Reconciling pH for Ammonia Biofiltration and Cucumber Yield in a Recirculating Aquaponic System with Perlite Biofilters

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Abstract. Integrating hydroponic and aquaculture systems (aquaponics) requires balanced pH for plants, fish, and nitrifying bacteria. Nitrification prevents accumulation of fish waste ammonia by converting it to NO_3^- -N. The difference in optimum pH for hydroponic cucumber (*Cucumis sativa*) (5.5 to 6.0) and nitrification (7.5 to 9.0) requires reconciliation to improve systems integration and sustainability. The purpose of this investigation was to: 1) determine the ammonia biofiltration rate of a perlite trickling biofilter/root growth medium in an aquaponic system, 2) predict the relative contribution of nitrifiers and plants to ammonia biofiltration, and 3) establish the reconciling pH for ammonia biofiltration and cucumber yield in recirculating aquaponics. The biofiltration rate of total ammonia nitrogen (TAN) removal was 19, 31, and 80 $\text{g}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ for aquaponic systems [cucumber, tilapia (*Oreochromis niloticus*), and nitrifying bacteria (*Nitrosomonas* sp. + *Nitrobacter* sp.)] with operating pH at 6.0, 7.0, and 8.0, respectively. With the existing aquaponic design (four plants/20 L perlite biofilter/100 L tank water), the aquaponic biofilter (with plants and nitrifiers) was three times more effective at removing TAN compared with plant uptake alone at pH 6.0. Most probable number of *Nitrosomonas* sp. bacteria cells sampled from biofilter cores indicated that the aquaculture control (pH 7.0) had a significantly higher (0.01% level) bacteria cell number compared with treatments containing plants in the biofilter (pH 6.0, 7.0, or 8.0). However, the highest TAN removal was with aquaponic production at pH 8.0. Thus, operating pH was more important than nitrifying bacteria population in determining the rate of ammonia biofiltration. Early marketable cucumber fruit yield decreased linearly from 1.5 to 0.7 kg/plant as pH increased from 6.0 to 8.0, but total marketable yield was not different. The reconciling pH for this system was pH 8.0, except during production for early-season cucumber market windows in which pH 7.0 would be recommended.

Aquaponics is the integration of hydroponic and aquaculture systems. Cucumber (*Cucumis sativus*) is an important hydroponic greenhouse crop (Tyson et al., 2001) with potential for production in aquaponic systems (Timmons et al., 2002). Worldwide

production of tilapia (*Oreochromis* sp.) exceeded 2.2 million metric tons in 2002 with 68% of that total coming from farmed aquaculture (Lim and Webster, 2006). Properly designed and managed hydroponic and aquaculture systems are considered environmentally responsible alternatives to field-grown vegetable production and wild-caught fisheries (Smither-Kopperl and Cantliffe, 2004; Timmons et al., 2002).

Aquaponics fits closely into the definition of sustainable agriculture in the 1990 USDA Farm Bill (Gold, 1999) because it combines plant and animal production, integrates natural biological cycles, and makes the most use of nonrenewable resources. It has the potential to become a near-zero agricultural discharge production system, significantly reducing wasteful effluent discharges to the

environment. However, with all its promise, limited information is available on nitrification rate in a perlite biofilter/root growth medium or on the interaction and competition between plant roots and nitrifying bacteria for ammonium. Also, there is no scientifically established reconciling pH for these operating systems, which contain plants, fish, and nitrifiers—each with their own optimum environmental requirements. Recommended pH for tilapia is 6.0 to 9.0 (Popma and Masser, 1999), for greenhouse cucumber is 5.5 to 6.0 (Hochmuth, 2001), and aquaculture biofilter nitrification was reported to be most efficient between 7.5 and 9.0 (Hochheimer and Wheaton, 1998).

One important subsystem of recirculating aquaculture systems is the biofiltration of fish waste ammonia through nitrification to maintain fish tank water quality (Gutierrez-Wing and Malone, 2006; Masser et al., 1999). This is necessary because 10% of the protein in fish feed becomes ammonia in the system water (Timmons et al., 2002) and unionized ammonia (NH_3) is toxic to fish at levels above 0.05 mg/L (Francis-Floyd and Watson, 1996). Nitrification is the biochemical conversion by bacteria of NH_3 to NO_3^- (Hagopian and Riley, 1998; Madigan et al., 2003; Prosser, 1986). It is a two-step process:

Primarily *Nitrosomonas* sp.

