Evaluating a commercial nitrifying bacteria product for enhancing nitrifier development in closed systems.

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High nitrite concentrations (up to 57 mg L-1 NO2-N) were recorded during a 2012 nursery study at the Texas A&M AgriLife Research Mariculture Lab with Litopenaeus vannamei in a 40 m3 greenhouse-enclosed super-intensive, bio-floc-dominated raceway system operated with no water exchange. Shrimp exposed to these high concentrations showed signs of stress with significant mortality. The high concentrations occurred due to the extended period of time required for nitrite-oxidizing bacteria (NOB) to become established in the system. Such nitrite spikes are commonly observed in closed intensive shrimp production systems as culture water matures.
Therefore a trial was conducted to test the efficacy of a commercial nitrifying bacteria product, **Fritz-Zyme® Turbo Start® 900 (Fritz Industries, Inc., Mesquite, Texas)**, for enhancing nitrifier development in simple closed systems.

METHODS

The trial was conducted at the Texas A&M AgriLife Research Mariculture Laboratory at Flour Bluff, Corpus Christi, Texas in April 2015. Thirty two 800 L cylindrical black polyethylene tanks housed under an open-sided shelter were used for the trial. Aeration was generated by a 3 HP regenerative air blower and supplied continuously through two air stones per tank. An equal volume (15% of tank volume) of assorted plastic media was added to each tank to provide surface area for bacterial growth. Each tank was covered with an opaque black plastic sheet to limit light penetration and evaporation. A 1-m length of 25 mm PVC pipe was assigned to each tank for manual mixing. The culture tanks and plastic media were disinfected with 10 ppm chlorine, neutralized with sodium thiosulfate, and rinsed with freshwater prior to the trial.

There were four replicates (tanks) of each of each of the following treatments:

A. Control (no bacterial inoculation) + Ammonia
B. Control (no bacterial inoculation) + Nitrite
C. Non-disinfected seawater + Ammonia
D. Non-disinfected seawater + Nitrite
E. Fritz-Zyme® Turbo Start® + Ammonia
F. Fritz-Zyme® Turbo Start® + Nitrite

Culture water was pumped from Laguna Madre (salinity ~ 35 ppt) to a 36,000 L storage tank, where it was mixed with municipal freshwater to reduce the salinity to 27 ppt and circulated for 24 h. On the first day of the trial, 760 L of culture water was pumped into each of the non-disinfected treatment tanks (Treatments C and D). The remaining water in the storage tank was disinfected with 20 ppm chlorine for 1 h, and then neutralized with sodium thiosulfate. Once no residual chorine remained, 760 L of culture water was pumped into each of the remaining trial tanks.

Once all tanks were full and initial water samples were taken, 20 mg L-1 PO4 (182.5 mL H3PO4 solution neutralized with NaOH to give 83.29 g PO4 L-1) was added to each tank. Then either 10 mg L-1 NH4 (22.65 g NH4Cl 99.5%) (Treatments A, C, and E) or 10 mg L-1 NO2 (11.51 g NaNO2 99%) (Treatments B, D, and F) were added to each tank, according to treatments. After 1 h, another set of water samples were taken to confirm phosphate, ammonia, and nitrite concentrations, and bacterial inoculations were added: Fritz-Zyme® Turbo Start® at 294 mL per tank (Treatments E and F). Each dose was measured in a graduated cylinder and poured directly into a tank and rinsed. Tanks were manually mixed with the individual mixing pipes after each chemical and bacterial inoculation.

The trial ran for 12 days with no water exchange. All tanks were mixed daily with the individual mixing pipes prior to taking water samples. Once the ammonia or nitrite concentrations of all four tanks in a treatment reached 0 mg L-1, those tanks were re-dosed with 10 mg L-1 NO2 (NaNO2¬ 97%) or 10 mg L-1 NH4 (NH4Cl 99.5%) as described above.

Temperature, salinity, pH and dissolved oxygen (DO) were recorded twice daily in each tank. When measuring water quality and taking water samples, the probe and operators hands were rinsed in municipal water between each treatment to avoid cross-contamination.

