The potential for inoculated nitrification bacteria to control ammonia and nitrite in shrimp ponds

By Dr David J. Drahos

Shrimp from treated ponds (L. vannamei and P. monodon)

During 2003-2004, two separate full-scale shrimp pond trials on the use of nitrification bacteria to control ammonia and nitrite levels were conducted at a commercial facility in central Thailand. Results indicated that this may be a new and vital tool for the farmer facing diverse problems associated with stress and disease. Subsequent effects on yield and feed conversion ratios also demonstrate that this can be a regular water management program for long term culture.

Overall shrimp health and survival is often hampered by high levels of ammonia and nitrite. This is particularly true for low saline ponds or even brackish water ponds. The ability of the shrimp farmer to control waste output from feed and shrimp in high intensity commercial ponds is often limited to indirect measures, such as feed reduction, alkalinity enhancement or alternative pond preparation methods. While such measures are important, a more direct and reliable means to control ammonia and nitrite using specific nitrifying bacteria can provide a valuable tool for both long term culture and short term emergency responses.

The ammonia/nitrite challenge

Healthy shrimp naturally produce ammonia as a waste product, which is subsequently converted first to nitrite then to relatively harmless nitrate by natural chemotropic nitrifying microorganisms typically present in ponds. However, problems arise when the rate of conversion does not keep up with ammonia production and the build up of toxic gases namely ammonia and nitrite begins to occur.

Stress on the shrimp from high ammonia or nitrite levels is well documented and known to cause both acute and chronic effects that can reduce disease resistance and dramatically impact yield. For example, nitrite is now recognized to affect long-term shrimp health even at levels as low as 0.45 ppm under brackish water conditions (Gross *et al.*, 2004). Problems can be especially detrimental, particularly under conditions of high density stocking, such as more than 80 PL/m^2 for *Litopenaeus vannamei* and more than 10 PL/m^2 for *Penaeus monodon* and rapid growth. Since naturally present nitrifying bacteria require a much longer time to increase in number than typical heterotrophic strains, the pond biology is simply overwhelmed during such critical periods of culture.

Opportunity for introducing nitrifying bacteria

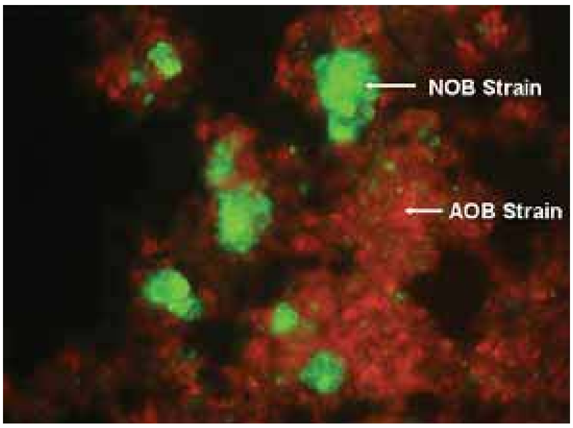
Naturally occurring nitrifying bacteria called nitrifiers can be found in most shrimp ponds at low levels. These organisms are unique in that they derive their energy from ammonia (NH_3) generated directly by the shrimp or indirectly by the activity of other microbial degradation processes in the pond.

Using CO_2 as their carbon source, the nitrifiers are relatively slow growing compared to most other heterotrophic bacteria in the pond. For intensive and even extensive shrimp culture, natural nitrifier populations are often too slow to respond to a rapid ammonia build-up in time to prevent the harmful effects on the shrimp. Furthermore, as ammonia is first converted to nitrite (NO_2) before the nitrite is finally metabolized to relatively harmless nitrate (NO_3), the build up of both ammonia and nitrite can occur quickly.

Using novel high-performance nitrifying stains

Recent intense efforts to identify natural nitrifying bacteria capable of rapid and effective ammonia/nitrite reduction in commercial-scale shrimp ponds have resulted in the discovery of a novel two-strain consortium with broad salt tolerance and good survival characteristics. FISH (fluorescent in situ hybridization) analysis (Figure 1) and full 16S rDNA sequence determination have confirmed that the consortium consists of a novel *Nitrosomonas eutropha* species and a *Nitrobacter winogradskyi* strain co-existing in a large flocculated biofilm structure readily suspended in a pond water column.

Figure 1. The novel nitrifier consortium



Field applications

Two commercial field trials involving a total of 11 treated and 8 non-treated commercial ponds, each of approximately 0.8ha were conducted over a two year period at SAM-D Farms in Chumphon, Thailand. The ponds were stocked with white shrimp *L. vannamei*. Post larvae from the same production batch were used for each trial and were certified pathologically clean by the Biotech Laboratory, Phuket, Thailand.

