

REFEREED PAPER

## RECONCILING WATER QUALITY PARAMETERS IMPACTING NITRIFICATION IN AQUAPONICS: THE PH LEVELS

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**Abstract.** Combining hydroponics and aquaculture into aquaponic systems requires reconciling water quality parameters for the survival and growth of plants, fish, and nitrifying bacteria. The plants and fish are grown as cash crops while bacteria are expected to oxidize ammonia (fish by-product) into nitrite and finally nitrate which may be used by plants. The objectives of this project were to determine nitrification activity response to pH between 5.5 and 8.5 in recirculating trickling biofilters containing perlite medium. Total ammonia nitrogen concentration decreased from 5 to 0 mg L<sup>-1</sup> in 12 (pH 8.5), 20 (pH 7.5), and 20-24 (pH 6.5) days after introduction of nitrifying bacteria to the perlite biofilters. Nitrite became measurable in the biofilter water at 8 (pH 8.5), 16 (pH 7.5), and 20-24 (pH 6.5) days after introduction of nitrifying bacteria. No nitrification occurred in the biofilters maintained at a pH of 5.5. These results indicate that ammonia conversion to nitrate in a perlite medium trickling biofilter startup cycle was significantly faster at pH 8.5 than at pH 7.5 and 6.5. The recommended pH for aquaculture systems is from 6.5 to 8.5 and for hydroponic systems is from 5.5 to 6.5. Results indicate the optimum pH for nitrification in this system is 8.5, however, the reconciling pH for aquaponics would likely be between 6.5 and 7.0 to optimize the production of the fish and plant cash crops.

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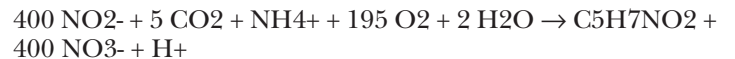
Aquaponics is an integrated system that links hydroponic plant production with recirculating aquaculture (Diver, 2000). The advantages of linking fish and plant culture together are shared startup, operating and infrastructure costs, fish waste nutrient removal by plants, reduced water usage, and increased profit potential by producing two cash crops (Rakocy, 1999; Timmons, et al., 2002). The potential of plants and fish for production in aquaponics has been investigated (Adler et al., 1996; Anonymous, 1997,1998; McMurtry et al., 1997; Rakocy et al., 1992,1997; Watten and Busch, 1984).

One of the most complex and important subsystems of recirculating aquaculture is the biofiltration and removal of fish waste. Recirculating systems must incorporate both solids removal and biological filtration into the water reconditioning process to achieve proper water quality for fish and plants (Harmon, 2001). Ammonia is the main excretion product from fish. Both un-ionized ammonia and nitrite can be toxic to fish at very low levels (Harmon, 2001; McGee and Cichra, 2000). In the process of nitrification, certain autotrophic bacteria (primarily *Nitrosomonas*) oxidize ammonia to nitrite and others (primarily *Nitrobacter*) oxidize nitrite to nitrate. The overall reaction of nitrification and cell biomass formation can be written as (Haug and McCarty, 1972):

*Nitrosomonas*



*Nitrobacter*



This nitrogen transformation eliminates ammonia from the water. Nitrate is not toxic to fish except at very high levels (96-h LC50 > 1000mg/L NO<sub>3</sub>-N; Colt and Tchobanoglous, 1976) and is the primary source of nitrogen for plants in hydroponic systems (Hochmuth, 1991; Resh, 1998).

Nitrate and ammonium are the most common forms of nitrogen taken up by vegetable crops (Cockx and Simonne, 2003). However, they should be regarded as two different nutrients because they affect plant metabolism differently. Plant nutrient uptake is a process that is electrically neutral. Uptake of NH<sub>4</sub><sup>+</sup> may depress uptake of the essential cations (K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>). The optimum nitrate to ammonium ratio for vegetables grown in hydroponics is 75:25. When ammonium is the dominant form of nitrogen available for plant uptake, a smaller plant will result. Thus where the nitrogen source in aquaponics comes primarily from the fish, the nitrification process is important for nitrate uptake by plants. The fish, the plants, and the nitrifying bacteria rely on the same recirculating water for optimum growth hence water quality parameters have to be favorable for all three organisms in a self-sustaining aquaponic system. The effects of water quality on nitrifying bacteria have not been investigated from the standpoint of conditions that can be present in aquaponic systems.

