AQUA EXPO 2017

CÁMARA NACIONAL DE ACUACULTURA

Título de la conferencia

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Aerobic nitrification and denitrification among commercially available heterotrophic strains in the genera *Bacillus* and *Pediococcus*

Introduction

- Aquaculture ponds receive hundreds of kg of feed per hectare.
- Three percent of applied feed (35% protein) becomes ammonia (Ebeling, 2006).
- Ammonia is toxic and can reduce survival and diminish production.

Introduction Cont.

- Traditionally autotrophic nitrifying bacteria have been relied upon to oxidize ammonia to less toxic nitrites and nitrates.
- Nitrifying bacteria are slow growing and difficult to get established in ponds. Their ability to function well is affected by temperature, pH, and alkalinity.
- Nitrites have been shown to inhibit the immune system of *Litopenaeus vannamei* (Tseng and Chen, 2004).

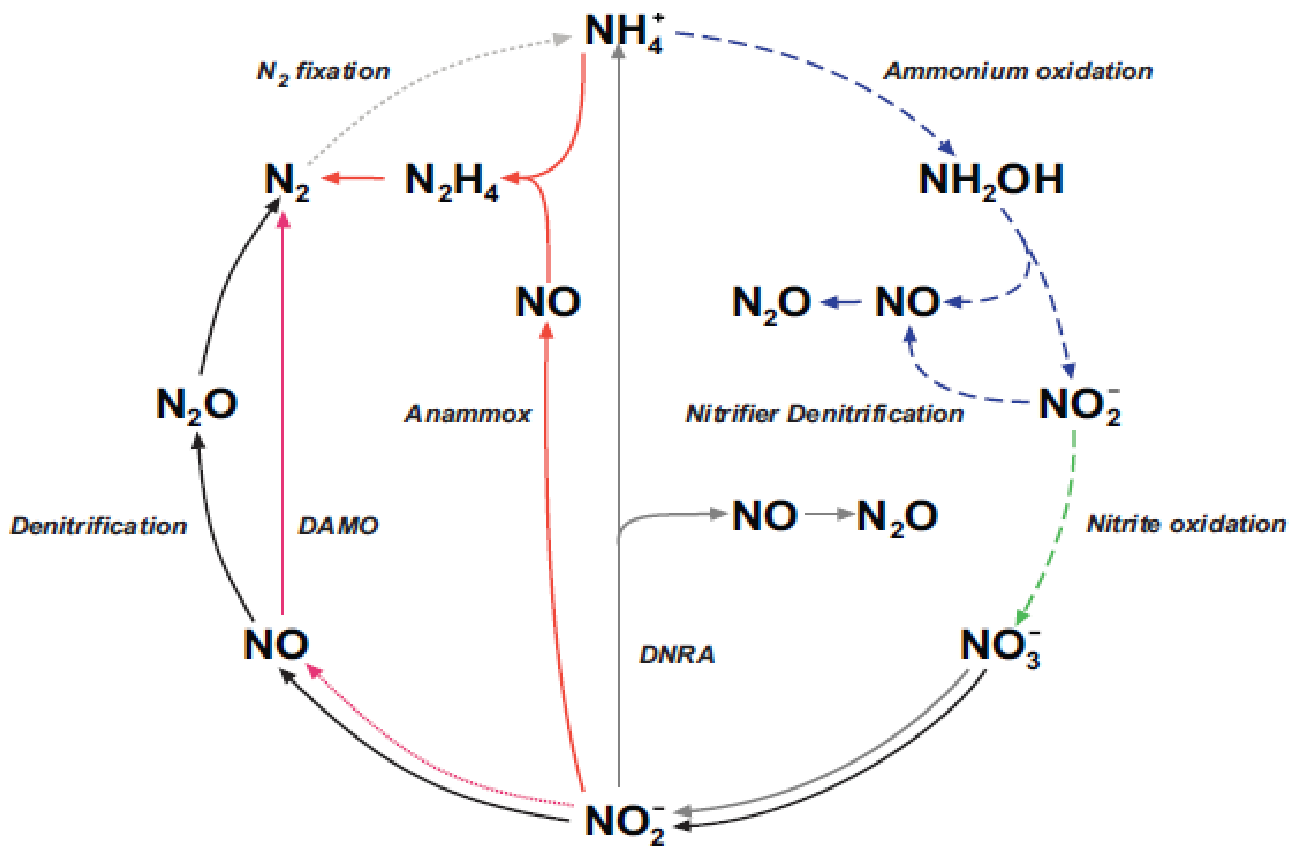
Introduction Cont.