Primarily *Nitrobacter* sp.

Nitrate, the end product of nitrification, is not toxic to fish except at very high levels (channel catfish, *Ictalurus punctatus*, 96-h $\text{LC}_{50} > 6200 \text{ mg}\cdot\text{L}^{-1} \text{ NO}_3\text{-N}$; Colt and Tchobanoglous, 1976). Nitrate is the primary source of nitrogen for plants in hydroponic nutrient solutions at concentrations from 50 to 280 $\text{mg}\cdot\text{L}^{-1} \text{ NO}_3\text{-N}$ (Resh, 2004). Hence, the understanding and management of the nitrification process in aquaponics is important for the maintenance of water quality and the production of nitrate nitrogen.

In water, ammonia exists in two forms, which together are called the total ammonium nitrogen (TAN) (Francis-Floyd and Watson, 1996) ($\text{TAN} = \text{NH}_4^+ + \text{NH}_3\text{-N}$). The equilibrium reaction is as follows (Campbell and Reese, 2002): $\text{NH}_4^+ \leftrightarrow \text{NH}_3 + \text{H}^+$. Plants absorb the ammonium (NH_4^+) form (Marschner, 2003) and the unionized ammonia (NH_3) is the substrate ion used for the nitrification reaction (Madigan et al., 2003; Prosser, 1986). Because in water they are in equilibrium, removal of either nitrogen form will result in a reduction of the other. Water temperature and pH will affect which form of ammonia is prevalent (Lim and Webster, 2006). At 22 °C, the unionized ammonia

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fraction is 0.46% and 4.4% of TAN for pH 7.0 and 8.0, respectively (Francis-Floyd and Watson, 1996). This represents nearly a 10-fold increase in unionized ammonia substrate for the nitrification reaction as pH increases one unit. Increased removal of ammonia by a trickling biofilter was found with increasing concentrations of ammonia in pond water (Rijn and Rivera, 1990) and removal rate was considered substrate-limited with respect to ammonia.

Plant uptake is one of the most widely recognized biological processes for contaminant removal in wastewater treatment wetlands (Debusk, 1999; Mitsch and Gosselink, 2000). Ammonium nitrogen removal efficiencies of 86% to 98% were reported from a constructed wetland system receiving aquaculture wastewater (Lin et al., 2002). In hydroponic greenhouse plant production systems receiving aquaculture wastewater (Adler et al., 1996), differences in nutrient removal rates of nitrate nitrogen and phosphorus were dependent 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. Aquaponic wastewater cleanup cost abatement alone can be a major factor in integrating hydroponic and aquaculture systems (Adler, 2001; Adler et al., 2000). Plant roots were found to be more competitive for ammonium than the ammonia-oxidizing bacterial species *Nitrosomonas europaea* (Verhagen et al., 1994). There may be a possibility for less reliance on nitrification in aquaculture biofilters for ammonia removal when sufficient plants are present in aquaponic systems. More work needs to be done to understand these relationships and thus optimize the biological efficiencies of the organisms through management practices.

The most probable number (MPN) technique is a method of estimating the numbers of microorganisms in foods, wastewater, enrichment cultures, or natural samples of water or soil (Madigan et al., 2003). A selective culture medium is prepared to target the growth of specific organisms or groups of organisms such as nitrifying bacteria. The MPN method has been used to enumerate nitrifying bacteria in sediments of aquatic environments (Feraý et al., 1999; Smorczewski and Schmidt, 1991) and terrestrial soils (Papen and von Berg, 1998; Prosser, 1986). It has also been used in a hydroponic system (Schwarz et al., 1999) and in soilless potting media (Lang and Elliott, 1997). MPN bacterial cell numbers can be used to compare the relative production environment effects on bacterial population growth provided initial cell inoculation numbers are the same among treatments.

A dichotomy exists between pH optima for hydroponic cucumber growth (pH 5.5 to 6.0) and nitrifying bacteria activity (pH 7.5 to 9.0). However, no scientifically based pH optima for aquaponics has been established. Plants and nitrifying bacteria both contribute to the biofiltration of fish waste in aquaponic systems. However, little is known about how

aquaponic cucumber and nitrifying bacteria are affected by operating outside their recommended pH ranges and how this affects fruit yield and ammonia biofiltration. The purpose of this investigation was to: 1) determine the ammonia biofiltration rate of a perlite trickling biofilter/root growth medium in an aquaponic system, 2) predict the relative contribution of plants and nitrifiers to the biofiltration of ammonia, and 3) establish the reconciling pH for ammonia biofiltration and cucumber yield in recirculating aquaponics.