The pH is one of the most important environmental parameters that can affect the activity of nitrifying bacteria. Recommended pH ranges for hydroponic systems are between 5.5 and 6.5 (Hochmuth, 1991) and for aquaculture systems are between 6.5 and 8.5 (Timmons et al., 2002). A wide range of pH optima have been reported from research on the effect of pH on nitrification rate. In substrates from terrestrial forest environments, increasing pH stimulated net nitrification while decreasing pH depressed it (Ste-Marie and Pare, 1999). Nitrification in aquaculture biofilters was reported to be most efficient at pH levels from about 7.5 to 9.0 (Hochheimer and Wheaton, 1998), and 7.0 to 8.0 (Masser et al., 1999). In a submerged biofilter investigation, a pH increase of one unit within a range of 5.0 to 9.0, produced a 13% increase in nitrification efficiency (Villaverde, et al., 1997). In another investigation with four different biological filters (under gravel, fluidized bed, non-fluidized bed, and gravel bed) nitrification slowed significantly or stopped when pH dropped below 6.0 (Brunty, 1995). The pH of approximately 7.8 produced the maximum growth rate of nitrifying bacteria for wastewater treatment processes (Antonioni et al., 1990). The causes of varying pH optima may be attributed to differences in substrate, effluent, or species of nitrifying bacteria present in the system.

The most common recirculating aquaponic systems to date employ either a media-filled raised bed, nutrient-flow technique (NFT), or floating raft system (Adler et al., 1996; Anonymous, 1997,1998; Diver, 2000; McMurtry et al., 1997; Rakocy et al., 1992,1997; Watten and Busch, 1984) for the plant growing area. Of those systems, the media filled bed has potential for providing for solids removal, biological filtration, and root zone space for plant production. Perlite is the most common plant growing medium used in hydroponic plant production in Florida (Tyson et al., 2001). It has also been investigated as a soilless culture alternative to soil fumigation with methyl bromide in field grown tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum* L) production (Hochmuth et al., 2002). However, perlite medium has not been investigated with respect to the activity of nitrifying bacteria in an aquaponic biofilter. The type of soilless media in which plants grow has been shown to significantly affect nitrifying bacteria counts (Lang and Elliott, 1997). The purpose of this investigation was to determine the nitrification activity response to pH ranging from 5.5 to 8.5 in a trickling biological filtration system containing perlite medium.

### Materials and Methods

Two experiments were conducted in 2004 in a Dutch style glass greenhouse with pad and fan cooling system at the Seminole Community College Horticultural Unit, Sanford, Fla. Sixteen perlite medium trickling biofilters were set up in a randomized block design with four treatments (pH 5.5, 6.5, 7.5, 8.5). Twenty liters of tap water were added to the 80-L plastic biofilter boxes which were kept closed during the experiment. Air vents in the upper section of the boxes allowed for natural ventilation and gas exchange. Screen colanders were placed above the water on plastic stools in each box and filled with 6.5 L of horticultural grade coarse perlite. Water was recirculated through the perlite with an aquarium pump at the average rate of 1.9 L min<sup>-1</sup>. Sodium bicarbonate and potassium hydroxide (Plant Food Systems, Zellwood, Fla.) were added to raise pH during experiment 1 and potassium hydroxide was used to raise pH in experiment 2. Phosphoric

acid (Plant Food Systems) was added to lower pH as needed during both trials. In addition, sodium bicarbonate was added when necessary to maintain recirculating water alkalinity above 50 mg L<sup>-1</sup> in experiment 1 and above 100 mg L<sup>-1</sup> in experiment 2.

Experiment 1 biofilter setup began on 20 Jan. with water and perlite added to the tanks and recirculating pumps installed. On 21 Jan., 'Proline' Aqua-Coat (Dechlorinator/Substrate Conditioner; Aquatic Eco-Systems, Apopka, Fla.) was added at 1.3 ml per tank. Ammonium chloride was added at 25 mg L<sup>-1</sup> resulting in 5.0 mg L<sup>-1</sup> total ammonium nitrogen concentration in the recirculating solution. 'Proline' Bio-Booster nutrient solution was added at 0.3 ml per tank. 'Proline' Freshwater Nitrifying Bacteria (Aquatic Eco-Systems) was added to the perlite at the rate of 2.5 mL L<sup>-1</sup> of tank water. The 'Proline' products are proprietary blends of water conditioner, nutrients, and nitrifying bacteria recommended for use when beginning new biofilter startup cycles in recirculating aquaculture. On 27 Jan., another 1.5 mL L<sup>-1</sup> of nitrifying bacteria was added to each tank in an effort to speed up the nitrification process. Total ammonia nitrogen (TAN = NH<sub>4</sub><sup>+</sup> - N plus NH<sub>3</sub> - N), nitrite nitrogen, nitrate nitrogen, pH, dissolved oxygen, soluble salts, salinity, and temperature measurements were taken every 4 d beginning on 21 Jan. Ammonium chloride (0.125 g) was added to the 8.5 pH treatment on 1 Feb., and to the other treatments on 9 Feb. One week after setup, aquarium heaters were installed in the boxes to maintain recirculating water temperatures between 26 and 31 °C. Upon completion of experiment 1, boxes and equipment were disassembled, triple rinsed, and dried prior to assembly for experiment 2.

