual colonies by streak plate method, each obtained isolate on a petridish and again incubated at 37^{o}C for 24-48 hrs.

2.2. Biochemical identification of *Bacillus* species: The isolated colonies were identified on the basis of morphology and Gram staining. The identification tests included hemolysis, starch hydrolysis, gelatin hydrolysis, citrate test, Voges–Proskauer (VP) test, and growth at different pH and temperature (Foysal and Lisa, 2018).

2.3. Screening of *Bacillus* for ability of NH_3, NO_2 và NO_3 removal: The identified *Bacillus* colonies were inoculated into 50 ml NB medium in a 100-ml flask cultured at 37^{o}C for 24 hrs in a shaker with 180 rpm. Then, a 5% of cellular culture was inoculated into 100 ml Heterotrophic Nitrification Medium - HNM (modified with NH_3, NO_2, NO_3 contents) in a 250-ml flask incubated at 37^{o}C for 24, 48 and 72 hrs in a shaker with 180 rpm. The NH_3, NO_2, NO_3 - containing HNM was prepared by making the same final concentration - 10 ppm. All the experiments were repeated thrice with the basal medium as control.

2.4. Removal tests of ammonia, nitrite and nitrate for the screened *Bacillus* strains: The screened *Bacillus* strains had ability to reduce NH_3, NO_2, NO_3 at the previous experiment that were inoculated into 50 ml NB medium in a 100-ml flask cultured at 37^{o}C for 24 hrs in a shaker with 180 rpm. Then, a 5% of cellular culture was inoculated into 100 ml NH_3, NO_2, NO_3 - containing HNM in a 250-ml flask incubated at 37^{o}C for 24, 48 and 72 hrs in a shaker with 180 rpm. The NH_3, NO_2, NO_3 - containing HNM was prepared by making the same final concentration - 10, 20, 40, 80 and 160 ppm (Yang et al., 2017; Xie et al., 2013).

2.5. Removal tests of ammonia, nitrite and nitrate for *Yucca schidigera* extract: *Yucca schidigera* extract powder - 40% Yucca Sarsaponin is from XiAnRainbow Biotech Co., Ltd. - No. 4, South of Tianhu Road, Leping City, Jiangxi Province, China. *Yucca schidigera* solutions were prepared by dissolving with deionized water at 6 dilutions - 1/2, 1/4, 1/8, 1/16, 1/32 and 1/64. Adding 1 ml of each *Yucca schidigera* prepared solution into 50 ml NH_3, NO_2, NO_3 - containing HNM in a 100-ml flask incubated at 37^{o}C for 24, 48 and 72 hrs in a shaker with 180 rpm. The final concentrations of NH_3, NO_2, NO_3 in HNM media are 10, 20, 40 and 80ppm.

2.6. Removal tests of ammonia, nitrite and nitrate for combination of *Yucca schidigera* extract and screened *Bacillus* strains: The experiment was carried out with the most effective dilution of Yucca and screened *Bacillus* strains which have the most effective ability to reduce NH_3, NO_2 or NO_3 in the previous experiments. Adding 1 ml of *Yucca schidigera* solution and 1 ml of *Bacillus* culture into 50 ml NH_3, NO_2, NO_3 - containing HNM in a 100-ml flask incubated at 37^{o}C for 24, 48 and 72 hrs in a shaker with 180 rpm. The HNM media were prepared by making the final concentration of NH_3, NO_2 and NO_3 at 10, 20, 40, 80 and 160 ppm, respectively.

2.7. NH_3 dertemination method: Adding 0.2 ml of phenol solution (11.1 % v/v), 0.2 ml of sodium nitroprusside (0.5 % w/v), 0.5 ml of oxydizing solution (Alkaline citrate and NaClO 5% - ratio 4:1) into tube contained 5 ml of sample. This solution was incubated in dark at least 1 hour and was measured at 640 nm absorbance using UV/VIS Spectrophotometer (Weatherburn, 1967).

2.8. NO_2 dertemination method: in general diazocoupling reactions for nitrite determination are based on the use of amines like sulphanilic acid and a specical coupling agent N-(1-Naphthyl)-Ethylenediamine dihydrocloride (Gayathri & Balasubramanian, 2001). Adding 1 ml of sulfanilic acid (1% v/v), 1 ml of N-(1-Naphthyl)-ethylenediamine dihydroclorua (0.1 % w/v) into tube contained 5 ml of sample. This solution was

incubated in dark at least 30 minutes and was measured at 540 nm absorbance using UV/VIS Spectrophotometer (Nerdy and Putra, 2018).

III. Result

3.1. Isolation and identification of *Bacillus* sp.: On nutrient agar, the colonies were identified as *Bacillus* sp. according to Bergey's manual for the identification of *Bacillus* species (Logan & De Vos, 2009; Wulff et al., 2002). 108 bateria strains were obtained from NA media, 20 of them are identified as *Bacillus* sp., namely, BIO_1 - BIO_5, BAL_1 - BAL_5, AQ_1 - AQ_{10}, based on the test results (Table 1), were selected for the further study.

Table 1: Biochemical charaterization of *Bacillus* sp. isolates.

Isolate	Gram	Spore formation	Shape	Motility	Starch hydrolysis	Gelatin hydrolysis	VP test	Citrate test	Growth in media with added NaCl				Growth at different pH				Growth at different temperatures (°C)			
									0%	5%	10%	20%	2	5	8	10	30	40	50	60
BIO_1 - BIO_5	+	+	Rod	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
BAL_1 - BAL_5	+	+	Rod	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
AQ_1 - AQ_{10}	+	+	Rod	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+

3.2. Screening of *Bacillus* for ability of NH_3 and NO_2 removal: There are 3 of 20 *Bacillus* strains have ability to effectively remove ammonia and nitrite included BIO_2, BAL_3 and AQ_1 (Figure 1 and Figure 2). Figure 1 showed that the remaining ammonia content was about 1.067 - 1.175 ppm after 24 hours in all 3 strains of *Bacillus* at 10 ppm of initial NH_3 level. The content of ammonia continued to be removed in 48 hrs (BIO_2 - 0.917 ppm, BAL_3 - 0.955 ppm and AQ_1 - 0.871 ppm) and 72 hrs (BIO_2 - 0.845ppm, BAL_3 - 0.873 ppm and AQ_1 - 0.256 ppm). Similar to the result of NH_3 removal in figure 1, figure 2 showed that almost nitrite content was effectively eliminated after 72 hrs in all 3 strains of *Bacillus* - BIO_2, BAL_3 and AQ_1 with remaining NO_2 contents - 0.063 ppm, 0.268 ppm and 0.086 ppm, respectively. BIO_2, BAL_3 and AQ_1 were selected for investigating for the threshold of ammonia and nitrite removal.