Materials and Methods

The growout phase of the experiment was conducted in a pad and fan greenhouse with polyethylene cover at the University of Florida Horticultural Sciences Department Teaching Park, Gainesville, FL, from 6 July to 12 Oct. 2006. Treatments were aquaponic systems with operating pHs of 6.0, 7.0, and 8.0 and controls consisting of a hydroponic system at pH 6.0 and an aquaculture system at pH 7.0. Aquaponic treatments consisted of cucumber, tilapia, and inoculation with nitrifying bacteria. The hydroponic control contained cucumber plants only, and the aquaculture control contained tilapia and nitrifying bacteria. The laboratory phase of the experiment to determine MPN estimates of the *Nitrosomonas* sp. bacterial cell populations in the biofilters was conducted from 12 Oct. to 20 Nov. 2006.

Each greenhouse plot consisted of a recirculating tank with a crate containing 20 L of perlite suspended above the tank. Recirculating tanks were placed 75 cm on center down a single row in the greenhouse running parallel to the north-south sidewalls. Each circular tank (180 L, model DM52; Aquatic Eco-systems, Apopka, FL) was filled with 100 L of well water on 6 July 2006. Biofilters consisting of rectangular plastic milk-carrying crates lined with natural burlap and filled with 20 L of horticultural-grade perlite were placed on top of the recirculating tanks on 13 July. One aquarium pump (model HX2500; Aquatic Eco-systems) was placed in the bottom of each tank and water was recirculated (beginning on 13 July) to distribution plates on top of the perlite in the biofilter and allowed to trickle down through the perlite back to the tanks at an average rate of 100 L·h⁻¹. An additional pump (model SP800; Aquatic Eco-systems) was added on 30 Aug. to each tank to increase water turnover rates through the biofilter to an average of 300 L·h⁻¹.

Phosphoric acid was used to lower water pH and potassium hydroxide was used to raise pH to treatment levels (target 6.0, 7.0, and 8.0). An ammonia surge test was conducted to determine ammonia volatilization rates from tanks at pH 6.0, 7.0, and 8.0 before inoculation of bacteria and introduction of fish and plants. Ammonium chloride (2.5 g/tank) was added and TAN measurements were taken on 13 July (all tanks reading 6 mg·L⁻¹ TAN) and 20 July. Water volume was

maintained constant. Ammonia volatilization loss in mg·L·d was determined by subtracting beginning and ending TAN and dividing by 7.

A complete hydroponic nutrient solution consisting of 200 mg·L⁻¹ NFT Vegetable Formula (Grower's Supply Center, Lynn Haven, FL) was added to each tank on 21 July. The NFT vegetable formula consisted of 6 mg·L⁻¹ nitrate nitrogen, 13 mg·L⁻¹ phosphorus, 45 mg·L⁻¹ potassium, 11 mg·L⁻¹ magnesium, 0.9 mg·L⁻¹ iron, 0.07 mg·L⁻¹ zinc, 0.1 mg·L⁻¹ manganese, 0.02 mg·L⁻¹ copper, 0.3 mg·L⁻¹ boron, and 0.02 mg·L⁻¹ molybdenum using potassium sulfate, monopotassium phosphate, magnesium sulfate, potassium nitrate, iron EDTA, zinc EDTA, manganese EDTA, copper EDTA, sodium borate, and sodium molybdate as nutrient sources. In addition to the hydroponic fertilizer blend, Ca(NO₃)₂, KNO₃, and MgSO₄ were added when needed based on plant nutritional status and water analysis to maintain plant nutrition and similar soluble salt concentrations among treatments. Sodium bicarbonate (NaHCO₃) and calcium carbonate (CaCO₃) were added to tank water during the trial to provide alkalinity.

Nitrifying bacteria were added to the biofilters (except the hydroponic control) on 26 July (170 mL, product no. 239211, Proline Freshwater Nitrifying Bacteria). The bacteria solution contained a mix of 50% *Nitrosomonas* sp. and 50% *Nitrobacter* sp. with a count of 6.7 × 10⁴ cells/mL according to the supplier (Aquatic Eco-systems). Another 50 mL per tank of Proline Freshwater Nitrifying Bacteria was added on 14, 15, and 16 Aug. to control an ammonia spike after the introduction of fish.

