RECONCILING pH FOR AMMONIA BIOFILTRATION IN A CUCUMBER/TILAPIA AQUAPONICS SYSTEM USING A PERLITE MEDIUM

By

RICHARD V. TYSON

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2007

To my beloved wife, Gladys, and to the loving memory of our parents: James and Alyce Tyson and Sandalio and Juaquina Moreno

ACKNOWLEDGMENTS

I wish to express my sincere appreciation and gratitude to Dr. Eric Simonne, chair, and Dr. Marion White, co-chair of my graduate advisory committee, for their encouragement, advice, guidance, and patience throughout my Ph.D. program. I would like to extend my deepest gratitude to the members of my advisory committee, Dr. Frank Chapman, Dr. Megan Davis, and Dr. Danielle Treadwell for their assistance, technical support, and guidance. I would also like to thank Dr. Elizabeth Lamb for her tireless reviews of my early work on the project.

My deepest gratitude also goes to my supervisors at the University of Florida and Seminole County Extension: Dr. Freddie Johnson, Dr. Dan Cantliffe, Barbara Hughes, and Suzy Goldman for their understanding and support of my Ph.D. program activities.

My sincere thanks is extended to those individuals who assisted in the setup and implementation of my research during the 2006 sabbatical at the University of Florida: Dr. Amarat Simonne, Mike Alligood, David Studstill, Aparna Gazula, and Wei-Yea Hsu.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

RECONCILING pH FOR AMMONIA BIOFILTRATION IN A CUCUMBER/TILAPIA AQUAPONICS SYSTEM USING A PERLITE MEDIUM

By

Richard V. Tyson

May 2007

Chair: Eric H. Simonne
Cochair: James M. White
Major: Horticultural Science

Integrated hydroponic and aquaculture (aquaponic) production requires balancing pH and water quality for the growth of 3 organisms: plants, fish, and nitrifying bacteria. To improve systems integration, a series of trials were conducted to 1) determine the optimum pH for nitrification and evaluate performance of perlite as a biofilter, 2) determine the effect of hydroponic nutrients on nitrification, 3) make predictions about the contribution of plants and nitrifiers to ammonia biofiltration, and 4) establish a reconciling pH for ammonia biofiltration and cucumber yield in aquaponics. Total ammonium nitrogen (TAN) removal and NO_2^--N accumulation in a trickling perlite biofilter increased as pH increased from 5.5 to 8.5. Aquaponic biofilter TAN removal rates were 19, 31, and 80 g/m^3/d for pH 6.0, 7.0 and 8.0, respectively. Nitrification was unaffected by plant nutrients in solution at optimum levels for hydroponic production. Nutrients may be tailored for plant production (with consideration for fish waste contributions) with no adverse impact on nitrifiers. Most probable number (MPN) sampling of biofilter cores indicated that aquaculture control at pH 7.0 with no plants had a higher (0.01% level) number of *Nitrosomonas sp.* biofilter bacteria compared to treatments containing plants in the biofilter. However, the highest ammonia biofiltration rate was aquaponic production (plant,

fish, bacteria) at pH 8.0. pH was a more important factor than bacteria population in the rate of ammonia biofiltration—most likely due to pH induced increases in unionized ammonia, the substrate for the nitrification reaction. Ammonia biofiltration increased 3.7 times at pH 6.0 when bacteria and plants were in the biofilter compared to plants alone. The vigor of tilapia (*Oreochromis niloticus*) feeding increased and mortality decreased as water pH increased from 6.0 to 8.0. Early marketable cucumber fruit yield decreased linearly as pH increased from pH 5.0 to pH 8.0. However, total marketable yield was unaffected by pH. The reconciling pH for ammonia biofiltration and cucumber yield should be pH 7.5 to 8.0 given the importance of pH and bacteria to the ammonia biofiltration rate, differences in fish vigor, and given that no difference in total cucumber fruit yield among treatments was found.

CHAPTER 1
INTRODUCTION

Importance of Hydroponic Vegetable Production, Aquaculture, and Integrated Aquaponic Systems

Hydroponics is a term used to describe the production of plants without soil. Plant roots grow in a nutrient solution with or without an artificial medium for mechanical support (Jensen, 1997). Greenhouse hydroponic vegetable production is expanding rapidly worldwide (Brentlinger, 1999; Cantliffe and VanSickle, 2000; Resh, 2004; Smither-Kopperl and Cantliffe, 2004; Steta, 2004). Recent estimates of production (in hectares) for selected countries are: Spain, 60,000; Israel, 12,141; Holland, 4,100; Mexico, 2,000; England, 1,722; Canada, 800; United States, 400. The value of the commercial hydroponic industry worldwide is currently estimated at 6 to 8 billion dollars (Hassall, 2001). Hydroponics is a very young science. It has been used on a commercial basis for only 50 years. However, greenhouse hydroponic vegetable production offers the potential for greater yields and more control of practices that can be environmentally sensitive compared to field grown production (Smither-Kopperl and Cantliffe, 2004).

Yield increases of 2.3 (lettuce, *Lactuca sativa*), 3.1 (pepper, *Capsicum annuum*) 4 and 8.7 (cucumber, *Cucumis sativus*), and 6 (tomato, *Solanum lycopersicum*) times field grown yields have been reported for hydroponic greenhouse production (Resh, 2004; Smither-Kopperl and Cantliffe, 2004). Greenhouse European cucumber yields were about 37.9 kg/m^2 per year during 1999 when spring and fall crops were combined at the Horticultural Research Unit in Gainesville, Florida (Shaw et al., 2000). This compares to 5.3 kg/m^2 of field grown cucumber yields (one crop) in the 1999–2000 growing season (Rhodes, 2001). Overall yield of hydroponic greenhouse vegetable crops increased an averaged of 5 times over field grown yields (Resh, 2004; Smither-Kopperl and Cantliffe, 2004).

Aquaculture, the commercial farm production of fish, shellfish and plant products, is the fastest growing sector of the world food economy, increasing by more than 10% per year. Nearly a third of the seafood consumed in the world today is a product of farmed aquaculture (Timmons et al., 2002). Consumer demand for aquaculture products is increasing as many wild fisheries stocks have reached, or are very close to, their maximum sustainable limits. The U.S. catfish (*Ictalurus punctatus*) industry has grown by 100 million kilograms in the last 7 years. The Atlantic salmon (*Salmo salar*) industry has been adding 50 million kilograms of new production each year for the last 10 years. 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).

Intensive recirculating aquaculture systems reuse relatively small volumes of water by circulating the water through biofilters to remove toxic waste products before returning the water to production tanks (Rakocy et al., 2006). These systems allow production of fish at much higher levels than extensive pond culture with carrying capacities of 60 kg/m^3 vs. 0.6 kg/m^3, respectively (Losordo et al., 1998). Recirculating aquaculture is an environmentally responsible alternative to fishing and virtually eliminates by-catch waste which occurs in wild fisheries. However, water discharge/replacement requirements of 5% to 10 % of recirculating water volume per day makes these systems subject to discharge restrictions due to concerns with environmental waste management (Timmons et al., 2002). Concentrations of organic matter, inorganic nitrogen and phosphorus in the waste water may be high requiring in-system or post-discharge treatment of effluents (Gutierrez-Wing and Malone, 2006; Shnel, 2002).