- In low salinity conditions, high nitrate levels reduce growth and shrimp marketability (Kuhn et al, 2010).
- Denitrification in shrimp ponds occurs very inefficiently (in anaerobic pockets of flocs or pond bottom).
- Feed application results in a steadily increasing concentration of inorganic nitrogen.

Introduction Cont.

- Heterotrophic bacteria can carry out aerobic nitrification as well as denitrification.
- Traditionally, heterotrophic bacteria aerobic nitrification rates have been considered slow.
- In 2005, it was reported that several strains of *Bacillus* are capable of much higher rates of aerobic nitrification and denitrification than previously known (Kim et al).
- Today, heterotrophic aerobic nitrification is an important phase of water treatment.

Microbial Nitrogen Cycle



Aerobic Pathway -----------

Anaerobic Pathway ———

Comparison of Bacteria Types

- Autotrophs take 8 to 24 hours to double populations.
- Autotrophic bacteria are very delicate and affected by low pH, high ammonia, low dissolved oxygen, and low alkalinity.
- Heterotrophs can double in 40 minutes.
- Heterotrophs are hardier than Nitrosomonas and Nitrobacter. Some form endospores.

Comparison of Bacteria Types

- This study isolated and evaluated a range of heterotrophic bacteria formulated to remove ammonia and nitrates under aerobic conditions.

- The objective was to determine the fate of nitrogen during bacterial metabolism.

- The study was carried out in laboratory conditions.

Materials and Methods

- Laboratory grade reagents and ammonium, nitrite, nitrate, and dissolved oxygen, test kits were used to carry out the study.
- Colonies of distinctly different bacteria were isolated and identified.
- Eight strains were isolated and screened for aerobic nitrification ability.

Materials and Methods

- Four strains were selected to be screened for denitrification capacity.
- The screening process was carried out in both minimal media and in untreated wastewater (sterilized and filtered).
- Reactor flasks were run in duplicates for each selected and identified strain.

Minimal Media Conditions

Nitrification

- Dextrose was used as a carbon source.
- Ammonium Phosphate was the Nitrogen source.

Denitrification

- Dextrose was the carbon source.
- Sodium Nitrate was used instead of ammonia.

Results

- The bacteria isolates were identified as members of phylum Firmicutes
- Genera: Bacillus, Pediococcus, and Lactobacillus.
- Plate culture of Pediococcus and Lactobacillus clearly demonstrated bacteria identity.
- 16s DNA sequencing was conducted on the Bacillus colonies for strain identification