Experiment 2 biofilter setup began on 3 Mar., with water and fresh perlite added to the tanks and recirculating pumps installed. Aquarium heaters were reinstalled. On 10 Mar., 'Proline' Aqua-Coat (Dechlorinator/Substrate Conditioner) was added at 1.3 mL per tank. Ammonium chloride was added at 25 mg L<sup>-1</sup>. 'Proline' Bio-Booster nutrient solution was added at 0.3 ml per tank. On 11 Mar., 'Proline' Freshwater Nitrifying Bacteria was added to the perlite at the rate of 10 mL L<sup>-1</sup> of tank water. Total ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, and pH measurements were taken every 4 d while dissolved oxygen, soluble salts, salinity, and temperature water quality data were taken every 8 d beginning on 11 Mar.

Total ammonia nitrogen (range 1.0-8.0 mg L<sup>-1</sup>), nitrite (low range, 0.1-0.8 mg L<sup>-1</sup>), chlorine, and alkalinity were measured with LaMotte Test Kits. Nitrite (high range, 0-150 mg L<sup>-1</sup>) was measured using a Hanna Ion Specific Meter. Nitrate was measured using a Cardy Ion Specific Meter (0-9,900 mg L<sup>-1</sup>). Dissolved oxygen, specific conductivity, temperature, and salinity were measured using a YSI Model 85 meter. Both experiments used a randomized block design with four replications. Data in Table 1 were analyzed using a Statistical Analysis System (SAS) software and Duncan's Multiple Range Test using a P value of <0.05. The pH data was measured using a Fisher Scientific AR15 Accumet Research pH meter. The pH data were analyzed with a Microsoft Excel program for mean and standard deviation.

### Results and Discussion

These experiments are based on typical startup characteristics for bringing a new biological filter system up to full capacity (Timmons et al., 2002). Relative nitrification activity

Table 1. Changes in TAN, N02-N, and N03-N concentrations in perlite medium trickling biofilters as affected by water pH.

Target pH	Day 0 <sup>c</sup>	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28	Day 32
Experiment 1									
Total ammonia nitrogen (mg L <sup>-1</sup> )									
8.5	5.0 a <sup>y</sup>	4.0 b	1.1 c	0 c	0 c	0 b	0 b	0 b	—
7.5	5.0 a	5.0 a	3.9 b	4.5 b	1.3 bc	0 b	0 b	0 b	—
6.5	5.0 a	5.0 a	4.4 a	4.9 a	2.5 b	0 b	0 b	0 b	—
5.5	5.0 a	5.0 a	4.5 a	4.9 a	4.6 a	4.1 a	3.6 a	3.0 a	—
		L**	L**	L**	L**	L**	L**	L**	
Significance <sup>x</sup>		Q**	Q**	Q**		Q**	Q**	Q**	
Experiment 2									
8.5	5.0 a	3.3 b	2.0 b	0 b	0 c	0 b	0 b	0 b	0 b
7.5	5.0 a	5.0 a	5.0 a	4.4 a	2.8 b	0 b	0 b	0 b	0 b
6.5	5.0 a	5.0 a	5.0 a	4.3 a	3.5 a	1.8 a	0.5 b	0 b	0 b
5.5	5.0 a	5.0 a	5.0 a	4.6 a	3.8 a	3.0 a	2.8 a	2.6 a	2.4 a
		L**	L**	L**	L**	L**	L**	L**	L**
Significance		Q**	Q**	Q**		Q**	Q**	Q**	Q**
Exp*pH P value <sup>w</sup>		0.01	0.09	0.14	0.21	0.01	0.11	0.83	
Experiment 1									
Nitrite nitrogen (mg L <sup>-1</sup> )									
8.5	0 a	0 a	0.9a	5.3 a	3.7 a	1.2 a	0 b	0 a	—
7.5	0 a	0 a	0 b	0.3 b	2.6 b	1.6 a	0.9 a	0 a	—
6.5	0 a	0 a	0 b	0.1 b	2.1 b	1.5 a	0.1 b	0 a	—
5.5	0 a	0 a	0 b	0 b	0 c	0 c	0 b	0 a	—
			L**	L**	L**	L**	Q**		
Significance				Q**		Q**			
Experiment 2									
8.5	0 a	0 a	0.6 a	4.5 a	2.0 ab	0.2 b	0 b	0 a	0 a
7.5	0 a	0 a	0 b	0.5 b	2.9 a	4.3 a	2.0 ab	0 a	0 a
6.5	0 a	0 a	0 b	0 b	0.2 b	1.2 ab	2.8 a	3.3 a	0.3 a
5.5	0 a	0 a	0 b	0 b	0 b	0 b	0 b	0 a	0 a
	L**	L**	L**	L**	L*	Q*	Q*		
Significance			Q**	Q**					
Exp*pH P value			0.71	0.78	0.29	0.17	0.07	0.07	
Experiment 1									
Nitrate nitrogen (mg L <sup>-1</sup> )									
8.5	3.8 a	3.0 a	1.5 a	5.0 a	2.0 a	8.3 a	7.8 a	8.8 a	—
7.5	2.0 b	2.0 b	0.3 b	3.0 b	1.3 b	4.3 b	3.0 b	5.0 b	—
6.5	2.0 b	2.0 b	0.3 b	3.0 b	0.3 c	3.8 b	3.0 b	4.0 c	—
5.5	2.0 b	2.0 b	0.5 b	3.0 b	0 c	2.8 c	1.0 c	2.5 d	—
	L**	L**	L**	L**	L**	L**	L**	L**	
Significance	Q**	Q**	Q*	Q*	Q*	Q*	Q**	Q**	
Experiment 2									
8.5	1.5 a	0 a	3.8 a	1.0 a	5.5 a	3.8 a	4.5 a	5.3 b	5.8 b
7.5	1.0 b	0 a	3.8 a	0.5 ab	4.5 b	3.0 ab	4.5 a	6.5 a	6.8 a
6.5	0 c	0 a	3.0 b	0.3 b	4.0 b	2.5 bc	2.8 b	5.0 b	6.5 ab
5.5	0 c	0 a	3.0 b	0 b	4.0 b	2.0 c	2.0 c	3.5 c	4.0 c
	L**		L**	L**	L**	L**	L**	L**	L**
Significance								Q**	Q**
Exp*pH P value	0.01	0.01	0.32	0.01	0.27	0.01	0.01	0.01	