Nile tilapia (*Oreochromis niloticus* from Harbor Branch Oceanographic Institution, Fort Pierce, FL) were stocked into treatment tanks (except hydroponic control) on 1 to 2 Aug. at a similar density of 15 fish per tank with a mean of 32.4 g/fish/tank and a size range from 8 to 122 g. Care was taken to ensure that the fish size distribution was similar in each tank. Density and size distribution were kept similar among treatments during the trial by periodic restocking to compensate for varying fish mortality among treatments. The fish feed contained 41% protein and was fed at a rate of 3 g/tank/d for the aquaponic treatments and the aquaculture control. The vigor of the initial fish feeding activity was monitored during five feedings beginning 20 Sept. through 30 Sept. and was rated as 1 = do not come to feed, 3 = half strike feed, and 5 = all strike feed. Final fish weights were obtained on 6 Oct.

Based on previous greenhouse trials (Hochmuth et al., 1996), 'Fitness' (Asgrow Seed Company, St. Louis) European cucumber seeds were planted into the perlite of the biofilter of each plot except the aquaculture control treatment on 4 Aug. Four seeds were planted on each side of the distribution plate for a total of eight seeds per plot. Plant germination was complete in all plots on 7 Aug. [0 d after germination (DAG)]. Starter fertilizer (400 mL of 2 g·L⁻¹ NFT vegetable

formula) was added to each tank biofilter around the young seedlings to stimulate growth 2 DAG. Plants were thinned to four plants per biofilter 7 DAG. Cucumber vines were trellised to overhead wires and pruned to a single leader stem. Fruit were harvested 36, 43, 50, and 58 DAG. Fruits showing poor tip fill or angled fruit greater than 45% were considered unmarketable.

Concentrations of NO_3^- -N, NO_2^- -N, NH_4^+ -N, and K^+ in recirculating tank water were measured weekly. Tank water TAN (low range 1.0 to 8.0 $\text{mg}\cdot\text{L}^{-1}$), nitrite nitrogen (low range, 0.1 to 0.8 $\text{mg}\cdot\text{L}^{-1}$), and alkalinity were measured with test kits (LaMotte Company, Chestertown, MD). TAN (high range 1.0 to 50.0 $\text{mg}\cdot\text{L}^{-1}$) and nitrite nitrogen (high range, 0 to 150 $\text{mg}\cdot\text{L}^{-1}$) were measured using an ion-specific meter (Hanna Instruments USA, Woonsocket, RI). Nitrate nitrogen and potassium were measured using an ion-specific electrode (Cardy meter, range 0 to 9900 $\text{mg}\cdot\text{L}^{-1}$; Spectrum Technologies, Plainfield, IL). Water pH was measured two to six times per week (mean of four measurements) using pH meter model WD-35624-86 (Oakton Instruments, Vernon Hills, IL). Water-dissolved oxygen, specific conductivity (electrical conductivity), temperature, and salinity were measured once every 2 weeks using a YSI Model 85 m (YSI, Yellow Springs, OH) during midday from 11 AM to 2 PM. Cucumber leaf petiole sap NO_3^- -N and potassium levels were measured beginning on 26 Aug. and every 2 weeks thereafter. One leaf petiole per plot from the most recently fully expanded leaf (from the sixth to eighth leaf below the growing point) was measured using ion-specific electrode Cardy meters (Hochmuth, 2003).

A second ammonia surge test was conducted after the last cucumber harvest to determine ammonia biofiltration rates from treatment tanks. Ammonium chloride (3 g/tank for all treatments except hydroponic control, which received 1g/tank as a result of the presence of residual TAN from applied fertilizer) was added at 11:30 AM on 10 Oct. TAN measurements were taken at 12:00 PM 10 Oct. and every 6 h thereafter for 24 h. Recirculating tanks were maintained at a constant water volume of 100 L. Ammonia biofiltration rates in $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ were determined by subtracting beginning and ending TAN except for aquaponic treatment pH 8.0, which was determined by subtracting beginning TAN from the first 6-h TAN measurement and multiplying by 4 because TAN was resolved to zero in this treatment at 12 h.