Aquaponics is an integrated system that links hydroponic plant production with recirculating aquaculture (Diver, 2006). The advantages of linking fish and plant culture are

shared startup, operating, and infrastructure costs, fish tank waste nutrient and water removal by plants, reduced water usage and waste discharge to the environment, and increased profit potential by producing two cash crops (Rakocy, 1999; Timmons et al., 2002). 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).

Statement of the Problem, Rational and Significance

Water and nitrogen budgets for conventional field grown vegetable crops are often formulated with the knowledge that a portion of these inputs will be lost to the environment through leaching, runoff (Hochmuth and Hanlon, 1995), denitrification and/or volatilization (Cockx and Simonne, 2003). Movement of fertilizer inputs, especially nitrogen, and buildup of phosphorus in the environment, may adversely impact natural ecosystems and the water resources they depend on (Mitsch and Gosselink, 2000). As a result, farmers are under tremendous pressure to reduce or eliminate nutrient-laden water discharges to the environment.

Despite significant progress in reducing phosphorus discharges in the Lake Apopka basin (Neal et al., 1996), 6,070 hectares of land for vegetable production was purchased and taken out of production several years after passage by the Florida legislature of the Lake Apopka Restoration Act of 1996 (Tyson et al., 1996). Since this area traditionally multiple cropped radishes (*Raphanus sativus*), sweet corn (*Zea mays*), and cole crops (various crops in the *Cruciferae* or mustard family), this translated into an average loss of 14,164 hectares of vegetable production and $50 million in farm gate value per year. Other agricultural areas of Florida are under discharge restrictions, including the Everglades Agricultural Area (EAA) covering 279,239 hectares in south Florida. Similar phosphorus reduction efforts are underway in the EAA (EPA, 2003).

Harvested vegetable acreage in Florida declined by 29,907 hectares (21%) during the ten year period from 1994–95 to 2003–04 (Bronson, 2005) and a third of the loss can be attributed directly to government buyouts over concerns with nutrient discharges to the environment. Designing and managing agricultural production systems for minimal discharge of water and nutrients to the environment such as aquaponics, protects groundwater quality, makes water permitting easier to obtain, and may help maintain the long term sustainability of agricultural enterprises. These designs will also reduce concerns about discharge of nutrients into coastal zones that could contribute to reef die-off and harmful algal blooms.

Additional land loss of agricultural production can be attributed to urban sprawl. Urban expansion in the United States claimed more than 400,000 hectares of cultivated lands per year between 1960 and 1990 (Heimlich and Anderson, 2001). Farms in metropolitan areas make up 33 % of all farms, 16 % of cropland, and produce a third of the value of U.S. agricultural output. Over 75 % of the land area of Florida is classified as either metropolitan core or metropolitan edge. The highest rates of population growth occur at the edges of metropolitan areas in predominantly rural counties. In order for farmers to adapt to rising land values and new residents, they need to change operations to emphasize higher value products, more diversification, intensive production on less land, and an urban marketing orientation (Heimlich and Anderson, 2001). Advances in technology, limitations in water quality and quantity, environmental regulations, and increasing input costs are driving the aquaculture industry towards similar more intensively managed systems (Fitzimmons, 2003; Guitierrez-Wing and Malone, 2006).

Hydroponics and recirculating aquaculture are intensive production systems that produce high value agricultural products. More diversification, conservation of resources, and total yield

increases are possible when these systems are integrated into aquaponic production systems. Developing aquaponic systems for minimal discharge of nutrients and water to the environment would allow sustainable agricultural production in and around metropolitan areas. However, this technology is new, and adoption is limited by a lack of basic production information (McMurtry et al., 1997).

Combining hydroponic and aquaculture systems requires reconciling water quality parameters for the survival and growth of plants, fish, and nitrifying bacteria. However, there are many unanswered questions regarding the optimum water quality parameters when the organisms present in aquaponics are grown together. In particular, a dichotomy exists between the optimum pH for plant nutrient availability in hydroponics (pH 5.5–6.5; Hochmuth, 2001a) and the optimum pH for nitrifying bacteria activity (7.5–9.0; Hochheimer and Wheaton, 1998). Aquaculture production is recommended to be maintained between pH 6.5 and 8.5 (Timmons et al., 2002). Aquaponics has the potential to be a sustainable zero agricultural discharge system (ZADS) since the waste by-products of aquaculture can be used by plants in hydroponic systems (Tyson, 2004). However, with all its promise as a sustainable alternative to conventional food production, there is limited information on how aquaponic system water quality impacts nitrification in perlite growth medium, little information on the plant/ nitrification interactions in root growth media biofilters, and how this interaction affects ammonia biofiltration and plant yield. In addition, it would be beneficial to add fertilizer nutrients to aquaponic production system water to optimize plant nutrient levels provided this does not adversely impact the fish and nitrifying bacteria but more science based information is needed before recommendations can be made. Information is lacking on the relative contribution of nitrifying bacteria and plants to system water ammonia biofiltration and the relative importance each has in the overall system

performance and economic yield. The reconciling pH for ammonia biofiltration in aquaponic systems will be determined by the amount of ammonia removed from system water by plants and nitrifying bacteria and the effect of pH on vegetable fruit yields.

Adoption of properly designed aquaponic systems will conserve our natural resources; water and the fossil fuels required to produce nitrogen and other plant nutrients. However, clear scientifically based recommendations on system management are required to remove information barriers to adoption of this technology. The overall goal of this research was to 1) determine the optimum pH for nitrification and evaluate performance of perlite as a biofilter/root growth medium, 2) determine the affect of hydroponic nutrients on nitrification, 3) make predictions about the contribution of plants and nitrifiers to ammonia biofiltration, and 4) establish a reconciling pH for ammonia biofiltration and cucumber yield in aquaponics.

CHAPTER 2
REVIEW OF LITERATURE

Introduction

Aquaponics is an integrated system that links hydroponic plant production with recirculating aquaculture (Diver, 2006; Timmons et al., 2002). The advantages of linking plant and fish culture include fish tank waste nutrient and water removal by plants, reduced water usage and waste discharge to the environment by both systems, and increased profit potential by producing two cash crops (Rakocy, 1997; Rakocy et al., 2006; Timmons, et al., 2002). The most common 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, 2006; McMurtry et al., 1997; Rakocy et al., 1997, 2006; Watten and Busch, 1984) for the plant growing area integrated with a recirculating aquaculture tank system (Timmons et al., 2002).