<sup>c</sup>Nitrifying bacteria introduced to the biofilters.

<sup>y</sup>Within columns, means followed by different letters are significantly different; four replicates.

<sup>x</sup>Linear and Quadratic effects were significant at the 5% (\*) or 1% (\*\*) level.

<sup>w</sup>P values for experiment x pH interaction.

is measured based on the time it takes after introduction of nitrifying bacteria to convert ammonia to nitrate. A significant experiment x pH interaction was present in enough data sets to warrant discussion by experiment.

Total ammonia nitrogen (TAN) decreased from 5 mg L<sup>-1</sup> to zero, 12 d after the introduction of nitrifying bacteria to the biofilters maintained at a target pH of 8.5 (Table 1). A

similar reduction in TAN for the target pH of 7.5 took 20 d and for pH 6.5 took 20 (Exp. 1) and 24 (Exp. 2) d. TAN did decline at pH 5.5 but since no subsequent nitrite buildup occurred, it is assumed that this loss was due to ammonia and nitrogen gas volatilization and not nitrification. Nitrite began to be measured in the biofilter water 8 (pH 8.5), 16 (pH 7.5), and 16-24 (pH 6.5) d after introduction of nitrifying bacteria.

No nitrite was measured in the biofilters maintained at a pH of 5.5. Nitrate readings were inconsistent but did indicate a trend towards increased nitrate buildup over time which would be consistent with the oxidation of ammonia to nitrate. The inconsistency may be due to the wide range of the Cardy Ion Specific Meter (0 to 9,900 ppm) and the low range of the nitrate measured. The conservation of nitrogen through the nitrification process from ammonia to nitrate was good. Overall, results indicate nitrifying bacteria activity in perlite medium trickling biofilters increased as pH increased and was greatest at pH 8.5.

Average water quality parameters during experiments 1 and 2 respectively were 7.4 and 7.0 mg L<sup>-1</sup> dissolved oxygen, 521 and 493 uS/cm specific conductivity, 0.25 and 0.24 ppt (parts per thousand) salinity, and 28.1 and 29.8 °C temperature. The use of sodium bicarbonate to raise pH in experiment 1 resulted in higher specific conductivity compared with experiment 2 where potassium hydroxide was used. Seasonally average greenhouse temperatures were higher during experiment 2 compared to experiment 1. Actual average (and standard deviation) pH values during experiment 1 were 8.6 (0.1), 7.3 (0.3), 6.4 (0.2), and 5.5 (0.2), and during experiment 2 were 8.4 (0.1), 7.4 (0.2), 6.4 (0.1), and 5.6 (0.1). Nitrification is an acid producing process requiring adjustment of recirculating water to maintain target pH levels. The measured pH was well within the target pH range of the treatments.