End-of-season MPN estimates of the *Nitrosomonas* sp. bacterial cell populations in the biofilters were made using the three-tube serial dilution method (Feng, 2001). Core samples of 200 mL perlite were taken from the middle of each biofilter on 12 Oct. Samples were mixed into a composite for each treatment and refrigerated at 5 °C. Nitrifier culture medium for *Nitrosomonas* sp. was prepared and autoclaved as described (ATCC, 2006) on 17 Oct. Ninety growout and 10 inoculation test tubes were prepared

for each of the five treatments. The ATCC liquid medium in the test tubes measured 0 $\text{mg}\cdot\text{L}^{-1}$ for NO_2^- -N and 5 $\text{mg}\cdot\text{L}^{-1}$ for NO_3^- -N before inoculation with nitrifying bacteria core samples. Growout test tubes were replicated three times with 30 test tubes per replicate per treatment. On 21 Oct., 200-mL subsamples of perlite from each treatment were mixed with 200 mL deionized water and blended in a laboratory blender (Hamilton Beech, Washington, NC) for 30 s. One milliliter of this blended solution was used to inoculate MPN test tubes. Tubes were shaken 8 h·d⁻¹ for 30 d and then measured for nitrifier activity. A measurement 0.1 $\text{mg}\cdot\text{L}^{-1}$ NO_2^- -N or greater or 10 $\text{mg}\cdot\text{L}^{-1}$ NO_3^- -N or greater was considered positive for *Nitrosomonas* bacteria activity (Eq. 1 and Eq. 2). NO_2^- -N (low range, 0.1 to 0.8 $\text{mg}\cdot\text{L}^{-1}$) was measured with a test kit (LaMotte Company) and NO_3^- -N was measured using an ion specific electrode (Cardy meter, range 0 to 9900 $\text{mg}\cdot\text{L}^{-1}$). One measurement for each replicate (three measurements per treatment) was recorded based on statistical tables (Feng, 2001) to determine bacteria cell population number.

Treatments were arranged in a randomized complete block design with four replications. Data were analyzed using analysis of variance (SAS Institute, Inc., 2004) and Duncan's multiple range test at the 0.05 level. Linear content over the nonrepeating equal interval pH units (excluding the hydroponic and aquaculture control treatments) were calculated (Gomez and Gomez, 1984).

Results and Discussion

Table 1 summarizes the season mean tank water TAN, NO_2^- -N, and CaCO_3 equivalent alkalinity as well as the operating pH ranges for each treatment. Nitrification is an acid-producing process (ammonia oxidation produces protons H^+ in Eq. 1) requiring adjustment of recirculating water to maintain target

total marketable yield when cucumbers were grown in hydroponic or aquaponic production systems at pH 6.0. These results indicate an early yield (first harvest) advantage in keeping nutrient solution pH between 6.0 and 7.0, but not an advantage for total season marketable yield. This would indicate that keeping the recirculating pH at 7.5 to 8.0 to accommodate nitrifying bacteria activity would negatively affect early but not total yield.

Nitrifying activity is commonly determined by measuring ammonia oxidation (substrate TAN loss), intermediate (nitrite) and end product (nitrate) accumulation, or oxygen uptake (Hagopian and Riley, 1998; Prosser, 1986). Ammonia biofiltration rate of the perlite trickling biofilters in aquaponic production was determined by measuring TAN loss during a 24-h period after introduction of ammonia to the system water. This ammonia surge test was conducted after the last cucumber fruit harvest. TAN loss from aquaponic system water was 3.8, 6.1, and 16 $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ for pH 6.0, 7.0, and 8.0 treatments, respectively (Table 4). This compared with TAN loss of 1.3 $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ for the hydroponic control and 5.9 $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ for the aquaculture control. TAN loss from the aquaponic treatments increased linearly at 6, 12, and 18 h after introduction of ammonia as the pH increased from 6.0 to 8.0. This linear increase in TAN loss may be the result of an increased unionized ammonia substrate (NH_3 ; Eq. [1]) concentration resulting from increasing pH (Francis-Floyd and Watson, 1996). Increased biofilter activity occurs with an increase in substrate (NH_3) concentration (Hagopian and Riley, 1998). During a similar ammonia surge test at the beginning of the experiment after system setup but before inoculation with bacteria, the maximum volatilization loss of TAN from these tanks was 0.39, 0.53, and 0.63 $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ for pH 6.0, 7.0, and 8.0, respectively. Because average

Table 1. System season average TAN, NO_2^- -N, CaCO_3 , and pH range in treatment tanks.