The two-strain consortium of nitrifying bacteria was applied as a liquid concentrate at 2 to 3 ppm rate over a 10 week period, starting at 4 weeks post stocking. A total of eight identical non-treated adjacent ponds serve as control ponds in the tests. However, shrimp in only two of the eight control ponds survived to harvest due to ammonia/nitrite stress and disease, possibly exacerbated by the relatively high stocking densities used (100-110 PL/m^2). Shrimp in all the treated ponds survived to harvest. Daily records were kept for ammonia-N, nitrite, alkalinity, pH, dissolved oxygen, temperature, total bacterial counts, applied feed and shrimp weight.

In the first trial, three ponds were treated with the nitrifier consortium and three adjacent ponds served as control ponds. In this test, shrimp in two of the control ponds succumbed to disease within 4 weeks of stocking whilst shrimp in the remaining control pond survived to harvest. Both ammonia (Figure 2) and nitrite (Figure 3) were controlled well in all 3 ponds receiving the nitrifier consortium, while significant spikes occurred in the control ponds during the 93 day culture period. It is clear that all of the ponds at this intensive stocking density (100 PL/m^2) were under significant stress, and the levels of ammonia and nitrite were all beginning to rise by week 4 except in the control pond that did survive to harvest. The addition of nitrifiers at this point did result in a relatively rapid ammonia and nitrite reduction which may have been a strong factor in overall survival. Similar rapid recoveries were also observed in the second trial.

Figure 2

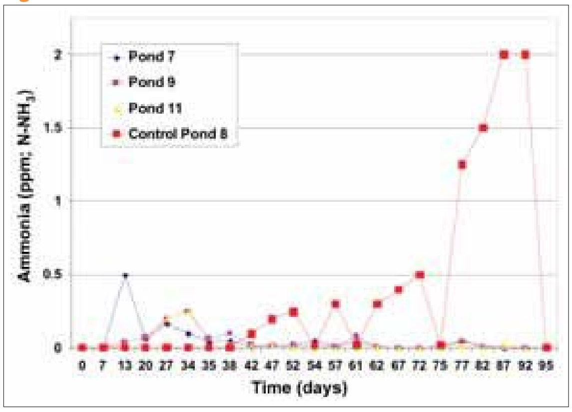
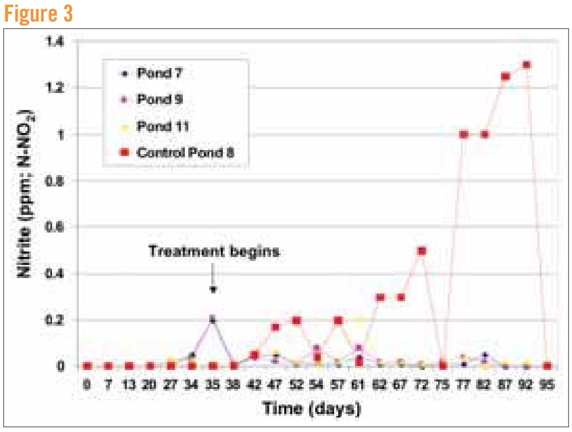


Figure 3



Trial 1. Changes in ammonia (Figure 2) and nitrite (Figure 3) levels in three treatment ponds and control pond (final surviving pond)

In the second trial, six ponds were treated with the nitrifier consortium, while five identical and adjacent non-treated ponds were the control ponds. Once again, shrimp in only one of the control ponds survived to harvest, while other control ponds succumbed to viral infections early in the grow-out period. The ammonia and nitrite levels in these ponds when compared with two control ponds are given in Figures 4 and 5, respectively.

Figure 4

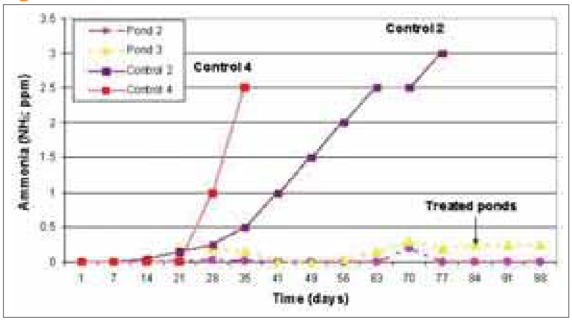
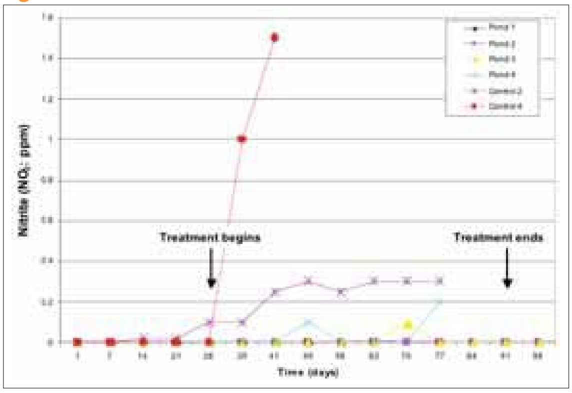