*Reconciling water quality parameters:* The pH recommendations for aquaculture systems range between 6.5 and 8.5 (Timmons et al., 2002). For a pH range between 2.0 and 7.0, ammonia in solution is completely present as NH<sub>4</sub><sup>+</sup> (De Rijck and Schrevels, 1999). However, as pH increases above 7.0, there is an increase in the un-ionized NH<sub>3</sub> form of ammonia and a decrease in the ionic NH<sub>4</sub><sup>+</sup> form. Un-ionized ammonia is the most toxic form for fish with 96-h LC50 varying by species from 0.08 mg/L NH<sub>3</sub>-N for pink salmon (*Oncorhynchus gorbuscha*) to 2.2 mg/L for common carp (*Cyprinus carpio*) (Timmons et al., 2002). The pH tolerances of plants can range from 5.0 to 7.6 depending on the species (Lorenz and Maynard, 1988). However, recommended pH ranges for hydroponic nutrient solutions tend to be slightly acidic (5.5 to 6.5 - Hochmuth, 1991; 5.8 to 6.4 - Resh, 1998) due to problems with plant nutrient solubility. At pH levels above 7.0 there can be reduced micronutrient and phosphorus solubility. If aquaponic recirculating water pH is maintained at levels optimum for nitrifying bacteria (8.5), plant uptake of certain nutrients may become restricted and un-ionized ammonia levels may become toxic to the fish.

Plant uptake is one of the most widely recognized biological processes for contaminant removal in wastewater treatment wetlands (Debusk, 1999). Ammonium nitrogen removal efficiencies of 86 to 98% were reported from a constructed wetlands system receiving aquaculture wastewater (Lin et al., 2002). In hydroponic greenhouse plant production systems receiving aquaculture wastewater, Adler (1996) found that differences in nutrient removal rates of nitrate nitrogen and phosphorus were dependant on plant numbers and effluent flow rate. If plant numbers are increased sufficiently, nutrient concentration can decrease to levels that may be too low to sustain plant growth. Plant roots were found to be more competitive for ammonium than the ammonium-oxidizing bacterial species *Nitrosomonas europaea* (Verhagen et al., 1994). There may be less reliance on nitrification for ammonia removal when sufficient plants are

present in aquaponic systems. However, since the optimum ratio of nitrate to ammonium nitrogen in hydroponic nutrient solutions is 75:25 (Cockx and Simonne, 2003), a source of nitrate-nitrogen would be needed for plant uptake either through nitrification or supplemental fertilization for optimum plant growth. Since certain plant nutrients can fall below sufficiency standards in aquaponics (McMurtry et al., 1990) without supplemental fertilization, methods to make up this deficit without adversely impacting fish and nitrifying bacteria need further investigation.

## Conclusions

Nitrification as measured by ammonia conversion to nitrate was significantly faster at pH 8.5 than at pH 7.5 or 6.5 in a perlite medium trickling biofilter startup cycle. The recommended pH for aquaculture systems is from 6.5 to 8.5 and for hydroponic systems is between 5.5 and 6.5. However, pH extremes should be avoided when reconciling pH between fish, plants, and bacteria since high alkaline conditions reduce the solubility of certain plant nutrients and increase the presence of the un-ionized (more toxic to fish) form of ammonia. It should be possible to maintain aquaponic water at a pH of 6.5-7.0, levels more conducive to hydroponic plant nutrient uptake and reduced un-ionized ammonia levels, without a significant buildup of ammonia in the recirculating water provided there are a sufficient number of plants present for uptake and reduction of nutrient loads in the system water and water flow rate through the root zone is adequate. Even though nitrification is slower at pH 6.5 than at pH 8.5, the increased uptake and utilization of ammonia by plants should make up for the reduced nitrifying activity. Plant nutrient availability could be enhanced by supplemental fertilization of the plant growing medium or by foliar application of specific elements.

Reconciling differences in optimum water quality for plants, fish, and nitrifying bacteria will be necessary to successfully integrate hydroponic and aquaculture systems. More information is needed on aquaponic systems containing soil-less media such as perlite and vermiculite. Also, the affects of pH and hydroponic nutrient concentration of the system water, as well as methods of plant nutrient application on nitrifying bacteria activity and growth and yield of plants and aquatic organisms need to be investigated more fully.

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