System ^y	pH	TAN ($\text{mg}\cdot\text{L}^{-1}$)	NO_2^- -N ($\text{mg}\cdot\text{L}^{-1}$)	CaCO_3 ($\text{mg}\cdot\text{L}^{-1}$)	pH	
					Low	High
Aquaponic	6.0	2.1	0.2	35	5.5	6.7
Aquaponic	7.0	0.7	1.8	42	6.5	7.5
Aquaponic	8.0	0.3	1.4	84	7.1	8.3
Hydroponic	6.0	3.0	0.0	39	5.5	6.8
Aquaculture	7.0	0.7	2.1	41	6.5	7.4

^zTAN = total ammonia nitrogen; NO_2^- -N = nitrite nitrogen; CaCO_3 = calcium carbonate.

^yAquaponic = with plants, fish, and nitrifying bacteria; hydroponic = with plants alone; aquaculture = with fish and nitrifying bacteria.

pH levels. Table 2 summarizes monthly average high and low greenhouse air temperatures, average tank water temperatures, and oxygen levels.

Early marketable cucumber yield decreased linearly from 1.52 to 0.67 kg/plant as pH increased from 6.0 to 8.0 (Table 3). Total marketable and cull yields were not significantly different among treatments ($P = 0.05$). There was no difference in early or

Table 2. Monthly average greenhouse air temperature, tank water temperature, and tank water oxygen level.

Month	Air temp (°C)		Tank water	
	High	Low	Temp (°C)	Oxygen (mg·L ⁻¹)
July	35	24	27	5.5
August	38	24	29	4.6
September	32	24	26	3.9
October	29	19	23	4.4

Table 3. Fitness cucumber fruit yield response to water pH and production system.

System ^y	pH	Early mark ^z		Total mark		Total cull	
		(kg/plant)	(no./plant)	(kg/plant)	(no./plant)	(kg/plant)	(no./plant)
Aquaponic	6.0	1.52 a ^x	3.3 a	3.64 a	8.3 a	0.44 a	3.3 a
Aquaponic	7.0	1.32 a	2.9 a	4.12 a	9.7 a	0.33 a	3.0 a
Aquaponic	8.0	0.67 b	1.8 b	3.54 a	8.8 a	0.33 a	3.0 a
		L ^{***w}	L [*]				
Hydroponic	6.0	1.57 a	3.3 a	3.63 a	8.4 a	0.53 a	3.9 a
Aquaculture	—	—	—	—	—	—	—

^zEarly = first harvest—36 DAG; total = four harvests—36, 43, 50, 58 DAG, kilograms and number of fruit per plant. Marketable fruit = 34 to 42 cm length, less than 45° angle, few blemishes; Cull = greater than 45° angle, poor tip fill, excess blemishes. Average of four plants per plot, four replicates.

^yAquaponic = with plants, fish, and nitrifying bacteria; hydroponic = with plants; aquaculture = with fish and nitrifying bacteria.

^xWithin columns, means followed by different letters are significantly different at the 0.05 level; four replicates.

^wpH effects of aquaponic systems were significantly linear at the 5% (*) or 1% (**) level.

DAG = days after germination.

Table 4. Twenty-four-hour total ammonia nitrogen (TAN) and nitrite nitrogen (NO₂⁻-N) concentrations in a perlite trickling biofilter after introduction of ammonium chloride.

		Hours after introduction of ammonia				
System ^z	pH	0 ^y	6	12	18	24
TAN (mg·L)						
Aquaponic	6.0	6.1 ab ^x	4.9 a	4.0 a	2.9 b	2.4 b
Aquaponic	7.0	6.4 a	3.4 b	2.1 b	1.3 c	0.3 c
Aquaponic	8.0	6.0 ab	2.0 c	0.0 c	0.0 d	0.0 c
			L ^{*w}	L [*]	L [*]	L [*]
Hydroponic	6.0	5.4 b	4.8 ab	4.5 a	4.4 a	4.4 a
Aquaculture	7.0	6.5 a	4.5 ab	2.1 b	1.4 c	0.6 c
NO ₂ ⁻ -N (mg·L)						
Aquaponic	6.0	0.0 a	0.0 b	0.0 c	0.0 a	0.0 a
Aquaponic	7.0	0.0 a	0.3 b	0.3 cb	0.1 a	0.1 a
Aquaponic	8.0	0.0 a	1.4 a	1.1 a	0.2 a	0.0 a
			L [*]	L [*]		
Hydroponic	6.0	0.0 a	0.0 b	0.0 c	0.0 a	0.0 a
Aquaculture	7.0	0.0 a	0.3 b	0.4 b	0.0 a	0.1 a

^zAquaponic = with plants, fish, and nitrifying bacteria; hydroponic = with plants; aquaculture = with fish and nitrifying bacteria.