Aquaponic system integration is hampered by the lack of information on a reconciling pH between the optimum pH for hydroponic plant production (5.5–6.5; Hochmuth, 2001a) and for rapid ammonia biofiltration of system water (7.5–9.0; Hochheimer and Wheaton, 1998). Recommended pH levels for aquaculture production are between 6.5 and 8.5 (Timmons et al., 2002). There is little information on the use of hydroponic media as biofilters of ammonia, especially newer, more commonly used media, such as perlite. In addition, it would be beneficial to add fertilizer nutrients to aquaponic production system water to optimize plant nutrient levels provided this does not adversely impact fish and nitrifying bacteria. A more flexible management strategy for these systems would be to supplement with plant nutrients, which would permit less reliance on the fish and nitrification to provide optimal plant nutrient levels. Information is also lacking on the relative contribution of nitrifying bacteria and plants to system water ammonia biofiltration and the importance each has in the overall system

performance. This will need to be determined to establish a reconciling pH for aquaponic system water.

This review will address the importance of pH and water quality in successful aquaponic production especially as they affect ammonia biofiltration and cucumber production. The review will identify the limits and dangers associated with managing pH and water quality with respect to the organisms present in the system – especially nitrifying bacteria and plants. It will emphasize nitrification, the biochemical reaction that converts NH_3 to NO_3^- and ammonia biofiltration (nitrification + plant removal of TAN). It will discuss the hydroponic subsystem, especially systems utilizing perlite medium, which may be used as biofilters for aquaponic systems. Further, the review will discuss limiting factors in plant nutrition and how aquaponic systems may be used to overcome these limits in order to improve system integration and sustainability.

The Dichotomy of pH in Aquaponics

The concept of pH was developed by a Danish biochemist named Soren Sorenson in 1909 to simplify working with solution hydrogen ion concentrations (Myers 2003). The pH of a solution is defined as the negative logarithm (base 10) of the hydrogen ion concentration (Campbell and Reese, 2002). A neutral pH in water based solutions has a pH of 7 ($-\log 10^{-7} = -(-7) = 7$) and occurs when an equal number of hydrogen (H^+) and hydroxyl (OH^-) ions are present. When the hydrogen ion concentration increases ($> 10^{-7}$ mol $\cdot$ L^{-1}) from neutrality, the pH decreases and the solution is termed acidic. When the hydrogen ion concentration decreases ($< 10^{-7}$ mol$\cdot L^{-1}$) from neutrality, the pH increases and the solution is termed alkaline. The pH controls a wide variety of solubility, oxidation–reduction and equilibrium reactions in hydroponics and aquaculture systems (Timmons et al., 2002; De Rijck and Schrevens, 1999).

Integrating hydroponics and recirculating aquaculture is hampered by differences in optimal pH recommendations for the nutrient solution (pH 5.5–6.5) which baths the plant root system (Hochmuth, 2001a) and the recirculating water (pH 7.0–9.0) of the fish production tanks (Hochheimer and Wheaton, 1998; Timmons et al., 2002). The former is recommended to maximize the availability of nutrients in solution for uptake by plants. The latter is determined by pH ranges conducive for production of fish and the pH range of more optimal activity of nitrifying bacteria in removing ammonia waste from recirculating waters. Recommendations on pH in plant and fish production are well established. However, scientific information is lacking in regards to pH in integrated hydroponic and recirculating aquaculture systems, especially as it relates to the biofiltration of ammonia by bacteria and plants.

Recommended pH ranges for the nutrient solution irrigation water in greenhouse hydroponic production tends to be slightly acidic (5.5–6.0, Hochmuth, 2001b; 5.5–6.5, Hochmuth, 2001a; 5.8–6.4, Resh, 2004) to avoid precipitation of Fe^{2+}, Mn^{2+}, PO_4^{3-}, Ca^{2+} and Mg^{2+} to insoluble and unavailable salts when pH > 7. Aquaponic recirculating water pH is recommended to be maintained in the range of 7.0 to 7.5 (Timmons et al., 2002) to balance the requirements of biofiltration of toxic fish waste ammonia from system water with the nutritional needs of plants. If aquaponic recirculating water pH is maintained at levels more optimum for nitrifying bacteria (7.5–9.0; Hochheimer and Wheaton, 1998), plant uptake of certain nutrients could become restricted and plant yield reduced. Reconciling pH optima for production of plants (5.5–6.5) and the growth of nitrifying bacteria (7.5–9.0) in aquaponic systems would significantly improve systems integration and sustainability. Integration in this context refers to systems that are connected or combined to function together as one unit, each dependant on the other in some way. Sustainability refers to the capacity of the combined units to use self

sustaining resources inherent to the systems in such a way that the resources are not depleted or the system harmed.

pH Affects Nitrification Activity

One of the most complex and important subsystems of recirculating aquaculture systems is the biofiltration and removal of fish waste ammonia through nitrification to maintain fish tank water quality (Gutierrez-Wing and Malone, 2006; Masser, et al., 1999). Nitrification is a biological process performed by nitrifying bacteria that maintains water quality in recirculating aquaculture systems and has been shown to transform 93%–96% of potentially toxic nitrogenous fish wastes (NH_3 -N) into relatively non-toxic NO_3^--N in biofiltration units (Prinsloo et al., 1999).

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*.

$NH_3 + 1\ ½\ O_2 \leftrightarrow NO_2^- + H_2O + H^+ + 84$ kcal mol^{-1} (Equation 2-1)

Primarily *Nitrobacter sp*.

$NO_2^- + ½\ O_2 \leftrightarrow NO_3^- + 17.8$ kcal mol^{-1} (Equation 2-2)

This nitrogen transformation eliminates ammonia from the water. Un-ionized ammonia nitrogen is toxic to fish at levels above 0.05 mg/L (Francis-Floyd and Watson, 1996) and is dependant on pH and temperature of culture water. Nitrate, the end product of nitrification, is not toxic to fish except at very high levels (channel catfish, *Ictalurus punctatus*, 96-h LC50 > 6,200 mg/L NO_3-N; Colt and Tchobanoglous, 1976), although some investigations suggest that prolonged exposure to 200 mg/L NO_3-N might decrease the immune response of some fish species (Hrubec et al., 1996). Nitrate is the primary source of N for plants in hydroponic nutrient solutions at concentrations from 50 to 280 mg/L NO_3 -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.

The pH is one of the most important environmental parameters that can affect the activity of nitrifying bacteria (Antoniou et al., 1990). A wide range of pH optima have been reported from research on the effect of pH on the process of nitrification. 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 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). In wastewater treatment processes, the pH of approximately 7.8 (Antoniou et al., 1990) produced the maximum growth rate of nitrifying bacteria and 8.4 (Peng et al., 2003) the greatest nitrification rate.