RESULTS AND DISCUSSION

Temperature, salinity, DO, and pH ranged from 20.39-25.16oC, 26.04-27.43 ppt, 7.06-9.86 mg L-1, and 7.70-8.14, respectively over the trial. These parameters were within established adequate ranges for nitrification.

Of the treatments dosed with ammonia (A, C, and E), the TAN concentration remained at initial levels in A and C. All TAN was consumed within five days in E (Fritz-Zyme® Turbo Start®), both after the initial and secondary doses. No TAN was detected in any treatments dosed with nitrite (Figure 1).

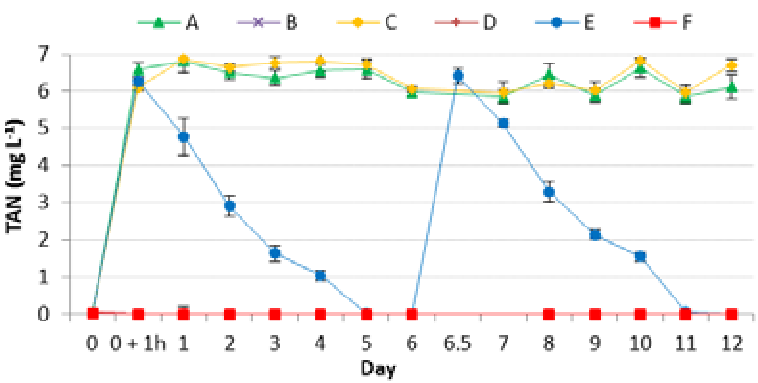

Figure 1. TAN concentrations (mean ± SD) over 12-days post-inoculation. A: Control (no inoculation) + Ammonia, B: Control (no inoculation) + Nitrite, C: Non-disinfected seawater + Ammonia, D: Non-disinfected seawater + Nitrite, E: Fritz-Zyme Turbo Start + Ammonia, F: Fritz-Zyme Turbo Start + Nitrite.

Corresponding with the reduction in TAN, the NO2-N concentration in E increased to 2.30±0.21 mg L-1 after two days, then declined to close to 0 mg L-1 by day 6. A lower NO2-N peak (0.65±0.02 mg L-1) followed the secondary ammonia dose, and all NO2-N was consumed within 3 days. Some NO2-N was detected in the other treatments dosed with ammonia on the final day of the trial (A: 0.23±0.07 mg L-1, C: 0.25±0.09 mg L-1), suggesting nitrification was commencing (Figure 2).

Of the treatments dosed with nitrite (B, D, and F), the NO2-N concentration declined only marginally over the trial in B and D (mean reduction of 0.12 and 0.22 mg L-1, respectively). The NO2-N concentration was close to 0 within three days (0.08±0.03 mg L-1) in F and was all consumed within five days after the initial dose. Following the secondary dose, all NO2-N was consumed within three days (Figure 2).

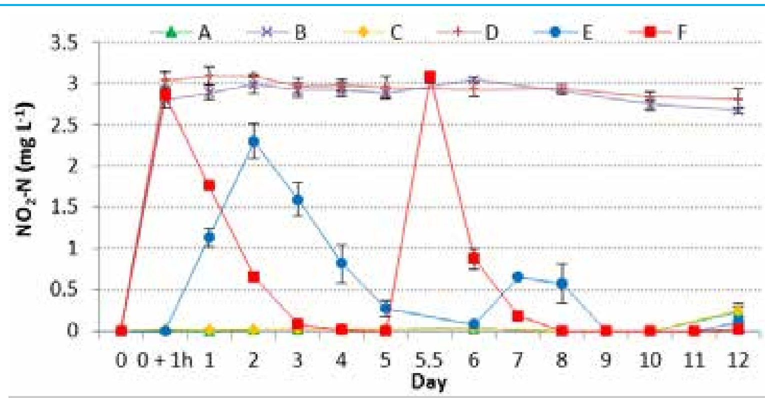

Figure 2. NO2-N concentrations (mean ± SD) over 12-days post-inoculation. A: Control (no inoculation) + Ammonia, B: Control (no inoculation) + Nitrite, C: Non-disinfected seawater + Ammonia, D: Non-disinfected seawater + Nitrite, E: Fritz-Zyme Turbo Start + Ammonia, F: Fritz-Zyme Turbo Start + Nitrite.