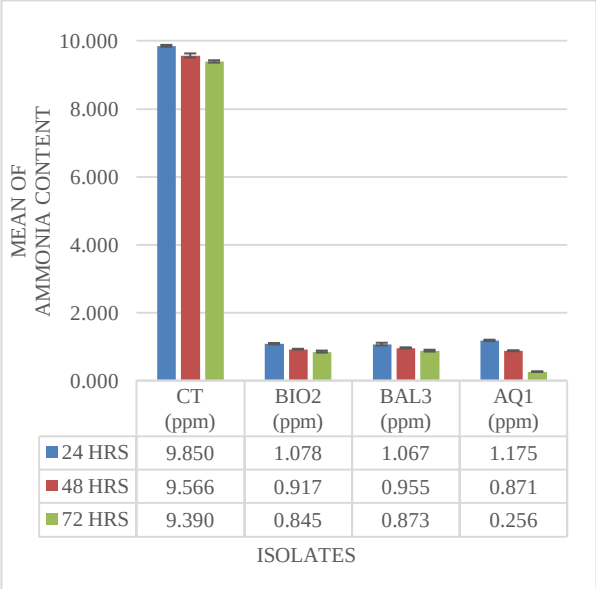


Figure 1: Ammonia removal tests for *Bacillus* strains

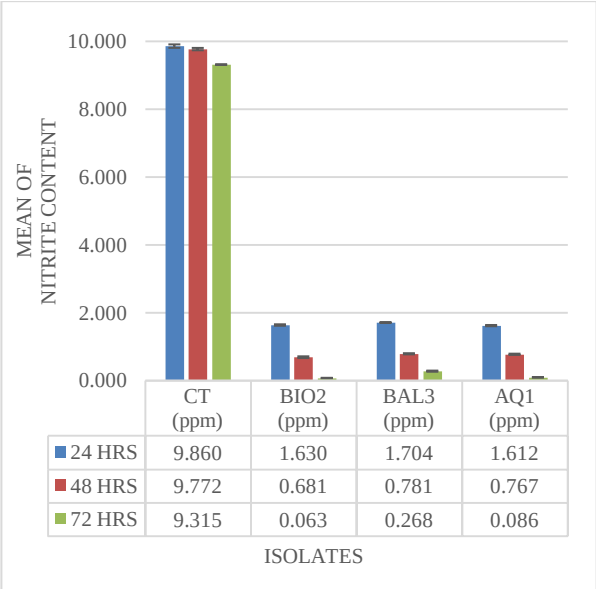


Figure 2: Nitrite removal tests for *Bacillus* strains

3.3. Investigating the threshold of screened Bacillus for ammonia and nitrite removal: Figures 3, 5, 7 and tables 2, 3, 4 showed that the remaining ammonia content is about 47 - 50% compared with the initial concentration of 20 ppm after 24 hours treated by *Bacillus* sp. BIO_2, BAL_3, AQ_1. After 48 hours, the remaining ammonia content was about 11 - 18%, and these strains removed completely 100% ammonia content within 72 hours. Similarly, at 40 ppm of initial NH_3 level, all three strains removed completely 100% ammonia content within 72 hours. However, when the inital NH_3 concentration was raised up 80 ppm, NH_3 content was removed almost 50% within 24 hours with treatment of BIO_2, BAL_3, AQ_1 - 44.47%, 41.65%, 42.11%, respectively. After 48 hours, ammonia content was removed only 8 - 10%, and the remaining ammonia content of BIO_2, BAL_3, AQ_1 treatments after 72 hours was 30.91% (24.686 ± 0.466 ppm), 28.87% (23.064 ± 0.661 ppm), 27.79% (22.241±0.328 ppm). At 160 ppm of initial NH_3 level, the remaining ammonia rate of BIO_2, BAL_3, AQ_1 treatments were rather high after 24 hours - 76.61% (122.778 ± 1.711 ppm). 77.06% (123.702 ± 1.277 ppm) và 76.07% (121.237 ± 0.856 ppm). After 48 and 72 hours, although ammonia content decreased statistically significantly compared with initial 24 hours of ammonia removal, the remaining ammonia rate of BIO_2, BAL_3, AQ_1 treatments was still so high after 72 hours that was 65.46% (104.910 ± 0.506 ppm), 64.82% (104.043 ± 0.435 ppm) và 67.60% (107.737 ± 0.911 ppm).

Table 2: Ammonia removal tests for BIO_2 strain

TREAMENTS	20 PPM	40 PPM	80 PPM	160 PPM
0 HR	20.021 ± 0.254^{d}	39.952 ± 0.185^{d}	79.859 ± 0.541^{d}	160.255 ± 0.985^{d}
24 HRS	10.101 ± 0.365^{c}	13.168 ± 0.158^{c}	35.516 ± 0.549^{c}	122.778 ± 1.711^{c}
48 HRS	2.370 ± 0.257^{b}	7.950 ± 0.067^{b}	27.667 ± 0.884^{b}	110.667 ± 0.650^{b}
72 HRS	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	24.686 ± 0.466^{a}	104.910 ± 0.506^{a}

Table 3: Ammonia removal tests for BAL_3 strain

TREAMENTS	20 PPM	40 PPM	80 PPM	160 PPM
0 HR	19.965 ± 0.189^{d}	40.102 ± 0.351^{d}	79.898 ± 0.325^{d}	160.521 ± 0.512^{d}
24 HRS	9.508 ± 0.196^{c}	14.173 ± 0.293^{c}	33.279 ± 0.317^{c}	123.702 ± 1.277^{c}
48 HRS	3.569 ± 0.481^{b}	5.357 ± 0.205^{b}	26.871 ± 0.718^{b}	113.534 ± 0.725^{b}
72 HRS	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	23.064 ± 0.661^{a}	104.043 ± 0.435^{a}

Table 4: Ammonia removal tests for AQ_1 strain

AQ1 (ppm)	20 PPM	40 PPM	80 PPM	160 PPM
0 HR	19.897 ± 0.321^{d}	40.022 ± 0.356^{d}	80.025 ± 0.514^{d}	159.369 ± 0.589^{c}
24 HRS	9.598 ± 0.218^{c}	13.879 ± 0.641^{c}	33.698 ± 1.031^{c}	121.237 ± 0.856^{b}

48 HRS	3.657 ± 0.061[b]	5.447 ± 0.450[b]	26.355 ± 0.678[b]	110.587 ± 0.621[a]
72 HRS	0.000 ± 000[a]	0.000 ± 0.000[a]	22.241 ± 0.328[a]	107.737 ± 0.911[a]