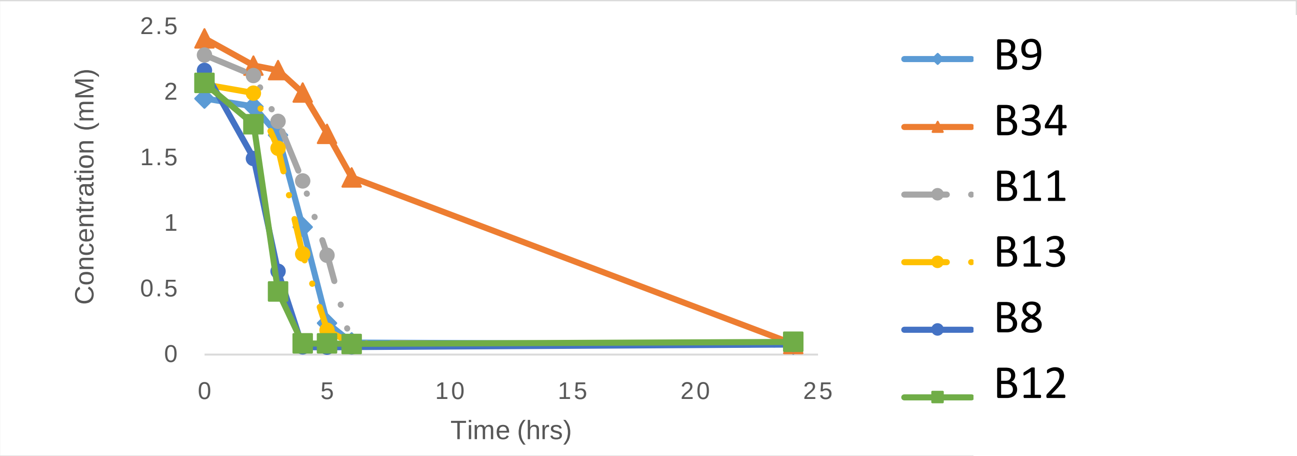
Results

Isolate	Gram Stain	Colony Morphology	Percent Similarity
B5	+	Cream, raised, irregular, mucoid, wrinkled	99.37
B6	+	Cream, raised, irregular, mucoid, wrinkled	99.65
B8	+	Creamy tan, flat, irregular	99.34
B9	+	Cream, raised, irregular, smooth	99.93
B10	+	Cream, raised, irregular, mucoid, wrinkled	99.11
B11	+	Cream, flat, irregular	96.00
B12	+	Cream, flat, irregular, wrinkled	100.00
B13	+	Cream, raised, irregular, mucoid, wrinkled	99.04

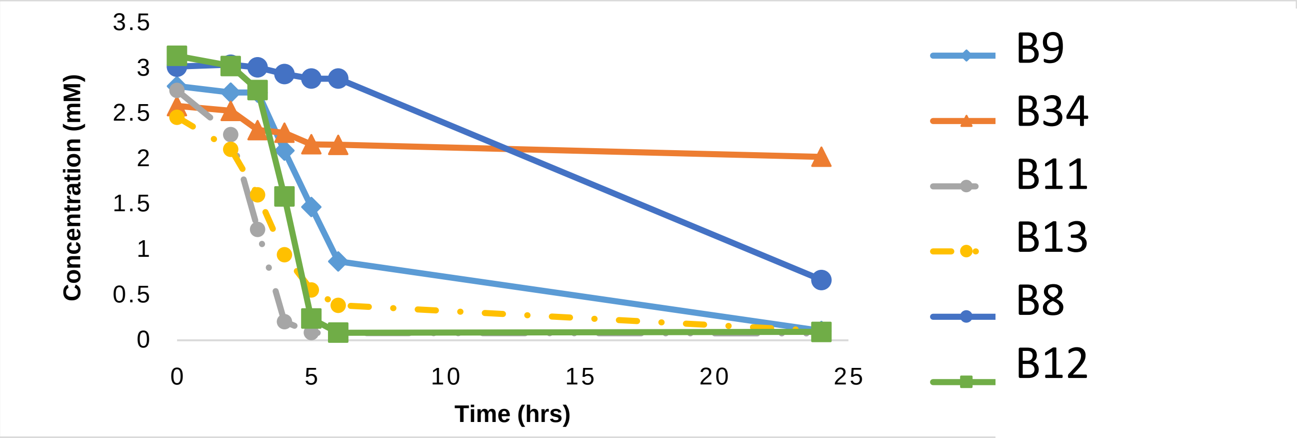
Results

Disappearance of ammonia over time in the presence of heterotrophic *Bacillus* isolates in:

Minimal Media



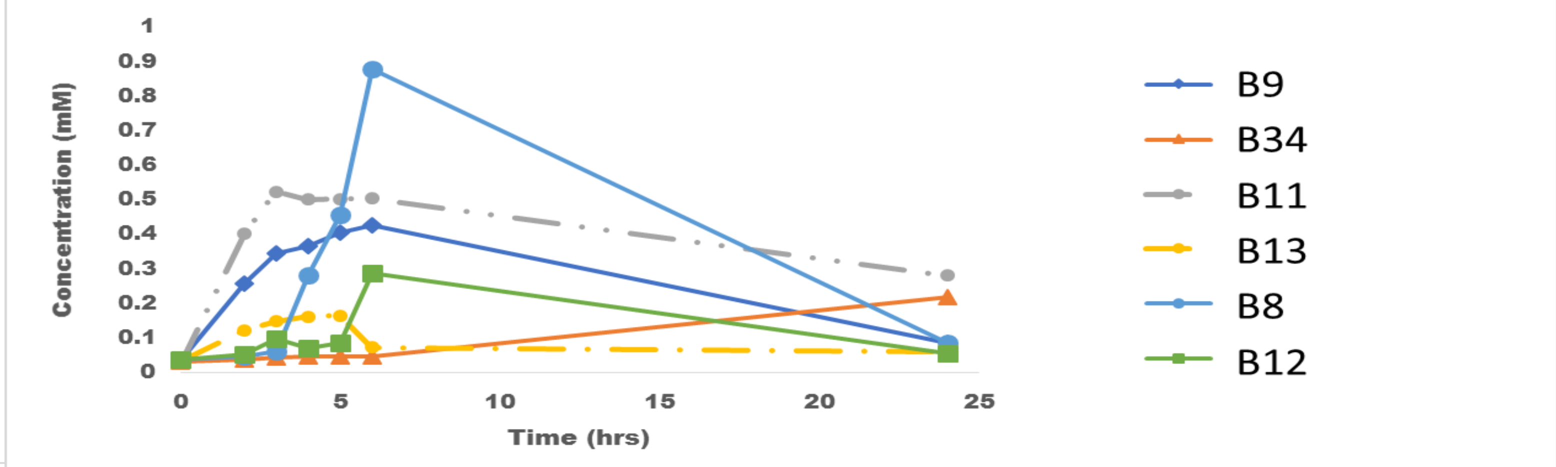
Wastewater Reactor Flasks



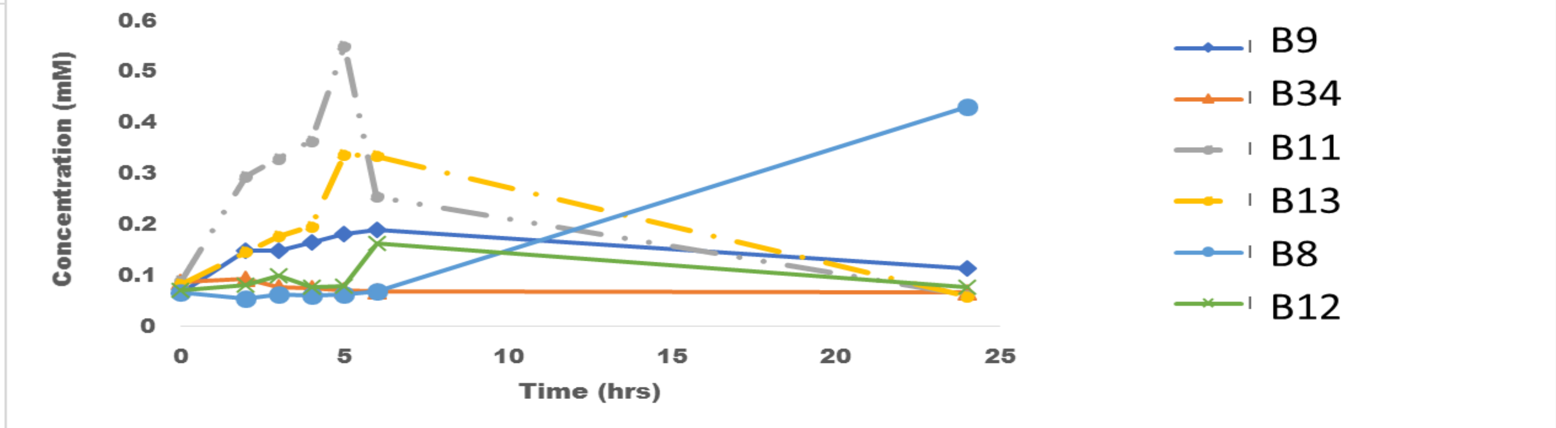
Results

Appearance of nitrite over time in the presence of heterotrophic *Bacillus* isolates in:

Minimal Media



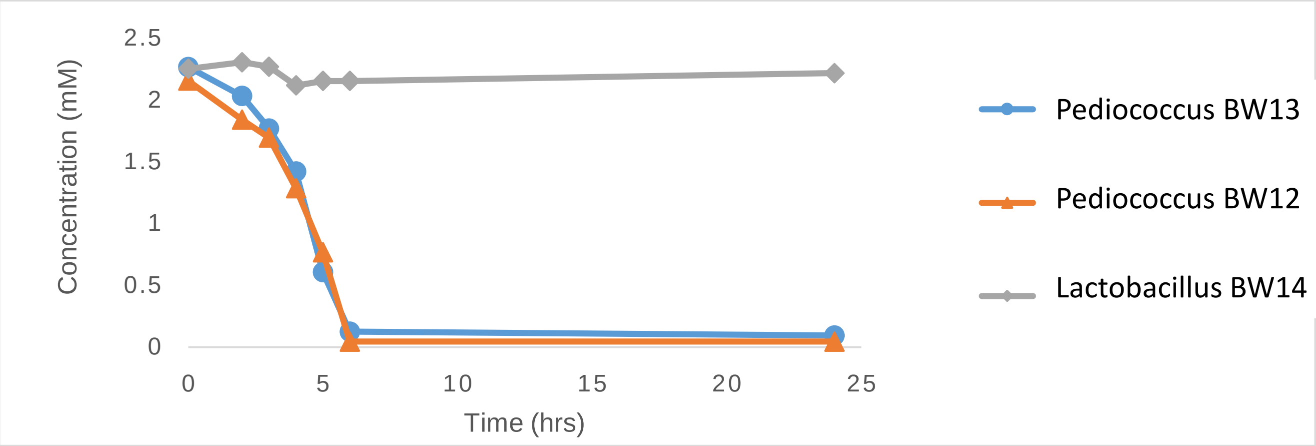
Wastewater Reactor Flasks



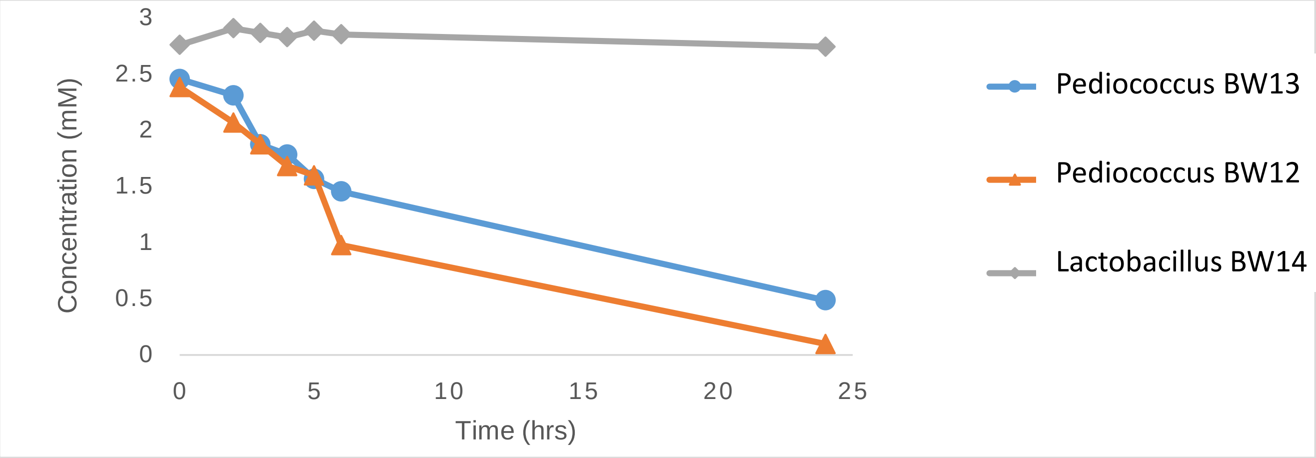
Results

Disappearance of ammonia over time in the presence of heterotrophic *Lactobacillus* and *Pediococcus* isolates in:

Minimal Media



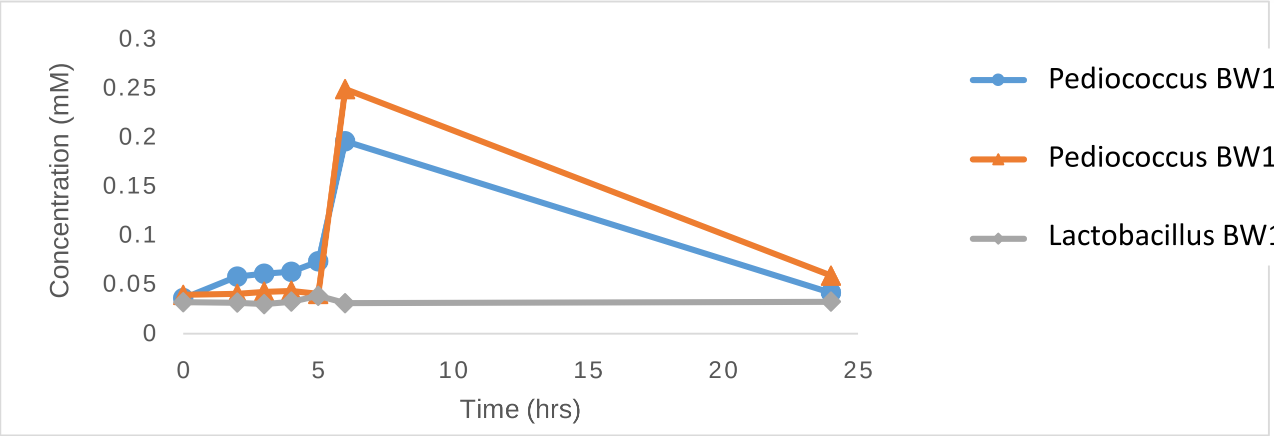
Wastewater Reactor Flasks



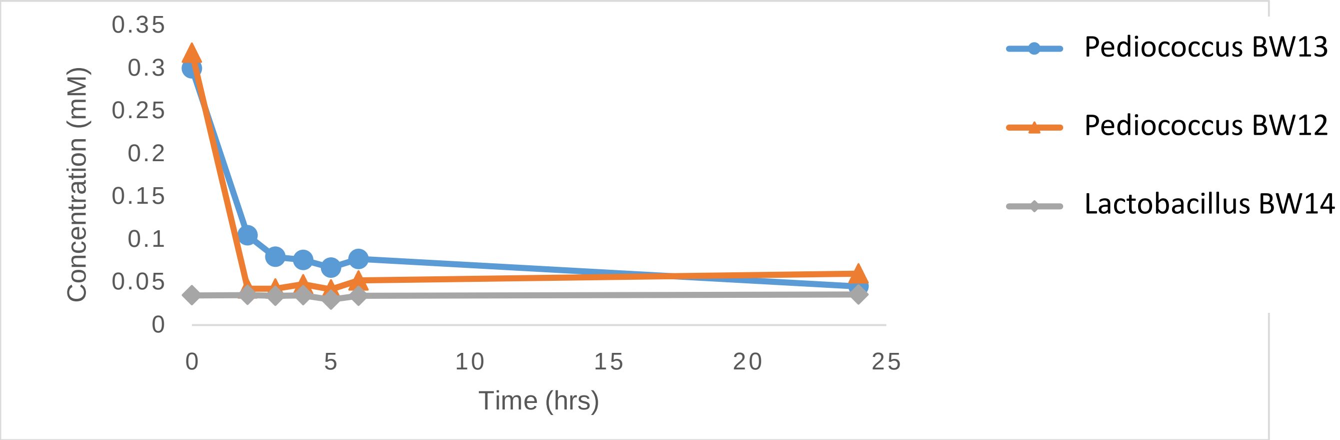
Results

Appearance of nitrite over time in the presence of heterotrophic *Lactobacillus* and *Pediococcus* isolates in:

Minimal Media



Wastewater Reactor Flasks



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Results

Ammonia metabolism in minimal media and wastewater reactor flasks.