No scientifically based optimum pH has been reported for aquaponic systems. The causes of varying pH optima may be attributed to differences in substrate, alkalinity, effluent, or species of nitrifying bacteria present in the system. The literature would support maintaining recirculating water pH between 7.5 and 8.5 for maximum waste ammonia biofiltration within the range of plant and fish production recommendations (pH 5.5–8.5) if this were the only consideration for determining pH. However, this pH range affects water quality parameters which may adversely impact plant production.

pH Determines Ammonia Equilibrium

In water, ammonia exists in two forms, which together are called the Total Ammonium Nitrogen (Francis-Floyd and Watson, 1996) or TAN. The equilibrium reaction is (Campbell and Reese, 2002): $NH_4^+ \leftrightarrow NH_3 + H^+$. Water temperature and pH will affect which form of ammonia is predominate (Table 2-1). To calculate the amount of unionized ammonia present, the TAN (TAN = NH_4^+-N + NH_3 -N) of a water sample must be multiplied by the factor (using the pH and temperature of that sample water) selected from Table 2-1. For example, at 28ºC, the percent of NH_3 increases by nearly a factor of 10 for each 1.0 increase in pH and is 0.2%, 2% and 18% of the TAN for pH 6.5, 7.5, and 8.5, respectively.

Un-ionized ammonia nitrogen (NH_3 – N) at concentrations as low as 0.02–0.07 mg/L have been shown to slow fish growth and cause tissue damage (Masser et al., 1999). The 96-h LC50 for un-ionized ammonia on fingerling channel catfish (*Ictalurus punctatus*) was 3.8 mg/L (Colt and Tchobanoglous, 1976). The 96–hour LC50's vary widely by fish species from as low as 0.08 mg/L NH_3 -N for pink salmon to 2.2 mg/L NH_3 -N for common carp (Timmons et al., 2002). The 72 h LC_{50} of NH_3 for tilapia *Oreochromis aurea* has been reported at 2.35 mg/L (Redner and Stickney, 1979; as referenced in Lim and Webster, 2006; original document not found). Safe pH ranges will depend largely on the species grown. Thus 5 mg/L TAN at pH 7 would be safe for pink salmon and common carp; but the same TAN at pH 8 would kill pink salmon, but not common carp. Within the range of pH 7 to 8, species sensitive to unionized ammonia should be grown closer to pH 7 to avoid damage.

pH and Nitrite Accumulation

The intermediate product of nitrification, nitrite (NO_2^-), may be toxic to both fish and plants at low levels. Nitrite at concentrations as low as 5 mg/L in nutrient solution damaged tobacco (*Nicotiana tabacum* L.) root tips (Hamilton and Lowe, 1981). Nitrite oxidation activity

was suppressed by elevated pH and ammonium concentrations when urea was used in a hydroponic tobacco float system resulting in the accumulation of toxic levels (30-70 mg/L) of nitrite (Pearce et al., 1998). Gila trout (*Oncorhynchus gilae*) exposed to nitrite at 10 mg/L or more for 96 h died (Fuller et al., 2003) and the 96 h LC50 for bass (*Morone sp*.) was 12.8 mg/L (Weirich et al., 1993). Nitrite is toxic to fish because it affects the blood hemoglobin's ability to carry oxygen (Timmons et al., 2002) and is called "brown blood disease."

Nitrite accumulation can occur due to the faster growth of ammonium oxidizers than nitrite oxidizers (most common in startup biofilter cycles) or due to the inhibition of *Nitrobacter* by free ammonia (Villaverde et al., 1997), which is more common under steady state conditions and was reported to start at pH above 7.5 and increasing asymptotically to 85% at pH 8.5. To avoid damage to plants and fish from nitrite accumulation under steady state conditions, a pH at or below 7.5 would seem to be an appropriate recommendation.

Plant Nutrient Considerations and pH

Plants require 17 elements (Table 2-2) for normal growth and development (Marschner, 2003). Carbon (C), hydrogen (H), and oxygen (O), required in the largest amounts, are supplied by air and water. The others come from soil and/or fertilizer: nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), sulfur (S), calcium (Ca), iron (Fe), copper (Cu), manganese (Mn), boron (B), molybdenum (Mo), zinc (Zn), chlorine (Cl) and nickel (Ni). Since hydroponic systems do not use soil, care must be taken to insure that all the essential elements necessary for plant growth are supplied to the system in some fashion, usually by adding water soluble fertilizer. The recommended amounts of major elements such as nitrogen and potassium vary depending on crop species, especially in regard to specific crops in hydroponic culture where nutrition can be managed (Hochmuth, 2001a; Hochmuth and Hochmuth, 2003). Cl and Ni are required in such small amounts and are available in the environment (soil or water) so that it is

usually not necessary to supply them in fertilizer applications. Some elements, although not required, are beneficial to the plant. Silicone, for example, is needed for stem support and gives resistance to fungal infection, especially in cucumber (Resh, 2004). Sodium, cobalt, selenium, and aluminum have also been found to be beneficial for certain plants and circumstances (Marschner, 2003).

pH affects the solubility of ions in solution and the ionic form of several elements (Epstein and Bloom, 2005; De Rijck and Schrevens, 1999). Acidity increases the solubility of sulfates and phosphates (Taiz and Zeiger, 2002). Increasing ion solubility facilitates their availability to roots. Rhizosphere pH may differ from bulk soil pH by up to two pH units (Marschner, 2003), because of the release from plant roots of H^+, OH^-, or HCO_3^- and the excretion of organic acids. Root uptake of ions is an electrically neutral process. Thus root induced changes in pH are the result of imbalances in the cation/anion uptake ratio resulting in net differences in the release of H^+, OH^-, or HCO_3^- by roots.

Recommended pH ranges for hydroponic production systems tend to be slightly acidic (5.5 to 6.5, Hochmuth, 2001a; 5.8 to 6.4, Resh, 2004) in order to provide a greater relative availability of most nutrients in solution. Precipitation of Fe^{2+}, Mn^{2+}, PO_4^{3-}, Ca^{2+} and Mg^{2+} to insoluble and unavailable salts can occur in nutrient solution culture at water pH levels above 7. Phosphorus deficiency causing yield reduction in hydroponic tomatoes (Wallihan et al., 1977) and iron deficiency with dry matter yield reduction in sorghum (*Sorghum bicolor*) grown in solution culture (Bernardo et al., 1984) occurred when pH levels were above 7. If aquaponic recirculating water pH is maintained at levels more optimum for nitrifying bacteria (7.5–9.0; Hochheimer and Wheaton, 1998), plant uptake of certain nutrients may become restricted and thus plant yield may be reduced.

It may be possible to overcome nutrient deficiency and increase crop yield under system water pH production conditions > 7.0 with the use of foliar application of certain plant nutrients. Foliar applications of Mg, Zn, and Mn fertilizers can effectively correct deficiencies of these nutrients in fruit and vegetable crops grown on calcareous soils (usually pH 7.4–8.4) in south Florida (Li, 2001). Overall yield increases of 33% occurred when strawberry cultivars were sprayed once per week with Fe when grown in calcareous (pH 8.2) soil (Zaiter and Saad, 1993).