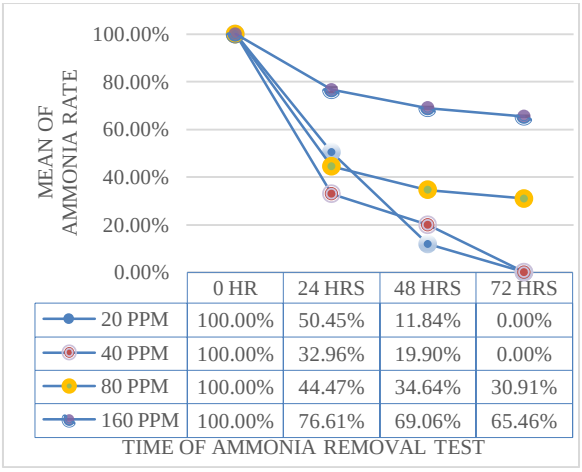


Figure 3: Ammonia removal tests for BIO_2 strain

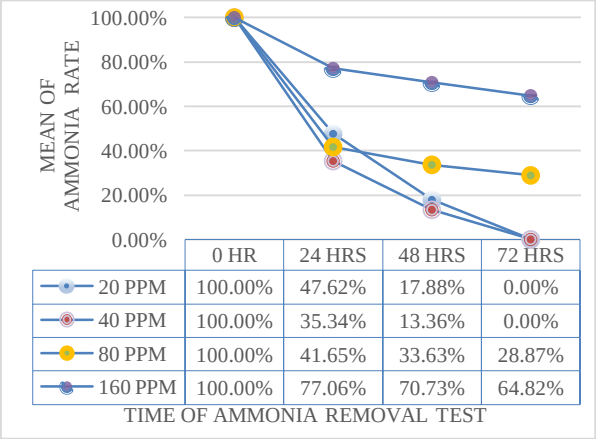


Figure 5: Ammonia removal tests for BAL_3 strain

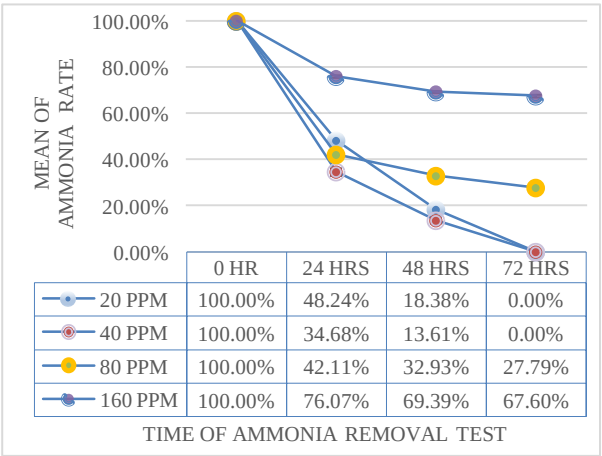


Figure 7: Ammonia removal tests for AQ_1 strain

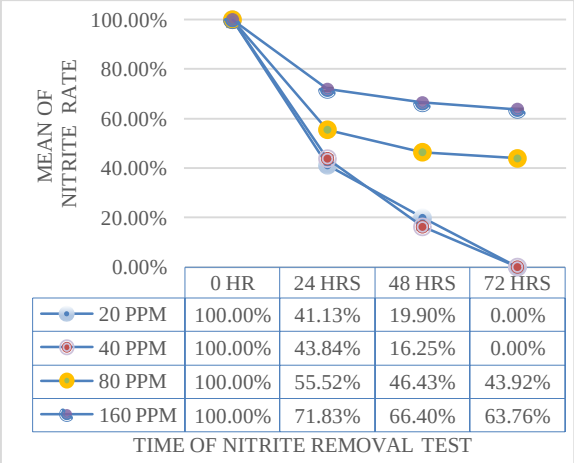


Figure 4: Nitrite removal tests for BIO_2 strain

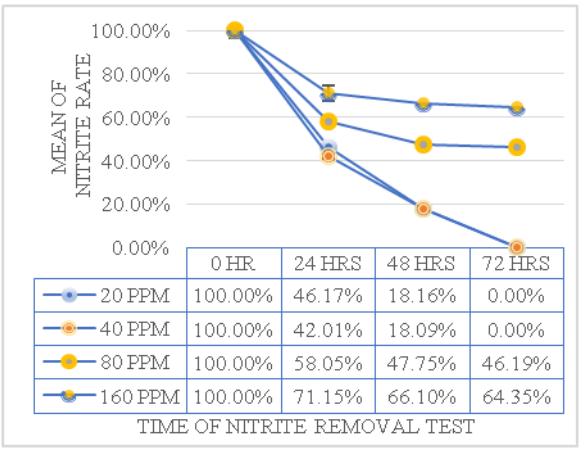


Figure 6: Nitrite removal tests for BAL_3 strain

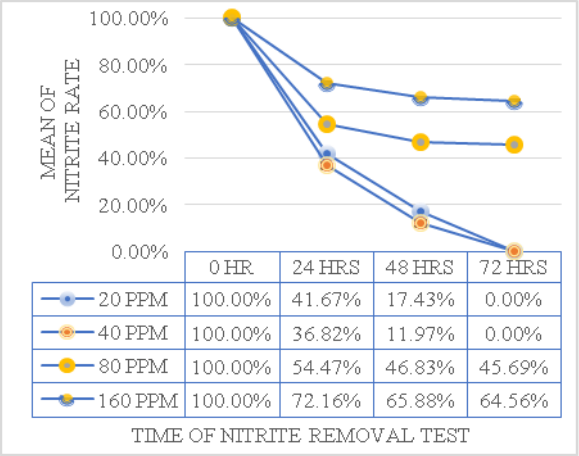


Figure 8: Nitrite removal tests for *Bacillus* sp. AQ_1

Figures 4, 6, 8 and tables 5, 6, 7 showed that the remaining nitrite rate was about 41 - 46% compared with the initial concentration of 20 ppm after 24 hours treated by BIO_2, BAL_3 và AQ_1. After 48 hours, the remaining nitrite rate was about 17 - 18%, and these strains removed completely 100% nitrite content after 72 hours. Similarly, at 40 ppm of initial nitrite level, these strains removed 100% nitrite content after 72 hours. However, at 80 ppm of initial nitrite level, nitrite content was removed almost 50% after 24 hours with BIO_2, BAL_3, AQ_1 treatments. After 48 hours, the nitrite content was removed only 5 - 9%, and the remaining nitrite content of BIO_2, BAL_3, AQ_1 after 72 hours were 43.92% (35.143 ± 0.769 ppm), 46.19% (37.396). ± 0.530 ppm), 45.69% (36.429 ± 0.849 ppm), the remaining nitrite content at 48 and 72 hours were not statistically

different. At 160 ppm of initial nitrite level, the remaining nitrite rates of strains BIO_2, BAL_3, AQ_1 were quite high after 24 hours, respectively 71.83% (114,798 ± 1,008 ppm), 71.15% (113,786 ± 5,978 ppm) and 72.16% (116,008 ± 0.663 ppm). The remaining nitrite rates of BIO_2, BAL_3, AQ_1 after 72 hours was 63.76% (101,892 ± 0.516 ppm), 64.35 % (102,914 ± 0.609 ppm) and 64.56% (103,778 ± 0.291 ppm). The above results showed that all three strains of BIO2, BAL3 and AQ1 were able to remove 100% of ammonia and nitrite with initial concentration of 40 ppm within 72 hours. With higher concentrations of ammonia and nitrite, these strains removed up to approximately 56 - 58 ppm ammonia and 43 - 45 ppm nitrite at initial concentrations of 80 ppm, 53 - 55 ppm ammonia and 57 - 59 ppm nitrite at initial concentrations of 160 ppm after 72 hours of testing.

Table 5: Nitrite removal tests for BIO_2 strain

TREAMENTS	20 PPM	40 PPM	80 PPM	160 PPM
0 HR	20.158 ± 0.599^{d}	40.215 ± 0.925^{d}	80.021 ± 0.925^{c}	159.817 ± 0.935^{d}
24 HRS	8.291 ± 0.352^{c}	17.631 ± 0.493^{c}	44.424 ± 0.504^{b}	114.798 ± 1.008^{c}
48 HRS	4.012 ± 0.191^{b}	6.534 ± 0.416^{b}	37.154 ± 0.444^{a}	106.117 ± 0.257^{b}
72 HRS	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	35.143 ± 0.769^{a}	101.892 ± 0.516^{a}

Table 6: Nitrite removal tests for BAL_3 strain

TREAMENTS	20 PPM	40 PPM	80 PPM	160 PPM
0 HR	19.898 ± 0.638^{d}	40.028 ± 0.365^{d}	80.958 ± 0.968^{c}	159.925 ± 0.859^{d}
24 HRS	$9.186 \pm 0.266_{c}$	16.817 ± 0.446^{c}	46.996 ± 0.672^{b}	113.786 ± 5.978^{c}
48 HRS	3.613 ± 0.392^{b}	7.243 ± 0.333^{b}	38.654 ± 0.439^{a}	105.715 ± 0.542^{b}
72 HRS	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	37.396 ± 0.530^{a}	102.914 ± 0.609^{a}

Table 7: Nitrite removal tests for AQ_1 strain

TREAMENTS	20 PPM	40 PPM	80 PPM	160 PPM
0 HR	19.923 ± 0.258^{d}	39.897 ± 0.487^{d}	79.725 ± 0.563^{c}	160.759 ± 0.899^{d}
24 HRS	8.302 ± 0.281^{c}	14.692 ± 0.561^{c}	43.430 ± 0.605^{b}	116.008 ± 0.663^{c}
48 HRS	3.472 ± 0.286^{b}	4.777 ± 0.614^{b}	37.338 ± 0.490^{a}	105.910 ± 0.551^{b}
72 HRS	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	36.429 ± 0.849^{a}	103.778 ± 0.291^{a}

3.4. Removal tests of ammonia, nitrite and nitrate for *Yucca schidigera* extract: Figure 8 and table 8 showed that at 10 ppm of initial ammonia level, 3 dilutions - 1/2, 1/4 and 1/8 of *Yucca schidigera* extract was able to effectively remove ammonia and nitrite, the remaining ammonia contents after 72 hours are 0.018 ± 0.003 ppm, 0.991 ± 0.136 ppm và 1.785±0.224 ppm, respectively. The other dilutions ineffectively removed ammonia. Therefore, these dilutions of *Yucca schidigera* extract were chosen for the next experiment with initial ammonia concentrations of 20, 40, and 80 ppm.

Table 8: Ammonia removal tests for *Yucca schidigera* Extract at an initial ammonia concentration of 10 ppm

TREATMENT	1/2	1/4	1/8	1/16	1/32	1/64
0h	9.821 ± 0.176^{a}	9.852 ± 0.212^{a}	9.931 ± 0.125^{a}	9.951 ± 0.316^{a}	9.889 ± 0.426^{a}	9.853 ± 0.282^{a}
24h	3.509 ± 0.316^{a}	4.551 ± 0.272^{b}	4.929 ± 0.186^{c}	5.944 ± 0.043^{d}	7.354 ± 0.032^{e}	8.829 ± 0.032^{f}
48h	1.701 ± 0.436^{a}	2.313 ± 0.137^{b}	3.420 ± 0.585^{c}	5.884 ± 0.028^{d}	6.298 ± 0.307^{d}	7.799 ± 0.147^{e}
72h	0.018 ± 0.003^{a}	0.911 ± 0.136^{b}	1.785 ± 0.224^{c}	4.422 ± 0.071^{d}	4.889 ± 0.446^{e}	7.019 ± 0.341^{f}

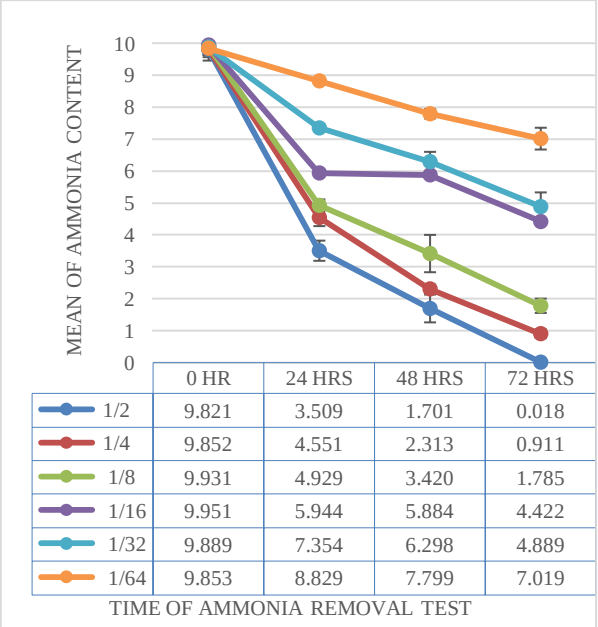


Figure 8: Ammonia removal tests for *Yucca schidigera* Extract at an initial ammonia concentration of 10 ppm

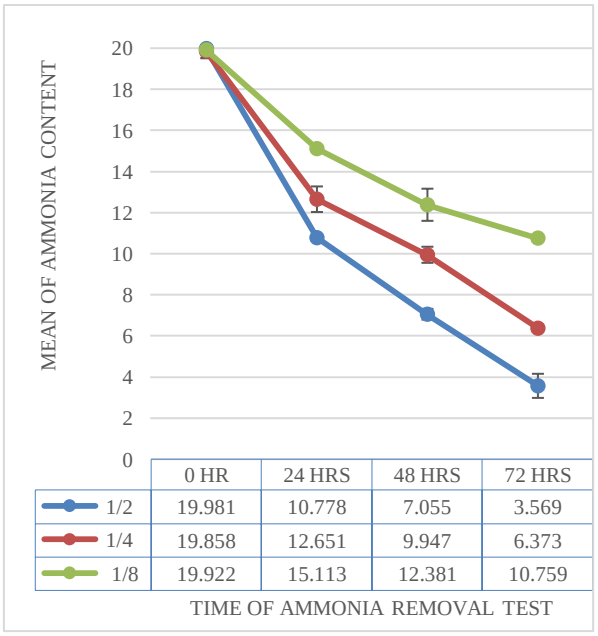


Figure 9: Ammonia removal tests for *Yucca schidigera* Extract at an initial ammonia concentration of 20 ppm

Figure 9 and table 9 showed that at 20 ppm of initial ammonia level, ammonia contents of 3 dilutions of *Yucca schidigera* were decreased statistically significantly at 24, 48, and 72 hours. However, the remaining ammonia content after 72 hours of 1/2, 1/4, 1/8 dilution of *Yucca schidigera* extract were 3.569 ± 0.587 ppm, 6.373 ± 0.183 ppm và 10.759 ± 0.147 ppm, respectively. Therefore, *Yucca schidigera* extract were able to remove 16.412 ppm (1/2 dilution), 13.485 ppm (1/4 dilution), and 9.163 ppm (1/8 dilution).