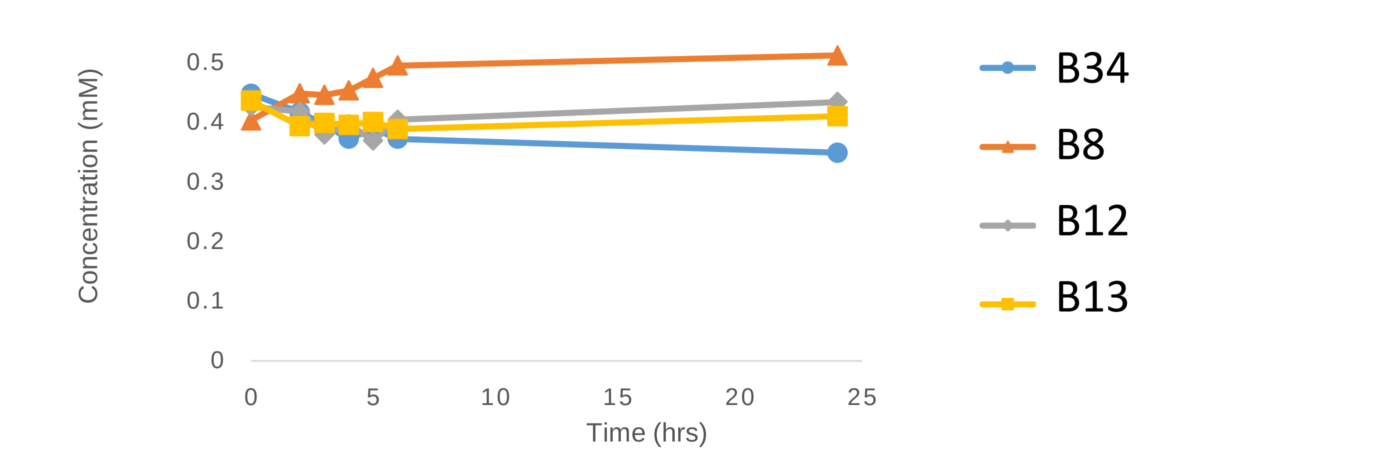
Rates of degradation (ppm hour/h) represent the highest hourly rate achieved by each organism.

	Minimal Media Reactors			Wastewater Reactors		
Isolate	Rate (ppm/hr)	DO (T=0)	DO (T=24)	Rate (ppm/hr)	DO (T=0)	DO (T=24)
Bacillus (B5)	3.59	6.6 ppm	4.2 ppm	4.26	7.9 ppm	5.8 ppm
Bacillus (B11)	2.99	7.2 ppm	6.3 ppm	4.58	6.7 ppm	5.8 ppm
Bacillus (B13)	3.53	8.0 ppm	6.2 ppm	2.89	6.2 ppm	5.6 ppm
Bacillus (B6)	1.65	8.2 ppm	4.2 ppm	4.79	5.8 ppm	4.4 ppm
Bacillus (B10)	4.90	7.9 ppm	2.4 ppm	5.65	5.5 ppm	5.0 ppm
Bacillus (B9)	3.21	6.0 ppm	5.6 ppm	2.72	5.0 ppm	4.8 ppm
Bacillus (B34)	1.45	6.9 ppm	4.6 ppm	0.927	7.0 ppm	6.5 ppm
Bacillus (B8)	3.76	6.1 ppm	5.4 ppm	0.321	6.0 ppm	3.8 ppm
Bacillus (B12)	5.58	6.1 ppm	5.3 ppm	5.89	5.9 ppm	5.6 ppm
Pediococcus BW13	3.55	8.4 ppm	5.6 ppm	0.947	7.1 ppm	6.8 ppm
Pediococcus BW12	3.16	7.6 ppm	6.5 ppm	2.72	6.9 ppm	5.4 ppm
Lactobacillus BW14	0.662	7.8 ppm	7.6 ppm	0.177	6.3 ppm	6.4 ppm