Balancing Aquaponic System Water pH

Where does the balance in pH lie for aquaponic system water? Is it weighted toward the plant component, the fish component, or the biofilter housing the nitrifying bacteria? How much do plants contribute to the biofiltration of ammonia and is that relevant to the overall system operation? If the plant contribution to ammonia biofiltration is significant then are the nitrifying bacteria and their special water quality requirements needed? Since unionized ammonia and nitrite are toxic to fish, and both increase with increasing pH, then what is the tipping point—the pH that the system should not go above? The adoption of aquaponics is hampered by a lack of scientifically based answers to some of these questions and the need to make sense of these dichotomies in pH. When this is accomplished, reasonable and understandable recommendations for system operation will make system adoption easier and more efficient.

Ammonia Biofiltration

Aquaculture Biofilters

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). Solids removal is accomplished when recirculating water passes through a material that intercepts suspended particles. A biofilter is simply a surface on which bacteria grow. Biological filtration can take place anywhere in the system where recirculating water comes in

contact with a surface to which nitrifying bacteria are attached–this may include tank walls, interior surfaces of pipes and even plant roots. However, to provide sufficient biofiltration activity to maintain optimum water quality in intensive recirculating aquaculture, where (TAN) loading can be high, separate biofilter equipment is currently required.

Biofilters used in recirculating aquaculture are of two main types: fixed film (attached growth) and suspended growth where microorganisms are maintained in suspension (Gutierrez-Wing and Malone, 2006). Suspended growth systems are not common because of their high level of management and reputation for instability. Thus, most of the biofiltration in recirculating systems are aerobic, fixed film biofilters. These biofilters basically consist of a porous solid phase on which nitrifying bacteria grow and extract nutrients from water passing over this solid phase (Wheaton, 1993). Water may enter the biofilter from the top, side or bottom and exit from the bottom, side or top, depending on design location relative to the water level of the fish tank. There are four basic types of biofilter designs: submerged bed, rotating disc, fluidized bed, and trickling (Tetzlaff and Heidinger, 1990). Submerged bed biofilters are characterized by tank water being pumped through a medium that is constantly underwater. The rotating disc biofilter consists of a series of parallel circular plates mounted on a shaft and rotating as a round drum with the lower part submerged and the upper part above water. The plates are the substrate the bacteria grow on. In a fluidized bed biofilter, water enters the bottom of a medium containing cylinder under high pressure and exits out the top after being acted on by the filter. In trickling filters water enters the top and flows down through the medium and keeps the bacteria wet but never completely submerged. Trickling filters are maintained above the water level of the fish tank.

Of all the water quality parameters which affect fish, ammonia is the most important after oxygen (Francis-Floyd and Watson, 1996). Ammonia is the main excretion product from fish and uneaten feed. It can quickly become a concern because of the buildup of un-ionized ammonia and nitrite, both of which can be toxic to fish at very low levels (Harmon, 2001; McGee and Cichra, 2000) as discussed previously. Ammonia is usually not a problem if the biological filters are properly sized for the loading rate and carrying capacity and if adequate water flow is maintained (Fowler, et al., 1994). Hockheimer and Wheaton (1998) recommend that the system water move through the biological filter at least 2–3 times per hour. However, Rakocy et al. (1997) were unable to detect any difference in tilapia growth rate, total weight, or survival between water exchange rates of 0.55 and 1.25 times per hour. Perhaps both of these flow rates were too low to detect a difference. McGee and Cichra (1999) recommend a 3:1 fish tank to biofilter volume ratio as being a more than sufficient design for biofilters.

The ammonia generation load is based on the fish feeding rate and could be assumed to be 10% of the protein in the feed becomes the ammonia-N generation rate (Timmons et al., 2002). The size of the biofilter depends on the amount of ammonia added to the system which is closely related to the feeding rate and efficiency of food utilization (Tetzlaff and Heidinger, 1990). Van Gorder (2000) indicated that feed levels change with fish size as fingerlings consume a much higher percentage of their body weight (5%–8%) than harvestable size fish (0.75%–3%). Another way to determine ammonia – N load is to consider that generally 2.2 to 6.6 kg of ammonia are produced for each 220 kg of feed. Thus, 220 kg of fish being fed 6.6 kg of fish feed per day (3% of body wt/d) produce 0.1 kg of ammonia per day. Chapman (2000) puts these feed levels at 6 to 15% of body weight for young fish (<25 g) and 1% to 3% of body weight for older fish (>25 g).

Trickling biofilters provide nitrification, aeration, and some carbon dioxide removal in one unit (Losordo, et al., 1999). The main disadvantage of trickling filters is that they are relatively large and biofilter media are expensive. The quantity of bacteria available to oxidize ammonia is limited by the surface area of the biofilter medium thus an important factor in biofilter design is to get the maximum amount of surface area into a given volume (Harmon, 2001). However, when particle size is reduced, filter clogging may increase and the ability of oxygenated water to mix well within the filter decreases. Clogging of the medium may occur if the solids are not pre-filtered. Volumetric nitrification rates of about 90 g total ammonia nitrogen (TAN)/m^3 per day can be expected with trickling filters (Losordo et al., 1999). When designing these filters into a recirculating system for nitrification (assuming 2.5 percent of the feed becomes TAN), a design criteria of 3.6 kg feed/day/m^3 of trickling medium should be used. Based on these numbers, and a feeding rate of 3% of fish body weight per day, 1 m^3 of trickling medium biofilter should support 120 kg of fish. With a carrying capacity at harvest of 60kg/m^3, 1 m^3 of trickling medium biofilter would be required for every 2000 L of fish tank water, a 1 to 2 ratio. 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.

Research on freshwater recirculating aquaculture biofilters should focus on cost competitiveness, low head and low energy use operation in support of large scale facilities (Gutierrez-Wing and Malone, 2006). The efficiency of biofilters need not be associated with use of expensive commercial biofiltration devices (Prinsloo, et al., 1999). When two types of trickling filters were compared, one containing PVC shavings (surface contact area of 1,220 m^2), the other a more sophisticated commercially available biofilter made up of Siporax porous

sintered glass cylinders (surface water contact area of 32,000 m^2), the PVC biofilter was more efficient at breaking down nitrogenous wastes (efficiency 96% and 93%, respectively). Efficiency of biofilter media consisting of hydroponic media such as perlite has not been scientifically established. This should be accomplished in order to more effectively integrate sustainable hydroponic and aquaculture systems.

Plants as Biofilters

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 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. Aquaponic wastewater cleanup cost abatement alone can be a major factor in integrating hydroponic and aquaculture systems (Adler, 2001; Adler, et al., 2000).

Plant uptake was insufficient to remove contaminants (Prinsloo et al., 1999) in one aquaponic trial due to a high ratio of fish to plants. Rakocy et al. (1997) were able to establish a balanced system by maintaining a large plant growing area relative to fish production area in a commercial scale aquaponics system. Rakocy (1999) indicated that sufficient nitrification occurs in lettuce floating raft systems when correct ratios of fish feed to plant growing area are maintained. In a tilapia/floating romaine lettuce aquaponic system, each square meter of hydroponic growing area removed 0.56 g of ammonia-nitrogen, 0.62 g of nitrite-nitrogen, 0.83 g of total nitrogen, and 0.17 g of total phosphorus per day.