Table 9: Ammonia removal tests for *Yucca schidigera* Extract at an initial ammonia concentration of 20 ppm

TREATMENT	1/2	1/4	1/8
0 HR	19.981 ± 0.232^a	19.858 ± 0.325^a	19.922 ± 0.413^a
24 HRS	10.778 ± 0.155^a	12.651 ± 0.622^b	15.113 ± 0.126^c
48 HRS	7.055 ± 0.261^a	9.947 ± 0.391^b	12.381 ± 0.782^c
72 HRS	3.569 ± 0.587^a	6.373 ± 0.183^b	10.759 ± 0.147^c

Figure 10 and table 10 showed that at 40 ppm of initial ammonia level, the remaining ammonia contents of 1/2, 1/4, and 1/8 dilution of *Yucca schidigera* extract after 72 hours were 15.941 ± 0.936 ppm, 20.977 ± 0.266 ppm

and 25.696 ± 0.296 ppm, respectively. In other words, the removed ammonia contents of yucca extract treatments were 23.937 ppm (1/2 dilution), 18.945 ppm (1/4 dilution) and 14.405 ppm (1/8 dilution). Therefore, the higher the initial ammonia content is, the greater yucca extract eliminates or metabolizes. Compared with 20 ppm of initial NH_3 level, at the same period and dilution, the more ammonia contents of these treatments were removed about 5 – 7 ppm.

Table 10: Ammonia removal tests for *Yucca schidigera* Extract at an initial ammonia concentration of 40 ppm

TREATMENT	1/2	1/4	1/8
0 HR	39.878 ± 0.524^a	39.922 ± 0374^a	40.101 ± 0.247^a
24 HRS	28.415 ± 1.608^a	30.592 ± 0.322^b	32.152 ± 0.476^c
48 HRS	25.285 ± 1.785^a	28.499 ± 0.725^b	30.283 ± 1.213^b
72 HRS	15.941 ± 0.936^a	20.977 ± 0.266^b	25.696 ± 0.296^c

Figure 11 and table 11 showed that at 80 ppm of initial ammonia level, the remaining ammonia contents of 1/2, 1/4, and 1/8 dilution of *Yucca schidigera* extract after 72 hours were 55.667 ± 2.543 ppm, 62.885 ± 2.876 ppm và 65.782 ± 0.371 ppm, respectively. The removed ammonia contents of yucca extract treatments were 24.314 ppm (1/2 dilution), 17.243 ppm (1/4 dilution) and 14.501 ppm (1/8 dilution). Compared with 40 ppm of initial NH_3 level, at the same period and dilution, the removed ammonia contents of these treatments were undifferent. Therefore, a certain content of *Yucca schidigera* extract is possible to remove only a corresponding amount of ammonia. In which, the 1/2 dilution of *Yucca schidigera* extract gave the highest results in the treatments with the ability to remove about 24.314 ppm of ammonia within 72 hours.

Table 11: Ammonia removal tests for *Yucca schidigera* Extract at an initial ammonia concentration of 80 ppm

TREATMENT	1/2	1/4	1/8
0 HR	79.981 ± 0.231^a	80.128 ± 0.353^a	80.283 ± 0.219^a
24 HRS	72.298 ± 0.331^a	73.560 ± 0.140^b	75.498 ± 0.673^c
48 HRS	66.248 ± 0.202^a	68.211 ± 1.607^b	72.392 ± 0.881^c
72 HRS	55.667 ± 2.543^a	62.885 ± 2.876^b	65.782 ± 0.371^c

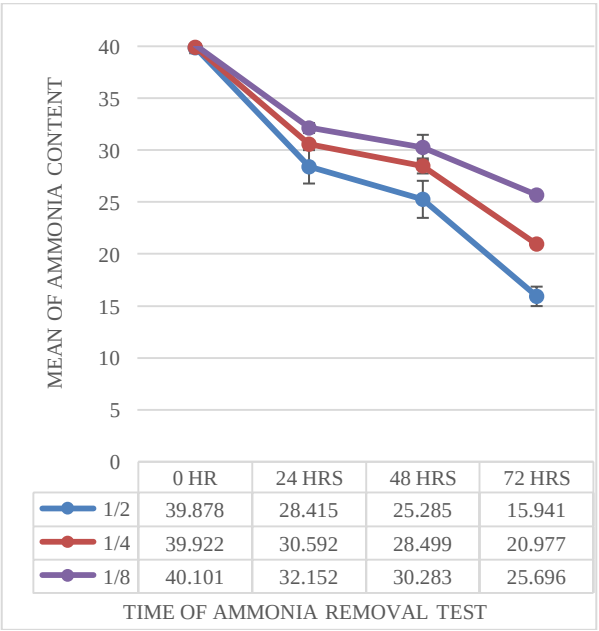


Figure 10: Ammonia removal tests for *Yucca schidigera* Extract at an initial ammonia concentration of 40 ppm

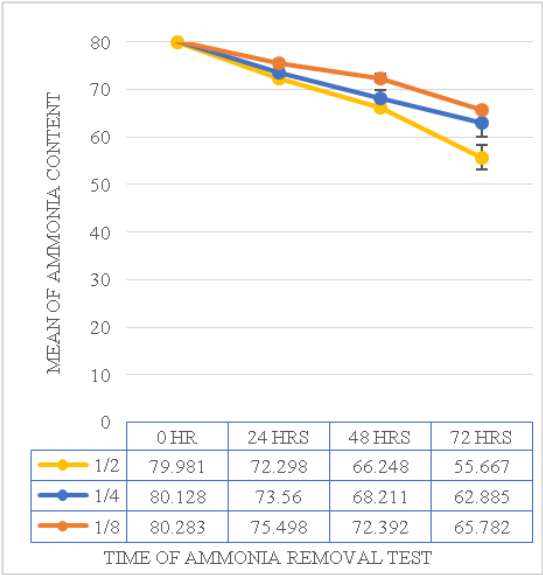


Figure 11: Ammonia removal tests for *Yucca schidigera* Extract at an initial ammonia concentration of 80 ppm

At 10 ppm of initial NO_2 level, figure 12 and table 12 showed that the ammonia contents were evidently decreased after 24 hours, the remaining nitrite contents were about 0.914 - 2.045 ppm. After 48 hours, the remaining nitrite contents were continuously reduced about 0.188 - 0.948 ppm. The 1/2 and 1/4 dilutions of *Yucca schidigera* extract removed completely 100% of nitrite contents after 72 hours, these results at 1/8, 1/16, 1/32, and 1/64 dilutions were 0.166 ± 0.053 ppm, 0.184 ± 0.046 ppm, 0.914 ± 0.096 ppm và 0.931 ± 0.043 ppm, respectively.