The rapid reduction in nitrite following the secondary ammonia and nitrite doses in the Fritz-Zyme treatments (E and F) indicates the nitrifying bacteria were well established. Typically nitrite oxidizing bacteria (NOB, predominantly Nitrobacter spp.) take longer to establish in a virgin system than ammonia oxidizing bacteria (AOB, predominantly Nitrosomonas spp.) and are more sensitive to poor water quality such as low DO. This trend has been regularly observed in the closed shrimp production systems at Texas A&M AgriLife Research Mariculture Laboratory at Flour Bluff, where nitrite concentrations peak higher and for longer than ammonia concentrations as virgin systems are maturing. However, in the present trial nitrite was eliminated faster than ammonia when a commercial nitrifying bacteria product (Fritz-Zyme) was added, indicating that both AOB and NOB were well established. This demonstrates the benefits of spiking bio-media with nitrifying bacteria. Use of non-disinfected seawater (C and D), presumably with diverse bacterial populations intact, did not accelerate nitrifier development compared to the disinfected control (A and B). Further, using non-disinfected water in closed intensive shrimp production systems presents a biosecurity risk and is not recommended.

NO3-N concentrations increased to the greatest degree over the trial in the two treatments with the most reduction in ammonia and nitrite- E (3.37±0.14 mg L-1), followed by F (1.53±0.02 mg L-1). Final NO3-N concentrations were significantly higher ($P<0.05$) in E and F than in all other treatments. Final NO3-N concentrations in other treatments dosed with nitrite (B: 0.81±0.01 mg L-1 and D: 0.72±0.06 mg L-1) were significantly higher ($P<0.05$) than in treatments dosed with ammonia (A: 0.23±0.01 mg L-1 and C: 0.17±0.02 mg L-1, E: 0.22±0.07 mg L-1) (Figure 3). Excluding E, where ammonia oxidizing bacteria (AOB) were evident immediately, the lower nitrate concentrations in ammonia-dosed treatments than in corresponding nitrite-dosed treatments indicates AOB had not yet metabolized any ammonia into nitrite for subsequent conversion into nitrate. In contrast, plentiful nitrite was available for oxidation into nitrate in the nitrite-dosed treatments.

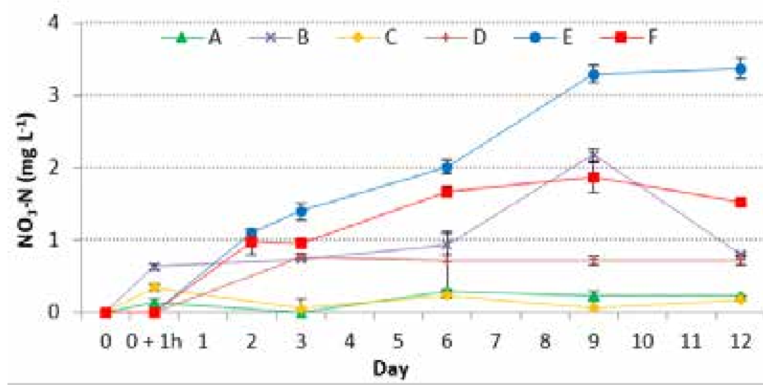

Figure 3. NO3-N concentrations (mean ± SD) over 12-days post-inoculation. A: Control (no inoculation) + Ammonia, B: Control (no inoculation) + Nitrite, C: Non-disinfected seawater + Ammonia, D: Non-disinfected seawater + Nitrite, E: Fritz-Zyme Turbo Start + Ammonia, F: Fritz-Zyme Turbo Start + Nitrite.

Nitrate is the end product of bacterial nitrification and will accumulate in closed systems without water exchange or a denitrification loop. The present data clearly indicates this nitrate accumulation as nitrification progresses. Nitrate concentrations stabilized in E and F once all ammonia and nitrite had been metabolized. Figure 4 demonstrates the progress of nitrification in treatment E, following a typical pattern of ammonia peak, followed by nitrite peak, followed by steady nitrate accumulation (until ammonia and nitrite exhaustion).