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 a possibility for less reliance on nitrification in aquaculture biofilters for ammonia removal when sufficient plants are present in aquaponic systems. The optimum ratio of nitrate to ammonium nitrogen in hydroponic nutrient solutions is 75:25 (Cockx and Simonne, 2003; Simonne et al., 1992). Consequently, a source of nitrate-nitrogen would be needed for plant uptake in aquaponics either through nitrification or supplemental fertilization for optimum plant growth. In addition, certain plant nutrients can fall below sufficiency standards in aquaponics (McMurtry et al., 1990; Rakocy et al., 1997; Seawright et al., 1998) without supplemental fertilization. Thus methods to make up this deficit without adversely impacting fish and nitrifying bacteria need further investigation.

Hydroponic Systems and Media

Hydroponics is the term used to describe the production of plants without soil. Plant roots grow in a nutrient solution with or without an artificial medium for mechanical support (Jensen, 1997). Most fruits and vegetables are grown in field soil. Soil serves two basic purposes: it acts as a reservoir for essential elements and water and it provides physical support for the plant (Johnson et al., 1985). Soilless culture (hydroponics) is an artificial means of providing plants with support and a reservoir for nutrients and water. The growing medium can be perlite, vermiculite, rockwool, peatmoss, coir, composted pine bark, sawdust, sand or gravel. Water only systems such as the nutrient flow technique and the floating raft system utilize artificial means of support for the plant. Many hydroponic systems have been developed and the technology is rapidly changing (Resh, 2004, Tyson, et al., 1999, 2001), but they have only been used commercially for the last 50 years. Most systems are housed inside a greenhouse (considered Controlled Environment Agriculture or CES) but they can be used outdoors.

The most common hydroponic systems in Florida today use some form of perlite medium which provides anchorage for the plant roots (Tyson, 2002). A nutrient solution is pumped through the medium and mineral elements are taken up by the roots. Other systems in use include the nutrient flow technique (NFT) where the nutrient solution trickles down a plastic or PVC trough, rockwool culture where slabs of fibrous rockwool anchor plants as nutrient solution drips through them, and floating hydroponics, where a raft containing plants floats in a nutrient solution. Commercial production of leafy salad crops in greenhouses containing floating raft systems have been used in Florida and Canada since the 1980's (Resh, 2004, Spillane, 2001).

Perlite is a generic term for naturally occurring volcanic glass or rock (Reed, 1996). When this material is heated, it pops like popcorn and expands from four to twenty times its original volume. The expansion process creates a white angular pearl like pebble that is light weight (32 kg/m^3) and adaptable for numerous applications such as low to high temperature and acoustic insulation, as fillers, as adsorption carriers, and light weight aggregate construction, among other uses (Anonymous, 2007). Table 2-3 describes a typical elemental analysis and physical properties of perlite. It also contains fluoride (17 mg/L, Reed, 1996). Certain ornamental plants such as dracaena (*Dracaena marginata*) have been shown to be sensitive to fluoride. Perlite has a high water holding capacity and high aeration properties (Hochmuth and Hochmuth, 2003). Medium to course grade horticultural perlite is recommended for use in hydroponic vegetable production. Perlite has become the most commonly used plant growing medium in the Florida greenhouse vegetable industry (Tyson, 2002, Tyson et al., 2001). Perlite has also been used successfully as a filter to remove gaseous ammonia and other waste gases (Flanagan, et al., 2002; Joshi, et al., 2000; Wright and Raper, 1998).

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, 2006; McMurtry et al., 1997; Rakocy et al., 2006, 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 and pepper (*Capsicum annuum* L) production (Hochmuth et al., 2002). The type of soilless media in which plants grow has been shown to significantly affect nitrifying bacteria counts (Lang and Elliott, 1997). However, perlite medium has not been investigated with respect to the activity of nitrifying bacteria in an aquaponic biofilter.

Overcoming Limiting Factors in Plant Nutrition

Yields of most crops in the United States have increased an average of 3% per year for the past 30 years (Wallace and Wallace, 1993). This has been accomplished in part by breeding and in part by improving the efficiency of inputs and overcoming limiting production factors such as plant nutrient and water stress. The production and use of fertilizers has played a large role in this increase in productivity. For example, a doubling of agricultural food production over the last 35 years was accompanied by a 7-fold increase in nitrogen fertilization (Tillman, 1999).

Nitrogen is an essential nutrient element and the fertilizer nutrient required in largest amounts by plants. It can accumulate to levels up to 5% of plant dry matter (Marschner, 2003). Nitrogen is an essential constituent of proteins and amino acids. It is assimilated into plants primarily in the form of nitrate (NO_3^-) and ammonium (NH_4^+). The manufacture of nitrogen fertilizer is expensive, requiring large amounts of non-renewable energy resources. In addition,

the uptake and assimilation of nitrogen in the plant is energy consuming for the plant, requiring 15 moles of ATP for the reduction of one mole of nitrate and 5 moles of ATP for the assimilation of one mole of ammonia.

Fluctuating nutrient environments require that organisms continually adapt with specific responses to either cope with nutrient limitations or adjust to oversupply (Grossman and Takahashi, 2001). Nitrogen limitation can influence the physiology and morphology of plants reducing cell division rates and metabolic activities. For example, a feedback control of photosynthesis is related to the carbon to nitrogen balance (Paul and Pellny, 2002). An adequate supply of building materials (C-skeletons) and energy is necessary to carry out many plant functions.

Recent developments in addressing the problem of limiting nutrients focus on manipulating gene expression to improve yield by improving absorption and nutrient efficiency (Good et al., 2004; Grotz and Guerinot, 2002). However, for the more traditional horticulturist, there may be ways of improving nutrient efficiency in the short run by manipulating production system design. In an integrated hydroponic and aquaculture system, nitrogen in the nutrient solution was reduced 3.5 times compared to traditional solution concentrations to produce lettuce in a 2.5 year continuous multiple cropping pilot project (Rakocy et al., 1997). It was suggested that this was the result of the nutrient solution constantly bathing the plants roots compared to intermittent applications, which may be the case in more traditional hydroponic applications. This was also suggested by much earlier work (Olson, 1950).

Olson (1950) was able to establish that nutrients were absorbed at a constant rate regardless of concentration, as long as the overall proportion and concentration of nutrients in solution remained nearly the same, and that the nutrient solution was thoroughly mixed. More

recent work (Schon and Compton, 1997b) illustrates the importance of irrigation frequency on the effects of nutrient solution concentration.