Table 12: Nitrite removal tests for *Yucca schidigera* Extract at an initial nitrite concentration of 10 ppm

TREATMENT	0 HR	24 HRS	48 HRS	72 HRS
1/2	9.969 ± 0.155^{a}	0.914 ± 0.040^{a}	0.188 ± 0.048^{a}	0.000 ± 0.000^{a}
1/4	9.872 ± 0.255^{a}	1.034 ± 0.013^{a}	0.249 ± 0.017^{ab}	0.000 ± 0.000^{a}
1/8	9.913 ± 0.212^{a}	1.041 ± 0.014^{a}	$0.278 \pm 0.029_{b}$	0.166 ± 0.053^{b}
1/16	9.951 ± 0.325^{a}	1.061 ± 0.041^{b}	0.304 ± 0.054^{b}	0.184 ± 0.046^{b}
1/32	9.972 ± 0.148^{a}	1.177 ± 0.063^{c}	0.920 ± 0.023^{c}	0.914 ± 0.096^{c}
1/64	9.894 ± 0.275^{a}	2.045 ± 0.030^{d}	0.948 ± 0.046^{c}	0.931 ± 0.043^{c}

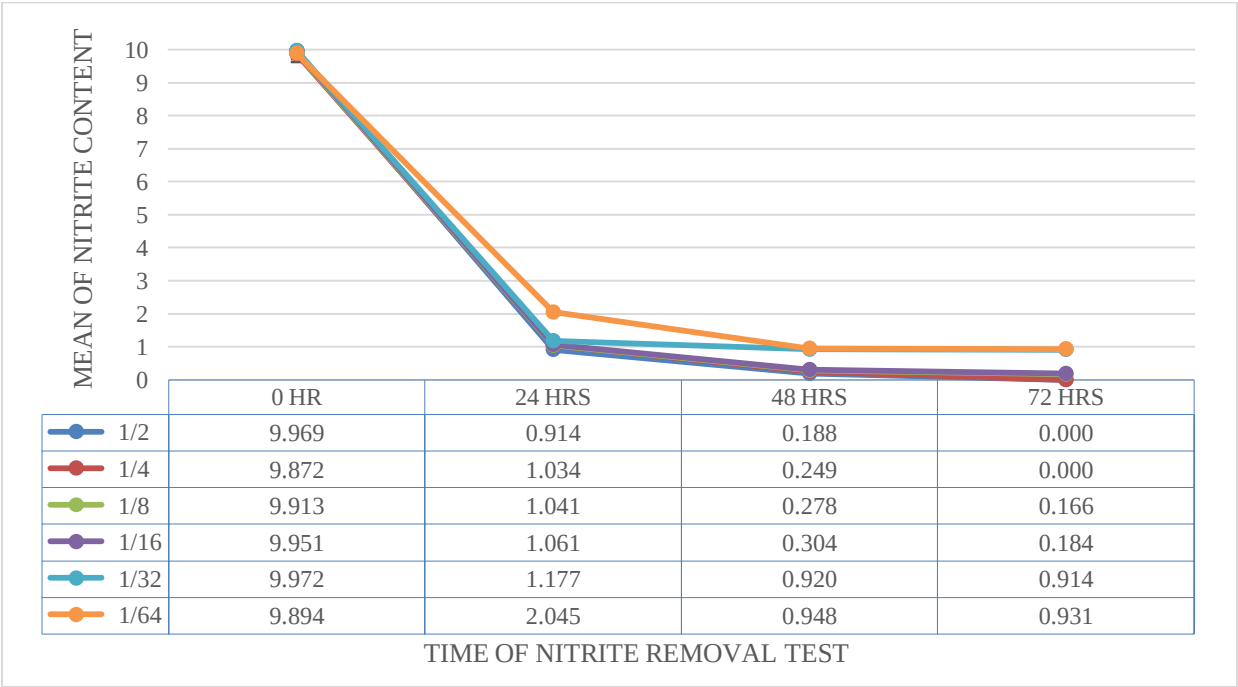


Figure 12: Nitrite removal tests for *Yucca schidigera* Extract at an initial nitrite concentration of 10 ppm

Figures 13, 14 and tables 13, 14 showed that the nitrite removal process had similarity in removal periods between 20 ppm and 40 ppm of initial NO_2 levels. After 24 hours, the remaining nitrite contents of treatments were about 13.862 – 17.946 ppm, equivalent to the removed nitrite contents of 1.971 - 6.124 ppm (20 ppm of initial nitrite level) and 2.008 - 6.748 ppm (40 ppm of initial nitrite level). After 72 hours, the remaining nitrite contents of treatments were about 7.923 - 13.072 ppm, equivalent to the removed nitrite contents of 6.845 - 12.063 ppm (20 ppm of initial nitrite level) and 9.145 - 12.081 ppm (40 ppm of initial nitrite level). These results also showed that a certain content of *Yucca schidigera* extract is possible to remove only a corresponding amount of nitrite. In the result, the 1/2 dilution of *Yucca schidigera* are able to remove maximally 12.081 ppm of nitrte after 72 hours. Thus, the 1/2 dilution of yucca extract was chosen for the next experiment.

Table 13: Nitrite removal tests for *Yucca schidigera* extract at an initial nitrite concentration of 20 ppm

TREATMENT	0 HR	24 HRS	48 HRS	72 HRS
1/2	19.986 ± 0.241^{a}	13.862 ± 0.451^{a}	11.211 ± 0.367^{a}	7.923 ± 0.221^{a}
1/4	20.023 ± 0.356^{a}	15.142 ± 0.292^{b}	12.322 ± 0.522^{b}	9.132 ± 0.243^{b}
1/8	19.925 ± 0.311^{a}	15.670 ± 0.310^{b}	13.568 ± 0.445^{c}	11.671 ± 0.559^{c}
1/16	20.101 ± 0.152^{a}	16.473 ± 0.369^{c}	$14.111 \pm 0.188c^{d}$	12.275 ± 0.277^{d}
1/32	19.955 ± 0.125^{a}	17.411 ± 0.505^{d}	14.660 ± 0.332^{d}	12.829 ± 0.129^{e}
1/64	19.917 ± 0.319^{a}	17.946 ± 0.091^{d}	15.377 ± 0.382^{e}	13.072 ± 0.115^{e}

Table 14: Nitrite removal tests for *Yucca schidigera* extract at an initial nitrite concentration of 40 ppm

TREATMENT	0 HR	24 HRS	48 HRS	72 HRS
1/2	39.895 ± 0.379^{a}	33.147 ± 0.893^{a}	29.751 ± 0.561^{a}	27.814 ± 0.286^{a}
1/4	39.935 ± 0.408^{a}	34.163 ± 0.788^{b}	30.867 ± 0.306^{b}	28.593 ± 0.413^{b}
1/8	40.214 ± 0.356^{a}	35.293 ± 0.320^{c}	32.076 ± 0.264^{c}	29.084 ± 0.316^{b}
1/16	40.106 ± 0.297^{a}	35.597 ± 0.436^{c}	32.343 ± 0.211^{c}	29.837 ± 0.352^{c}
1/32	39.981 ± 0.354^{a}	37.364 ± 0.517^{d}	32.630 ± 0.753^{c}	30.342 ± 0.184^{cd}
1/64	39.992 ± 0.211^{a}	37.984 ± 0.141^{d}	33.693 ± 0.264^{d}	30.847 ± 0.204^{d}

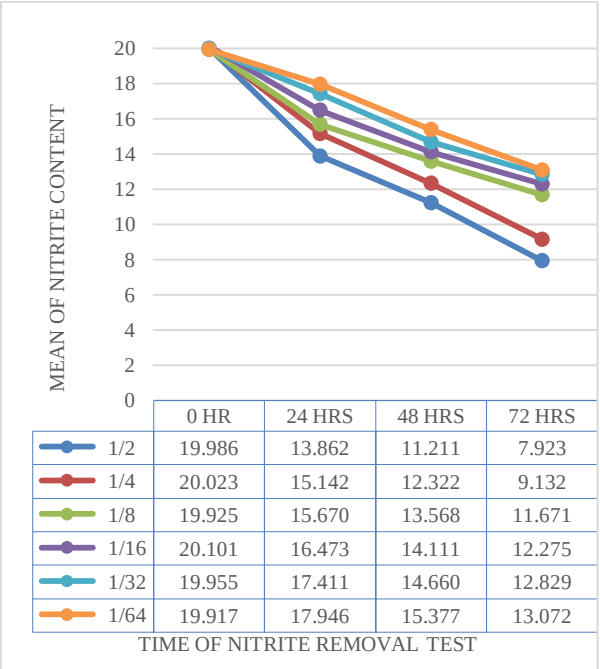
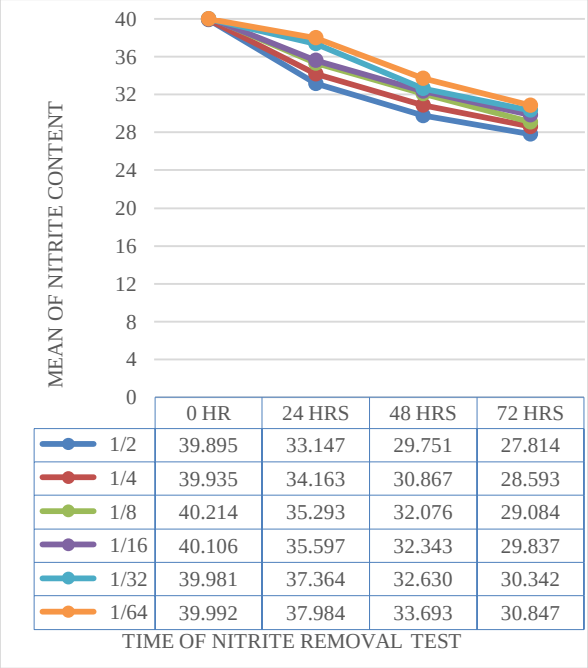


Figure 13: Nitrite removal tests for *Yucca schidigera* Extract at an initial nitrite concentration of 20 ppm