^yAmmonium chloride introduced into the recirculating tanks.

^xWithin columns, means followed by different letters are significantly different at the 0.05 level; four replicates.

^wpH effects of aquaponic systems were significantly linear at the 1% (*) level.

greenhouse temperatures were 6 °C cooler during the second ammonia surge test and volatilization decreases with decreasing temperatures, it is likely that the actual ammonia biofiltration is very near the measured TAN loss in Table 4 for each treatment.

Considering TAN loss as a function of biofilter media volume (20 L), these results convert to 19, 31, and 80 g·m⁻³·d⁻¹, respectively, for the aquaponic treatments pH 6.0, 7.0, and 8.0 (Fig. 1). This compares to 6 g·m⁻³·d⁻¹ for the hydroponic control and 29 g·m⁻³·d⁻¹ for the aquaculture control. The recommended ammonia biofiltration rate for trickling biofilters in aquaculture production is 90 g·m⁻³·d⁻¹ (Losordo et al., 1999). Thus, at pH 8.0, the perlite trickling biofilter/root growth medium is functioning at near optimum for trickling biofilters. Comparing 6 g·m⁻³·d⁻¹ (TAN removal with cucumber plants only) and 19 g·m⁻³·d⁻¹ (with plants and nitrifying bacteria) at pH 6.0 indicates that the aquaponic biofilter was three times more efficient as plants alone in the biofilter in removing ammonia from aquaponic system water under these conditions. In addition, there was no significant difference ($P = 0.05$)

in TAN loss between the aquaculture control and the aquaponic treatment when both were at pH 7.0 (Fig. 1), which again emphasizes the importance of nitrification over plant uptake for biofiltration of ammonia. More work should be done to test the nitrification/plant biofiltration relationship on TAN re-

Fig. 1. Perlite trickling biofilter 24-hour total ammonia nitrogen loss as influenced by pH and production system. Note: 6.0-aqon, 7.0-aqon, 8.0-aqon = aquaponic system pH with plants, fish, and nitrifying bacteria; 6.0-hc = pH hydroponic control with plants; 7.0-ac = pH aquaculture control with fish and nitrifying bacteria. Error bars represent $\pm$ SE (n = 4).

moval during early-season active stages of plant growth, because the current test was done after the last cucumber harvest when plants were fully mature but not actively growing.

Recirculating aquaculture systems are usually intensively managed with maximum carrying capacities of 60 g fish per liter of water for systems with oxygen injection and 30 g·L⁻¹ for natural air-aerated systems (Masser et al., 1999; M. Davis, personal communication). The ammonia biofiltration rate of the designed biofilter in this experiment (perlite volume: tank water volume ratio of 1:5) was sufficient to oxidize ammonia from a naturally aerated system at maximum carrying capacity with production water at pH 8.0, but not when operating at pH 7.0 or 6.0. Thus, the perlite medium biofilter volumes would most likely need to be increased to compensate for reduced biofiltration rates at lower operating water pH levels.

Results of MPN bacterial cell counts from biofilter core sampling indicate that the aquaculture control without plants in the biofilter had a significantly higher ($P = 0.01\%$) number of *Nitrosomonas* sp. bacteria compared with treatments containing plants in the biofilter (Fig. 2). In other work, numbers of nitrifying bacteria (*Nitrosomonas europaea*) were reduced 200-fold in the presence of plants roots compared with without them (Verhagen et al., 1994). In the current trial, the aquaponic system at pH 8.0 had the highest ammonia biofiltration rate of all treatments (Fig. 1). Hence, MPN bacteria counts now (Fig. 2) were not a good indicator of biofilter performance (Fig. 1). This indicates that pH of system water was a more important factor in determining efficiency of microbial activity in the biofilter rather than bacterial species density. The increased activity was most likely the result of pH-induced increases in unionized ammonia substrate available for the nitrification reaction [Eq. 1] as the pH increases. It would be beneficial to take MPN counts over time during the growing season to more accurately predict the systems effect on their numbers and activity. The hydroponic control was not inoculated with nitrifying bacteria, but the MPN test indicated a low

Fig. 2. Most probable number (MPN) of *Nitrosomonas* sp. bacteria in perlite trickling biofilters as influenced by pH and production system. Note: 6.0-aqon, 7.0-aqon, 8.0-aqon = aquaponic system pH with plants, fish, and nitrifying bacteria; 6.0-hc = pH hydroponic control with plants; 7.0-ac = pH aquaculture control with fish and nitrifying bacteria. Error bars represent $\pm$ SE (n = 3).