Irrigation frequency for greenhouse hydroponic cucumbers is usually determined by the use of the weighing lysimeter system (Schon and Compton, 1997b). The length of each irrigation event is determined by the amount of time needed to obtain some leaching fraction (LF) that can range from 15% to 40% of the applied irrigation (Schon and Compton, 1997a). The LF is defined as the volume of nutrient solution leached, divided by the total volume of solution delivered. This leaching process ensures the replenishment of nutrients in the media to recommended levels. The frequency of the irrigation event will be determined by the loss of weight (due to water uptake) of the bag, pot, or slab containing the growing media. When the water loss from the substrate reaches a level that may result in loss of plant turgor, irrigation is initiated. In this way the plant receives sufficient water to prevent deficit stress. Under this regime, with a 20% LF in perlite media pots, irrigation frequency increased from twice per day (for 3 min. each) early in the growth season, to 10 times daily (for 10 min. each) during the cucumber harvest season (Chaverria et al., 2005).

However, despite regimes of commercially recognized, commonly used irrigation frequencies described above for greenhouse cucumbers, nitrate-N concentrations in rockwool media slabs can drop below the depletion level (<10 mg/L N) just prior to harvest (Schon and Compton, 1997b). This N depletion from substrate occurred even when the nutrient solution concentrations ranged from 90 to 175 mg/L N; this low level can reduce crop yields. Increased N concentrations were recommended (between 225 and 275 mg/L N) for the nutrient solution so that N did not drop below depletion level in the substrate between irrigation events.

In soils, nutrients move to the surface of roots by diffusion and bulk flow of the soil solution resulting from transpiration (Taiz and Zeiger, 2002). Concentration gradients can form in the soil solution as nutrients are taken up by the roots and the concentration of nutrients at the root surface is lowered compared to the surrounding area. This can result in a nutrient depletion zone near the root surface. The capacity for continuous growth by roots however, extends this region of nutrient uptake beyond the depletion zone. Thus, optimum nutrient acquisition by plants depends on the capacity of their root systems not only to absorb nutrients, but also to grow into fresh soil.

In hydroponic production, the media volume is finite and nutrient depletion can be recovered only in the next irrigation event. The results described above, from Schon and Compton (1997b), indicate that N depletion does occur at lower N nutrient solution concentrations, and that irrigation frequencies adequate to prevent water stress are not necessarily adequate to prevent nutrient depletion except at high N nutrient solution concentrations. Therefore, it seems logical to propose that more frequent flushing of the media with lower concentrations of N would obviate N depletion between irrigation events. If this flushing was continuous, there would be no appreciable depletion of nutrients in the root zone. This reasoning could be applied to all nutrients in the nutrient solution and may provide an avenue for production of plants at pH levels > 7.0. The constant recirculation of the nutrient solution across the root zone would obviate low nutrient concentrations of ions with potential for precipitation (Fe^{2+}, Mn^{2+}, PO_4^{3-}, Ca^{2+} and Mg^{2+}) to insoluble and unavailable salts at pH levels > 7.0.

These data suggest that optimum plant yields may be maintained when lower nutrient solution concentrations are constantly provided to the root system. Further investigation is

needed to establish the reasons why this occurs. This reduction in nutrient solution concentration requirement may be due to plant conservation of resources since the constant water and nutrient supply negates the need for the production of exploratory roots or it may be due to the increased turgor pressure (pushing growth) associated with a constant supply of water to the transpiration stream, or both. In addition, the relationship between nitrogen, sucrose, and carbon supply, and the matrix of processes mediating plant growth that utilize those resources may be simplified, when a constant – sufficient and not excessive or restrictive - supply of N and other nutrients are available to the plant. Since N is the major mineral nutrient assimilated by plants, considerable savings could be obtained by changing practices to insure optimum yield with reduced N input. The end result should be reduced nutrient solution concentrations required for optimum yield and significant saving to growers, consumers, and society.

Aquaponics: the Potential for Sustainability

Aquaponics fits closely into the definition of sustainable agriculture as defined by the 1990 Farm Bill, Title XVI, Subtitle A, Sec. 1603. Aquaponics is 1) "an integrated system of plant and animal production practices" using vegetables with aquaculture species, 2) "having a site-specific application" in greenhouse production units. It will 3) "over the long term satisfy human food needs" and "enhance environmental quality" by producing crops using environmentally friendly practices that minimize water and nutrient waste discharges to the environment. Aquaponics will 4) "make the most use of nonrenewable resources" by conserving nitrogen fertilizer, produced from non-renewable fuels, and water. It will 5) "integrate natural biological cycles" by using nitrifying bacteria in the process of nitrification to convert harmful ammonia fish waste to usable, safe, nitrate nitrogen for plants. Aquaponics will 6) "sustain the economic viability of farm operations" and "enhance the quality of life for farmers…and society as a

whole" by producing food in a sustainable agricultural production method and in an environmentally bio-rational manner without wasteful discharge to the environment.

In hydroponic systems, nutrients are precisely controlled and spoon fed into the plant growing area as needed and recommended from research trials. Optimum yields are obtained by adjusting nutrient amounts depending on the crop variety and stage of growth of the plant. In aquaponic systems, most nutrients are part of the aquaculture waste stream and dependent on daily feed amounts based on fish weight and density. However, the nutrients available in wastewater alone are insufficient to maintain maximum plant productivity (Rakocy et al., 1997; Seawright et al., 1998) which is an important factor when producing in expensive greenhouse facilities. More research into plant nutrient management in aquaponics is needed before widespread system adoption can be realized. There is no single ratio of plant biomass to fish biomass that results in equilibrium concentrations of most of the essential plant nutrients (Seawright et al., 1998). Thus optimum plant yield in aquaponics requires nutrient management and supplementation. However, aquaponics does significantly reduce the need for fertilizer inputs, especially applied nitrogen (Rakocy et al., 1997). Nitrogen is an essential nutrient element and the fertilizer nutrient required in largest amounts by plants (Marschner, 2003). The manufacture of nitrogen fertilizer is expensive, requiring large amounts of non-renewable energy resources. Understanding and management of the nitrification process in aquaponics is critical for the maintenance of water quality, the production of nitrate nitrogen, and the management of nutrients for plant production in these integrated systems.

Water and Nitrogen Budgets

Designing agricultural production systems for minimal discharge of water and nutrients to the environment protects groundwater quality and makes water permitting easier to obtain. It also would protect coastal waters from harmful algal blooms. A zero-discharge tilapia

recirculating system has been successfully evaluated (Shnel, 2002) and used a combination of a trickling biofilter, fluidized bed reactor, and a sedimentation/digestion basin to maintain system water quality during a 331-day grow-out period. The ideal scenario would be a zero agricultural discharge system (ZADS) where inputs become a harvestable product with no discharge to the environment. One potential ZADS production arrangement combines hydroponic plant production and recirculating aquaculture systems into what is known as aquaponics. The potential for plants to use the by-products of aquaculture and keep recirculating water clean have been documented (Adler, 1996; Adler et al., 2000; Lin et al., 2002).