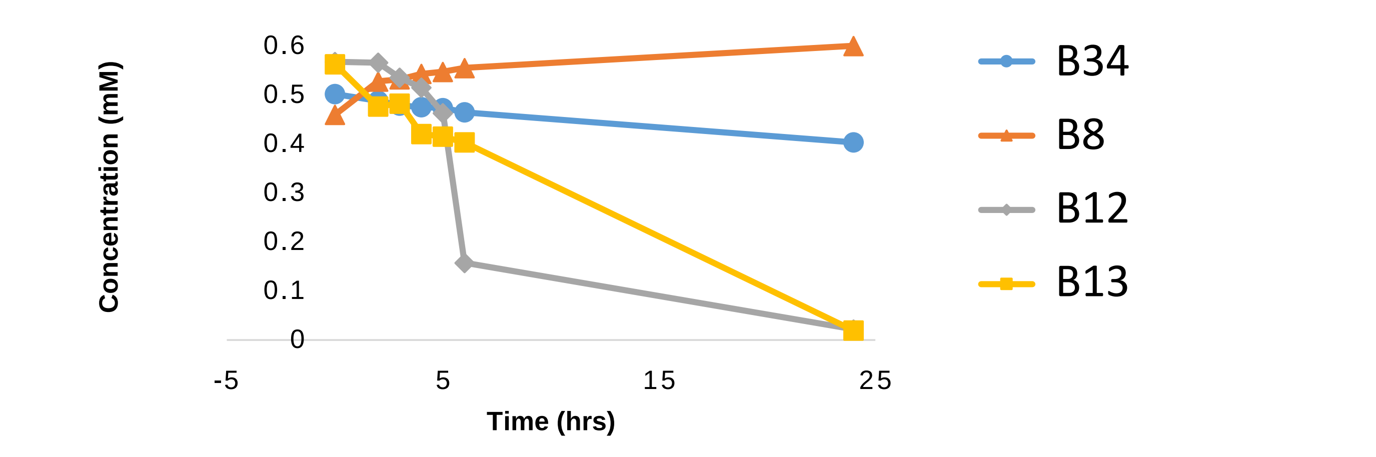
Results

Disappearance of nitrate over time in the presence of heterotrophic *Bacillus* isolates in:

Minimal Media



Wastewater Reactor Flasks



Results

Nitrate metabolism in minimal media and wastewater reactor flasks.

	Minimal Media Reactors			Wastewater Reactors		
Isolate	Rate (ppm/h)	DO (T=0)	DO (T=24)	Rate (ppm/h)	DO (T=0)	DO (T=24)
(B34)	1.93	7.7 ppm	6.4 ppm	0.834	7.0 ppm	6.1 ppm
(B8)	-2.79	8.5 ppm	6.8 ppm	-4.23	8.0 ppm	5.2 ppm
(B12)	0.539	7.7 ppm	5.8 ppm	18.9	7.9 ppm	4.7 ppm
(B13)	0.196	7.3 ppm	5.4 ppm	1.32	7.0 ppm	4.9 ppm

Discussion

- All bacterial isolates with the exception of Lactobacillus BW14 showed aerobic nitrification ability at high dissolved oxygen levels.
- Higher wastewater nitrification rates were observed for
  - Bacillus (B5, B11, B13),
  - Bacillus (B6,B10), and
  - Bacillus (B12).
- Higher nitrification rates were observed in minimal media for
  - Bacillus (B9, B34),
  - Lactobacillus BW14,
  - Pediococcus BW12, and
  - Pediococcus BW13.
- Differences in rates may be due to carbon source affinity, or availability of other micronutrients.

Discussion

- The highest rate of nitrification in both minimal media and in wastewater was achieved by Bacillus (B12).

- All reactor flasks experienced nitrite spikes subsequent to ammonia degradation.

- In some cases, there was a delay in the process.
  - Preferential conversion of ammonia to biomass
  - Delay in conversion of ammonia to nitrite.

Discussion

- Neither water quality analysis, or nitrogen balance indicated generation of nitrates, subsequent to nitrite degradation.

- This suggests that gaseous N_2O may be the metabolic result.

- Mass balance analysis indicates there is a reduction of nitrogen up to 1.4 mg/L depending on species of bacteria.

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Conclusion

- Nitrification and denitrification under aerobic conditions with heterotrophic bacteria is a more secure option than relying on autotrophic nitrifying bacteria .
- Ammonia, nitrites, and nitrates can have detrimental effects on shrimp, so it is important to reduce inorganic nitrogen.
- If inorganic nitrogen levels are not reduced, effluent leaving the ponds contaminates source water for future cycles.

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