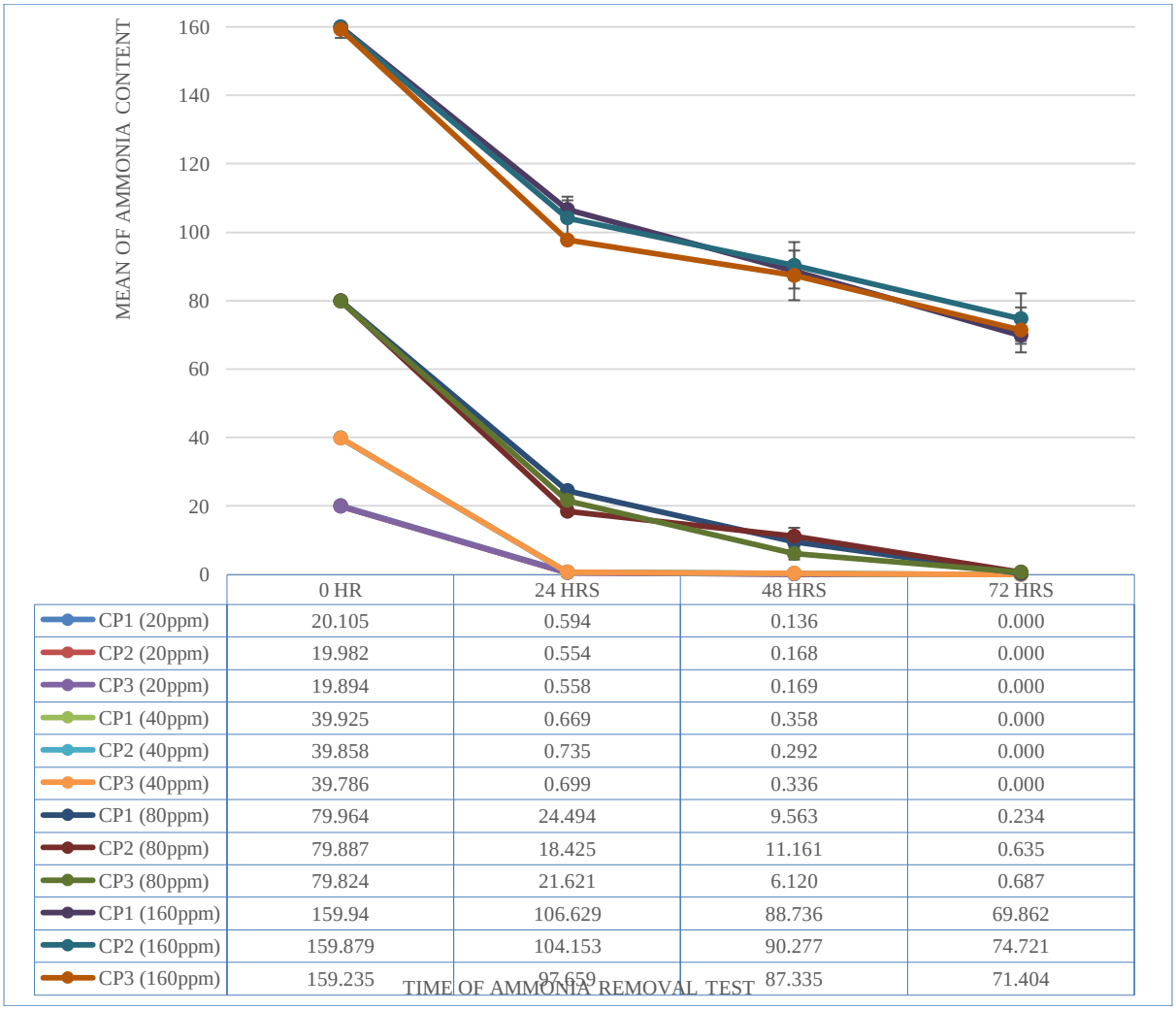


	0 HR	24 HRS	48 HRS	72 HRS
1/2	39.895	33.147	29.751	27.814
1/4	39.935	34.163	30.867	28.593
1/8	40.214	35.293	32.076	29.084
1/16	40.106	35.597	32.343	29.837
1/32	39.981	37.364	32.630	30.342
1/64	39.992	37.984	33.693	30.847

Figure 14: Nitrite removal tests for *Yucca schidigera* Extract at an initial nitrite concentration of 40 p

3.5. Removal tests of ammonia, nitrite and nitrate for combination of *Yucca schidigera* extract and screened *Bacillus* strains: From the results of 3.3 and 3.4 experiments, three treatments were chosen for this experiment including CP1 – *Bacillus* sp. BIO_2 strain combined with 1/2 dilution of yucca extract, CP2 - *Bacillus* sp. BAL_3 strain combined with 1/2 dilution of yucca extract, and *Bacillus* sp. AQ_1 strain combined with 1/2 dilution of yucca extract. Figure 15 and table 15 showed that ammonia contents of 20 mg and 40 mg of initial ammonia levels were reduced below 1.000 ppm after 24 hours and completely removed 100% after 72 hours in all treatments. At 80 ppm of initial ammonia level, the remaining ammonia contents of CP1, CP2, and CP3 after 24 hours were 24.494 ± 0.768 ppm, 18.425 ± 1.133 ppm, 21.621 ± 1.816 ppm, respectively. After 48 hours, the remaining ammonia content of CP3 treatment was 6.120 ± 1.816 ppm which was statistically lower and significantly different from CP1 and CP2 treatments with the remaining ammonia contents of 9.563 ± 0.564 ppm và 11.161 ± 2.434 ppm, respectively. All three treatments of CP1, CP2, and CP3 removed almost 100% of ammonia contents after 72 hours with the remaining ammonia contents of 0.234 ± 0.137 ppm, 0.635 ± 0.218 ppm và 0.687 ± 0.915 ppm, respectively. At 160 ppm of initial ammonia level, after 24 hours, the remaining ammonia contents of CP1, CP2, and CP3 treatments were 106.629 ± 2.631 ppm, 104.153 ± 6.167 ppm và 97.659 ± 0.632 ppm, respectively. Ammonia content of this level was removed very slowly, the removed ammonia contents of three treatments were about 85 – 90 ppm after 72 hours, and there was no statistical difference between treatments.

Figure 16 and table 16 showed that at 20 ppm and 40 ppm of initial nitrite levels, the nitrite contents of three treatments were removed almost completely after 72 hours. At 80 ppm of initial nitrite level, the remaining nitrite contents of CP1, CP2, CP3 after 24 hours were 46.216 ± 3.697 ppm, 40.406 ± 3.499 ppm, 79.935 ± 4.477 ppm, respectively. After 72 hours, CP1 removed 100% of nitrite content, CP2 and CP3 removed almost 100% of nitrite contents with 0.013 ± 0.003 ppm và 0.003 ± 0.001 ppm, respectively. At 160 ppm of initial nitrite level, the remaining nitrite contents of CP1, CP2, CP3 after 24 hours were 104.093 ± 9.532 ppm, 104.039 ± 5.245 ppm và 104.200 ± 5.311 ppm, respectively. After 72 hours, the remaining nitrite contents of CP1, CP2, CP3 were 65.163 ± 1.159 ppm, 71.838 ± 6.819 ppm, 68.901 ± 7.758 ppm, respectively. Therefore, the combinations of CP1, CP2, CP3 were able to remove maximally 85 – 90 ppm of ammonia and 87 – 94 ppm of nitrite within 72 hours.