Table 5. Tilapia mean weight, initial feeding activity, and overall mortality as influenced by water pH and production system.

System ²	pH	Mean stocking wt. g/fish	Mean final wt. g/fish	Initial ³ feeding activity	Fish ⁴ mortality
Aquaponic	6.0	37 a	79 a	1.6 b ^w	18.5 a
Aquaponic	7.0	39 a	72 a	2.3 ab	8.0 b
Aquaponic	8.0	37 a	65 a	4.0 a L ^{*v}	3.4 b L [*]
Hydroponic	—	—	—	—	—
Aquaculture	7.0	38 a	66 a	3.0 ab	8.8 b

²Aquaponic = with plants, fish, and nitrifying bacteria; hydroponic = with plants; aquaculture = with fish and nitrifying bacteria.

³Initial feeding activity rating: 1 = do not come to feed; 3 = half strike feed; 5 = all strike feed.

⁴Average number dead at end of growing season.

^wWithin columns, means followed by different letters are significantly different at the 0.05 level; four replicates.

^vpH effects of aquaponic systems were significantly linear at the 1% (*) level.

level of bacteria—2 cells/mL of perlite. Therefore, some unintentional or low-level natural inoculation occurred during the growing season. To maximize the ammonia biofiltration rate, system operating pH should be maintained near 8.0 provided it does not adversely impact the plant or fish yield.

Although no differences in tilapia growth were expected among treatments resulting from their adaptation to wide ranges of water quality conditions (Chapman, 2000; Lim and Webster, 2006; Popma and Masser, 1999; Watanabe et al., 1997), significant differences in initial feeding activity and fish mortality ($P = 0.05$) were observed (Table 5). Initial feeding activity, or the vigor response of fish when feed is first thrown into the recirculating tanks, increased and fish mortality decreased as pH increased from 6.0 to 8.0. The vigor of fish feeding activity is a reflection of the general health and stress level of the fish (Lim and Webster, 2006). These results imply that fish were healthier, under less environmental stress, and more likely to survive as pH increased from 6.0 to 8.0. Fish density and feeding rate were kept the same among the aquaponic and aquaculture control treatments so that ammonia generation by the fish would not be a variable in the biofiltration rate. This required periodic restocking of these tanks as a result of variable mortality among treatments. Final total fish biomass was 316, 324, and 325 g for the aquaponic treatments at pH 6.0, 7.0, and 8.0, respectively. This compared with 317 g for the aquaculture control. Mean final fish weights (g/fish) were not significantly different among these treatments (Table 5). Ammonium and nitrate-containing fertilizers were periodically added to the hydroponic control treatment to keep the level of these nutrients similar to those being generated in the aquaponic and aquaculture control treatments. This ensured that pH would be the main variable affecting the ammonia biofiltration rate.

Conclusion

Biofilter removal of TAN increased linearly in a perlite trickling biofilter/root growth medium and occurred at the rate of 19, 31, and 80 $\text{g}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ for aquaponic systems

operating at pH 6.0, 7.0, and 8.0, respectively. Maximum TAN removal ($80\text{ g}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ at pH 8.0) was similar to reported trickling biofilter removal rates in aquaculture. The difference in TAN loss at pH 7.0 between the aquaculture control (tilapia and nitrifying bacteria) and the aquaponic system (tilapia, nitrifying bacteria, and cucumber) were not significant. Thus, under these experimental conditions, nitrification was more important than plant uptake for biofiltration of ammonia. MPN analysis of *Nitrosomonas* sp. bacteria populations indicated a significantly higher population of bacteria in biofilters without plant roots. However, the highest TAN loss occurred in the aquaponic system produced at pH 8.0. Thus, operating pH was more important than nitrifying bacteria population in system TAN loss. Fish vigor increased as pH increased from 6.0 to 8.0. Early marketable aquaponic cucumber yield decreased linearly as pH increased from 6.0 to 8.0. However, there were no differences in total marketable cucumber yields among treatments. Results indicate that given the importance of pH in biofilter activity and no difference in total cucumber yields in a pH range of 6.0 to 8.0, then recirculating aquaponic systems using cucumber and tilapia with high densities of fish should be managed with pH closer to those favoring nitrification (7.5 to 8.0) to efficiently convert waste ammonia to nitrate nitrogen.

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