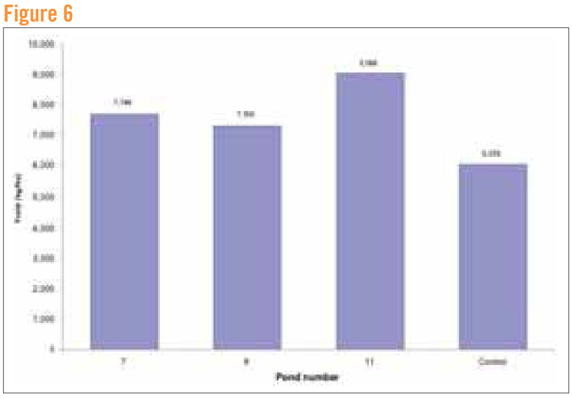
Figure 5



Trial 2. Changes in ammonia (Figure 4) and nitrite levels (Figure 5) in two ponds treated with the nitrifier consortium and two control ponds

In trial 1, shrimp yields in treated ponds were around 25 to 50% more than that obtained from the control ponds (Figure 6). The shrimp yield from trial 1 and 2 are summarised in Figure 7. The average pond yields in each trial are compared against the one surviving control pond from each study.

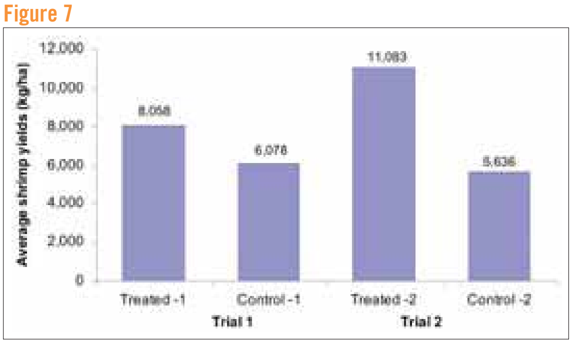
Figure 6



Comparison of shrimp yields (kg/ha) in treatment ponds in trial 1

Shrimp pond at SAM-D farms, Chumphon, Thailand

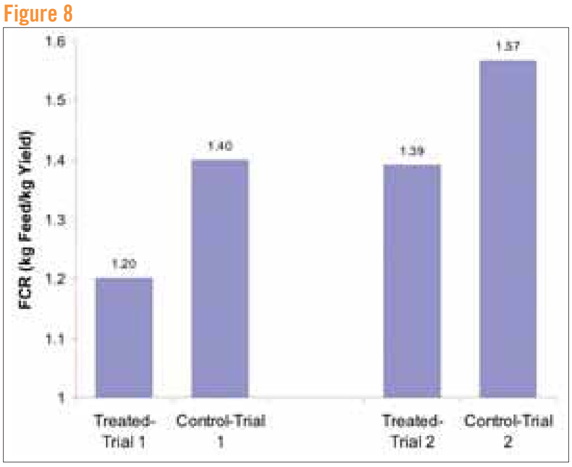
Figure 7



Improvements in average shrimp yields ((kg/ha) in treatment ponds in trial 1 and 2

Another indication of the overall shrimp health and value is provided by calculating feed conversion ratio (FCRs) defined as the kg amount of feed required per kg of shrimp yield obtained. As shown in Figure 8, there were significant improvements (i.e. lower FCRs) in ponds treated with the nitrifier consortium in both trials as compared with non-treated control ponds.

Figure 8



Improvements in average FCR in treated ponds in the trial 1 and 2

Conclusion

This capacity to reliably impact toxic ammonia and nitrite build up in large-scale commercial shrimp culture may provide a new and vital tool to the farmer facing diverse problems associated with stress and disease, even when using the best feed and pond preparation programs.

The control of ammonia levels was often noted in these trials within days of the application of the nitrifier consortium. Thus, an opportunity may exist to introduce nitrifiers on an emergency basis when higher daily ammonia or nitrite readings are noted, particularly during the most critical growth periods. For some treated ponds in these studies, it was also possible to extend the length of the grow out period to up to 113 days, which provided a notable increase in shrimp size and subsequent value.

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Reference

Gross, A., Abutbul, S., and Zilberg, D. 2004. Acute and chronic effect of nitrite on white shrimp, *Litopenaeus vannamei*, cultured in low-salinity brackish water. J. World Aquculture Society: 35: 315-321.

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