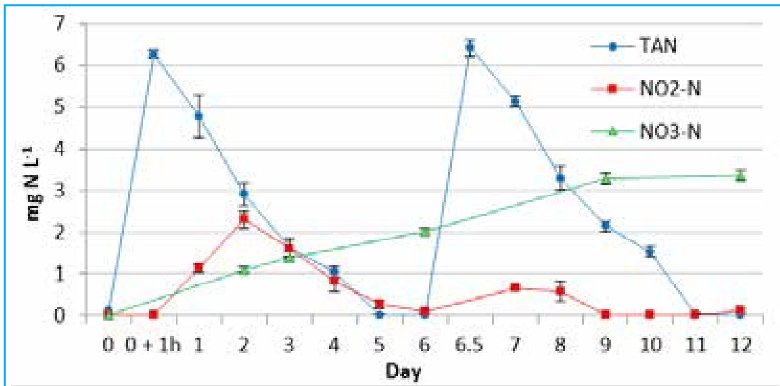

Figure 4. TAN, NO2-N and NO3-N concentrations in Treatment E (Fritz-Zyme Turbo Start + Ammonia) over the trial.

Alkalinity decreased significantly over the trial in E, from 119.62±6.54 to 35.02±2.92 mg L-1 CaCO3, with minimal change in the other treatments (Figure 5). Final alkalinity was significantly higher ($P<0.05$) in C and D than in all other treatments. There was a stronger positive correlation between final alkalinity and TAN (Pearson correlation: 0.994) than with NO2-N (Pearson correlation: 0.540). This correlation and the minimal change in alkalinity in the nitrite-dosed Fritz-Zyme treatment (F) compared to the ammonia-dosed treatment (E) suggest ammonia metabolism consumes more alkalinity than nitrite metabolism. The strong negative correlation between final alkalinity and NO3-N (Pearson correlation: -0.901) simply demonstrates that nitrification, with nitrate as the end product, consumes alkalinity.

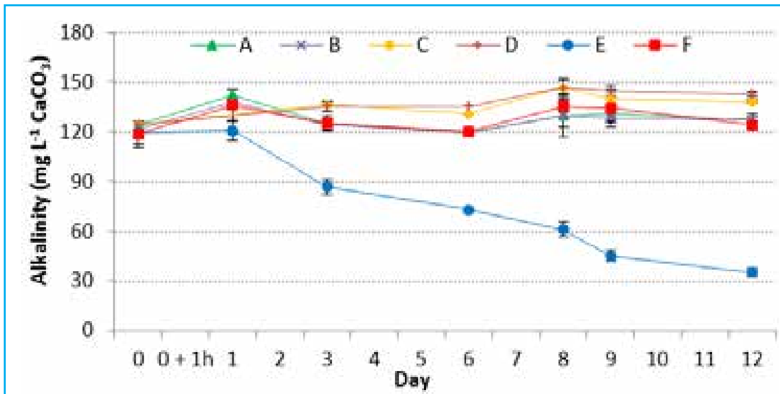

Figure 5. Alkalinity (mean ± SD) over 12-days post-inoculation. A: Control (no inoculation) + Ammonia, B: Control (no inoculation) + Nitrite, C: Non-disinfected seawater + Ammonia, D: Non-disinfected seawater + Nitrite, E: Fritz-Zyme Turbo Start + Ammonia, F: Fritz-Zyme Turbo Start + Nitrite.

CONCLUSION

This trial demonstrates the benefit of adding a commercial nitrifying product to establish nitrifying bacteria in a closed system. **Fritz-Zyme® Turbo Start® 900** greatly accelerated AOB and NOB establishment. Further, ammonia or nitrite inoculations are equally effective for establishing nitrifiers in conjunction with a nitrifying product. Alkalinity is consumed during the nitrification process and should be restored before spiked water is transferred to larger systems. This trial has demonstrated a viable system for establishing nitrifying bacteria, which can then be transferred to larger closed culture systems used for super-intensive shrimp culture.

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