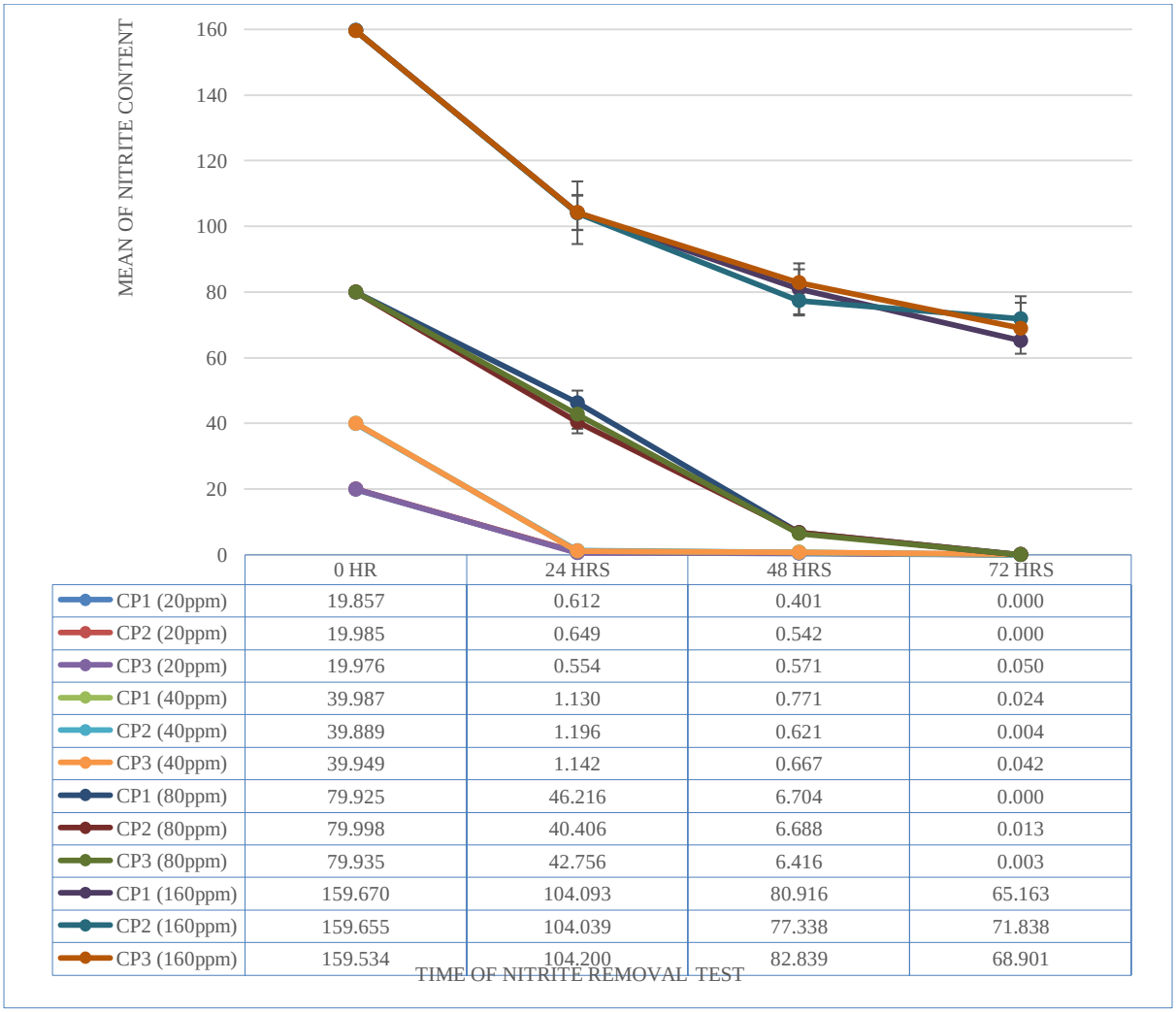


	0 HR	24 HRS	48 HRS	72 HRS
CP1 (20ppm)	20.105	0.594	0.136	0.000
CP2 (20ppm)	19.982	0.554	0.168	0.000
CP3 (20ppm)	19.894	0.558	0.169	0.000
CP1 (40ppm)	39.925	0.669	0.358	0.000
CP2 (40ppm)	39.858	0.735	0.292	0.000
CP3 (40ppm)	39.786	0.699	0.336	0.000
CP1 (80ppm)	79.964	24.494	9.563	0.234
CP2 (80ppm)	79.887	18.425	11.161	0.635
CP3 (80ppm)	79.824	21.621	6.120	0.687
CP1 (160ppm)	159.94	106.629	88.736	69.862
CP2 (160ppm)	159.879	104.153	90.277	74.721
CP3 (160ppm)	159.235	97.659	87.335	71.404

Figure 15: Ammonia removal tests for combination of BIO_2, BAL_3, AQ_1 and *Yucca schidigera* extract

Table 15: Ammonia removal tests for combination of BIO_2, BAL_3, AQ_1 and *Yucca schidigera* extract

TREATMENT	0 HR	24 HRS	48 HRS	72 HRS
CP1 (20ppm)	20.105 ± 0.125^a	0.594 ± 0.043^a	0.136 ± 0.082^a	0.000 ± 0.000^a
CP2 (20ppm)	19.982 ± 0.064^a	0.554 ± 0.028^a	0.168 ± 0.056^a	0.000 ± 0.000^a
CP3 (20ppm)	19.894 ± 0.276^a	0.558 ± 0.041^a	0.169 ± 0.036^a	0.000 ± 0.000^a
CP1 (40ppm)	39.925 ± 0.070^a	0.669 ± 0.031^a	0.358 ± 0.101^a	0.000 ± 0.000^a
CP2 (40ppm)	39.858 ± 0.350^a	0.735 ± 0.028^a	0.292 ± 0.036^a	0.000 ± 0.000^a
CP3 (40ppm)	39.786 ± 0.206^a	0.699 ± 0.025^a	0.336 ± 0.045^a	0.000 ± 0.00^a
CP1 (80ppm)	79.964 ± 0.843^a	24.494 ± 0.768^c	9.563 ± 0.564^b	0.234 ± 0.037^a
CP2 (80ppm)	79.887 ± 0.710^a	18.425 ± 1.133^a	11.161 ± 2.434^b	0.635 ± 0.018^a
CP3 (80ppm)	79.824 ± 0.841^a	21.621 ± 1.816^b	6.120 ± 1.816^a	0.687 ± 0.015^a
CP1 (160ppm)	159.940 ± 0.613^a	106.629 ± 2.631^b	88.736 ± 2.755^a	69.862 ± 1.699^a
CP2 (160ppm)	159.879 ± 0.505^a	104.153 ± 6.167^b	90.277 ± 6.787^a	74.721 ± 7.375^a
CP3 (160ppm)	159.235 ± 2.526^a	97.659 ± 0.632^a	87.335 ± 7.251^a	71.404 ± 6.569^a



	0 HR	24 HRS	48 HRS	72 HRS
CP1 (20ppm)	19.857	0.612	0.401	0.000
CP2 (20ppm)	19.985	0.649	0.542	0.000
CP3 (20ppm)	19.976	0.554	0.571	0.050
CP1 (40ppm)	39.987	1.130	0.771	0.024
CP2 (40ppm)	39.889	1.196	0.621	0.004
CP3 (40ppm)	39.949	1.142	0.667	0.042
CP1 (80ppm)	79.925	46.216	6.704	0.000
CP2 (80ppm)	79.998	40.406	6.688	0.013
CP3 (80ppm)	79.935	42.756	6.416	0.003
CP1 (160ppm)	159.670	104.093	80.916	65.163
CP2 (160ppm)	159.655	104.039	77.338	71.838
CP3 (160ppm)	159.534	104.200	82.839	68.901

Figure 16: Nitrite removal tests for combination of BIO_2, BAL_3, AQ_1 and *Yucca schidigera* extract

Table 16: Nitrite removal tests for combination of BIO_2, BAL_3, AQ_1 and *Yucca schidigera* extract

TREATMENT	0 HR	24 HRS	48 HRS	72 HRS
CP1 (20ppm)	19.857 ± 0.655^{b}	0.612 ± 0.051^{a}	0.401 ± 0.062^{a}	0.000 ± 0.000^{a}
CP2 (20ppm)	19.985 ± 0.548^{c}	0.649 ± 0.071^{a}	0.542 ± 0.063^{b}	0.000 ± 0.000^{a}
CP3 (20ppm)	19.976 ± 0.894^{b}	0.554 ± 0.033^{a}	0.571 ± 0.049^{b}	0.050 ± 0.004^{a}
CP1 (40ppm)	39.987 ± 0.224^{b}	1.130 ± 0.057^{a}	0.771 ± 0.041^{a}	0.024 ± 0.005^{a}
CP2 (40ppm)	39.889 ± 0.267^{b}	1.196 ± 0.027^{a}	0.621 ± 0.017^{a}	0.004 ± 0.001^{a}
CP3 (40ppm)	39.949 ± 0.537^{b}	1.142 ± 0.042^{a}	0.667 ± 0.113^{a}	0.042 ± 0.006^{a}
CP1 (80ppm)	79.925 ± 0.544^{b}	46.216 ± 3.697^{a}	6.704 ± 0.874^{a}	0.000 ± 0.000^{a}
CP2 (80ppm)	79.998 ± 0.364^{b}	40.406 ± 3.499^{a}	6.688 ± 0.537^{a}	0.013 ± 0.003^{a}
CP3 (80ppm)	79.935 ± 0.192^{b}	42.756 ± 4.477^{a}	6.416 ± 0.704^{a}	0.003 ± 0.001^{a}
CP1 (160ppm)	159.670 ± 0.734^{b}	104.093 ± 9.532^{a}	80.916 ± 7.758^{a}	65.163 ± 1.159^{a}
CP2 (160ppm)	159.655 ± 1.244^{b}	104.039 ± 5.245^{a}	77.338 ± 4.546^{a}	71.838 ± 6.819^{a}
CP3 (160ppm)	159.534 ± 0.562^{b}	104.200 ± 5.311^{a}	82.839 ± 4.028^{a}	68.901 ± 7.758^{a}

IV. Discussion

In aquaculture, toxic gas is a matter of concern, especially NH_3 and NO_2. Ammonia and nitrite with high concentration are able to diffuse across the gills into the bloodstream of aquatic animals, which makes pH of blood high, causing metabolic disorders in the body including inhibition of oxygen transport in the bloodstream, disturbance of the osmotic pressure of cells, neural inhibition, changing blood composition to slow growth of aquatic animals, reducing immune response, being very susceptible to diseases such as white feces, early motarlity syndrome (EMS), black gill syndrome, white spot syndrome, infectious myonecrosis desease. Ammonia can be toxic to shrimp and fish at concentration above 1.5 ppm (Crab et al., 2007). LD_{50} (Lethal dose, 50%) of shrimp and fish for nitrite concentrations in the range 0.1 - 10 ppm (Philips et al., 2002). Therefore, this

study was implemented for isolation of *Bacillus* sp. and investigation of combination of *Yucca schidigera* and isolated *Bacillus* sp. to find out an effective bioremediation of ammonia and nitrite removal in aquaculture.

Bacillus sp. is one of the potential microorganisms to control biosecurity, improve water quality, especially to control toxic gases NH_3 and NO_2 in aquaculture. Previously, some studies showed that *Bacillus subtilis* (Chen and Hu, 2011; Meng et al., 2009), *Bacillus licheniformis* (Meng et al., 2009) and *Bacillus cereus* (Lalloo et al., 2007) expressed strong ability in nitrite removal. Microbiological research showed that *Bacillus* spp. is capable of using nitrite and nitrate as an alternative electron acceptors and a carbon source (Nakano et al., 1998; Hoffmann et al., 1998).

This study showed that three of 108 *Bacillus* strains, isolated from Can Gio Mangrove Forest, were so efficient in ammonia and nitrite removal. They were able to remove approximately 55 ppm of ammonia and 59 ppm of nitrite within 72 hours. This result is higher than Xie et al.'s (2013), *Bacillus amyloliquefaciens* HN only removed maximally 20 ppm of nitrite within 48 hours and almost impossible to remove above 10 ppm of ammonia when the experiments were carried out on HNM medium for ammonia and nitrite removal (Xie et al., 2013). However, this result is lower than Yang et al.'s (2017), *Bacillus* sp. K5 were able to remove 87.8 ppm of ammonia within 24 hours on sodium citrate medium with C/N ratio of 15 (Yang et al., 2017), and Zang et al.'s (2020), *Bacillus* sp. SC16 removed 60.5 ppm ammonia within 48 hours on DM medium (Denitrifying medium). This proves that every different *Bacillus* strain is capble of removing different levels of ammonia and nitrite. Especially, the ability of ammonia and nitrite removal may be changed when *Bacillus* sp. was cultured in different nutrient media.

Other studies of nitrifiers, ammonia – oxidizing bacteria (AOB) and nitrite – oxidizing bacteria (NOB) participates in steps of nitrification in water and soil. They play a important role in the nitrogen cycle of the earth (Zhang et al., 2018). In the results of Reddy et al.'s study, three AOB strains of *Nitrosomonas europaea* ATCC 19718, AOB-12 and AOB-21 only removed maximally about 25 ppm of ammonia (*N. europaea* and AOB-12) and 70 ppm (AOB-21) after 72 hours at 200 ppm of initial ammonia level (Reddy et al., 2017). Other results of Ma et al.'s study, at the optimal temperature for nitrification, *Nitrobacter winogradskyi* only removed 7.21 ppm of nitrite after 4 days at 10 ppm of initial nitrite level. At the optimal initial pH, this strain oxidized completely nitrite after 12 days at 20 ppm of initial nitrite level. The peak nitrification rate was observed on day 6 – 8, the maximum nitrification rate, 3.18 ppm h^{-1}, was found at 500 ppm of initial nitrite level. Other days at this level, the nitrification rates of *Nitrobacter winogradskyi* only below 1 ppm h^{-1} (24 days of challenge) (Ma et al., 2014). These results showed that the nitrite and ammonia removal ability of *Bacillus* were similar to AOB and NOB strains'. For the nitrification rate, *Bacillus* sp. metabolizes nitrite more effectively than NOB – *Nitrobacter winogradskyi*. Chemilitotrophic bacteria biotransform ammonia to nitrite through nitrification and denitrification carried out by ammonia oxidizing bacteria (AOB) of the genus *Nitrosomonas* and *Nitrosococcus* or ammonia oxidizing archaeas (AOA) such as *Nitrosopumilus maritimus* which possess the *amo*A gene encoding an enzyme monoxygenase ammonia. This enzyme oxidizes ammonia in hydroxylamine (Ferrera and Sanchez, 2016; Geets et al., 2007). The *hao* gene encodes hydroxylamine oxidase which catalyzes the oxidation of hydroxylamine to nitrite that is also considered a nitrification marker gene. The next step is the oxidation of nitrite to nitrate carried out by nitrite oxidizing bacteria (NOB) of the genus *Nitrobacter* (Klotz and Stein, 2008) based on *nxrB* gene that encodes the enzyme nitrite oxido- reductase (Chen et al., 2017). Denitrification is the most important process for the nitrogen cycle, since nitrogen is produced through microbial respiration by converting nitrates and nitrites to oxide nitrous and dinitrogen, respectively (Dominguez et al., 2019). Some aerobic denitrifiers of the genus *Alcaligenes* and *Thiobacillus* possess key genes such as *nirS*, *nirK* and *nosZ* encoding enzymes – nitrite reductase containing cytochrome cd1, nitrite reductase containing copper (Cu) and the enzyme nitrous oxide reductase, respectively (Simon, 2002). Additionally, many studies showed that enzyme hydroxylamine oxido-reductase, which participates in the oxidation of ammonia to nitrite, was present only in AOB or AOA. However, this enzyme has been detected in *Bacillus* sp. N31 which is highly effective in nitrogen removal (Huang et al., 2017). *Bacillus* sp. is a heterotrophic microorganism in nitrification processes, including ammonia oxidation and nitrite oxidation (Hayatsu et al., 2008). The study of Dominguez et al. detected *nxrB* gene from *Bacillus* strains SM5 and EBA-P for the first time. The primers, which were used in Dominguez's study, were pre-designed for the *nxrB* gene of *Nitrobacter* sp. (Geets et al., 2007). This finding showed that the gene found in the *Bacillus* sp. could be closely related to the *nxrB* gene of *Nitrobacter* (Dominguez et al., 2019). In addition, the *nirK* gene was identified in *Bacillus* strains SM4 and EBA-P, and the nirS gene in SM5. This result was similar to Gao's study showed that the nirK gene has been present in *Bacillus firmus* (Gao et al., 2018). The *hao* gene was purified for the first time from *Bacillus* sp. K5 (Yang et al., 2017) and *Bacillus* sp. SM4, SM5, BM6 and EBA-P (Dominguez et al., 2019). Therefore, in the present study, *Bacillus* strains of BIO_2, BAL_3 and AQ_1 are capable of removing ammonia and nitrite that could be related to their possession of key genes encoding enzymes for heterotrophic nitrification and denitrification processes. Some aerobic denitrifers are also capable of performing heterotrophic nitrifcaton (Ji et al., 2015) which would explain the ammonia and nitrite removing of BIO_2, BAL_3 and AQ_1.

Yucca schidigera is a plant that has the potential to reduce ammonia in aquaculture through yucca extract (Santacruz-Reyes and Chien, 2012; Castillo-Vargasmachuca et al., 2015; Khalil et al., 2015; Yu et al., 2015; Hassan et al., 2017). Many researchers around the world have focused on investigating the effectiveness of yucca extract on growth rate of fish and improved water quality. Yucca extract can reduce NH_3 in both freshwater and saltwater (Santacruz-Reyes and Chien, 2010a,b). Additionally, yucca extract is able to effectively reduce nitrite in Pacific red snapper juvenile fish culture (Calisto - Vargasmachuca et al., 2015). The result of *Yucca schidigera* in ammonia and removal test showed that 1/2 dilution of yucca extract was able to remove about 12.081 ppm of nitrite and 24.314 ppm of ammonia within 72 hours that was the most effective compared with other dilutions of yucca extracts. This result showed that the removed ammonia content depends on the used content of yucca extract that was consistent with the results of Santacruz-Reyes and Chien (2010a), and Yu et al. (2015). The authors Headon and Dawson (1990) hypothesized that ammonia removal process is based on certain components of the yucca extract, which converts ammonia into other form of nitrogen. The other one is that yucca extract's saponin and glyco-components bind ammonia (Wacharonke, 1993; Santacruz-Reyes and Chien, 2010a).

The combination of yucca extract and *Bacillus* sp. BIO_2, BLA_3, AQ_1 showed the results of stronger ammonia and nitrite removal. These combinations are able to remove ammonia and nitrite within 72 hours ranging 85 – 90 ppm of ammonia and 87 – 94 ppm of nitrite. Thus, compared with the results of *Bacillus* sp. or yucca extract, this combination not only has resonance value between the two effects of yucca extract and *Bacillus* sp., but also more effective than using either. Based on the previous hypotheses of yucca's mechanism of action, this study's results indicated that ammonia and nitrite probably bound or were absorbed in yucca substances. Yucca extract positively combined with *Bacillus* sp. in ammonia and nitrite removal. Therefore, the combination of *Bacillus* spp. and yucca extract can be used for experiments in shrimp and fish culture to more fully evaluate the effects of this combination on water parameters and growth rates of shrimp and fish.

V. Conclusion

This study showed that there are 3 strains of *Bacillus* sp. BIO_2, BAL_3, AQ_1, isolated from Can Gio Mangrove Forest, have good ability to remove ammonia and nitrite. The yucca extract is able to absorb ammonia and nitrite, and the removed ammonia and nitrite content is proportional to the used yucca extract. *Yucca schidigera* extract not only does not inhibit *Bacillus* sp., but also enhances ammonia and nitrite removal when these elements are combined.

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