Two major components of both hydroponic and aquaculture systems are water and nitrogen. Most recirculating aquaculture systems replace 5% to 10% of system water daily to prevent the buildup of toxic levels of ammonia and other fish by-products and provide makeup water for evaporation and for backwashing filters (Masser et al., 1999). The irrigation requirement for a field grown watermelon crop in southwest Florida is about 4 liters of water per meter squared of growing area per day (Kovach, 1984). Greenhouse crops require as much as 1.9 liters of water per plant per day (Hochmuth, 2001a). Given recommended greenhouse plant densities (Marr, 1995), water use would be about 4.5 liters per meter squared per day. A single plant can use between 0.5 to 5 liters of water per day depending on its size, maturity, and the growing season or temperature. If we assume an average of 3 liters of water use per plant per day, 100 plants could satisfy the water replacement requirements of a recirculating aquaculture tank containing 3000 (at 10%) or 6,000 liters (at 5% replacement).

The main applied nutrient in plant production – nitrogen – could be supplied by fish in an aquaponic system (Rakocy et al.,1997). Sufficient nitrification to convert 75% of the ammonia to nitrate would be preferred since the recommended NO_3^- to NH_4^+ ratio in hydroponics is 75:25

(Cockx and Simonne, 2003; Simonne et al., 1992). One hundred kilograms of fish could produce an average of 90 g of ammonia nitrogen per day being fed at 3% body weight and 3% of feed becoming ammonia-N (Losordo et al., 1998). Nitrogen requirements of hydroponic vegetable plants can range from 1 to 20 g per plant per season (Hochmuth, personal communication, 2005) depending on the crop species (lettuce, cucumber, pepper or tomato – ranked low to high nitrogen requirement). A fish production rate of 90 g of N per day would support 4,050 lettuce plants (45 day crop and 1 g of N requirement per plant) or 1,080 cucumber plants (120 day crop and 10 g of N requirement) or 1,215 pepper or tomato plants (270 day crop and 20 g of N requirement).

Rakocy (1997) was able to obtain a 1.5% system volume daily water replacement with rain water as the sole source of water for a tilapia and lettuce aquaponic system. Also, potassium, calcium, or iron concentrations may fall below sufficiency standards in aquaponics without supplemental fertilization (McMurtry et al., 1990; Rakocy et al.,1997). Plant nutrient applications in aquaponics as well as the goal of zero discharge to the environment of water and nutrients need further investigation in order to improve systems integration and sustainability.

Further Systems Integration

The difficulty in finding a median environment between plant, fish, and nitrifying bacteria culture in aquaponics has resulted in less integration of the systems than would be ideal for maximizing space and infrastructure thus reducing the potential overall profitability of aquaponics. Most serious aquaponic trials to date actually use two systems, one hydroponic, one aquaculture, connected by pipes with water recirculating through both (Adler et al., 1996; Diver, 2006; Jones, 2001; Rakocy et al., 1997; Rakocy et al., 2006; Timmons et al., 2002). The addition of a hydroponic system does usually eliminate the need for a separate biofilter for the aquaculture component (Rakocy, 1999, Anonymous, 1997) since the plant growing area can

provide biofiltration. However, there are conflicting recommendations on the ratio of plant growing area to fish rearing surface area to maintain a balanced system—from 1:1 to 10:1. This may be due to the different hydroponic subsystems used with gravel (1:1) and float (2:1 to 10:1) being examples. Gravel media probably contains more space per meter squared for nitrifying bacterial growth to occur compared to floating systems. The use of gravel culture is no longer very common (Tyson et al., 2001) and has been replaced by lighter media such as perlite.

The most common hydroponic systems in Florida today use some form of perlite medium which provides anchorage for the plant roots (Tyson, 2002). Perlite is light weight and thus would facilitate plant production above aquaculture tanks. It should have the characteristics necessary to make a good biofilter (low density and high porosity), but this has not been scientifically shown. If hydroponic and aquaculture systems were integrated vertically in space, higher returns per unit area of space would be possible, which is important when producing inside expensive greenhouse structures. Gross monetary returns for hydroponic tomatoes of $\$47/m^2$ of greenhouse space can be expected (Smith et al., 2003) during a ten month production period. Similar gross returns of multiple cropped hydroponic greenhouse cucumbers are possible. Despite the advantages of high yield and potential gross returns, the cost of producing hydroponic vegetables on a per kilogram basis is usually higher than the cost of the field grown product and thus requires a greater return in the marketplace to be profitable (Hochmuth and Belibasis, 1991; Olson et al., 2006). Tilapia production harvest densities of 60 g/L (Rakocy, 1999) can be expected during a similar (ten month) period. A value of $1.65/kg for whole tilapia, with gross returns of $\$44/m^2$ of tank surface area is possible using rectangular recirculating tanks filled with water to a level of 0.61 m. Using the vertical space above the fish tanks for growing plants would significantly increase returns per square meter of greenhouse

space. Assuming yields are similar to when these crops are grown alone, ($47 tomatoes or cucumbers + $44 for tilapia (assume 50% space utilization in the greenhouse for the fish tanks with walkways between) then $91 per square meter of greenhouse space per year may be possible for a tomato/tilapia or cucumber/tilapia combination. Smith (2003) indicates that small positive changes in price and yield can significantly improve cash flows and gross margins for tomato greenhouse enterprises. The addition of another cash crop with the cost savings of increased systems integration (sharing equipment and structures) should improve profitability by reducing cost of production.

Conclusion and Objectives

In summary, water quality parameters affected by operating pH of aquaponic system water can provide potentially toxic conditions for fish and plants, and affect ammonia biofiltration rates of system water. No scientifically based information is available on pH effects on ammonia biofiltration rate in perlite medium or on the effect of hydroponic nutrient solution concentration on nitrification. Reconciling the pH optima for ammonia biofiltration and plant yield will increase aquaponic system integration and sustainability.

The goal of this project was to establish a reconciling pH for ammonia biofiltration and cucumber (*Cucumis sativus*) yield in an aquaponic system containing a perlite trickling biofilter / root growth medium. In addition, based on trial results, perlite will be evaluated as a medium for aquaponic biofilters. The relative contribution of plants and nitrifiers to the biofiltration of ammonia will also be assessed. Specific objectives were to:

1. Determine the optimum pH for nitrification in a trickling biofilter containing perlite medium within the range of recommended pH's for hydroponic (5.5 to 6.5) and recirculating aquaculture (6.5 to 8.5) systems (Chapter 3).
2. Determine the nitrification rate response in the designed biofilter to hydroponic nutrient solution, NO_3^--N concentrations, and to pH levels near optimal for plants (6.5) and nitrification (8.5) (Chapter 4).

3. Establish a pH range for optimum production of greenhouse cucumber in the designed biofilter system and determine if foliar applied nutrients could provide plant rescue of nutrient deficiency at high pH (Chapter 5).

4. Determine the ammonia biofiltration rate and evaluate a perlite trickling biofilter/root growth medium in aquaponic production (Chapter 6).

5. Make predictions about the relative contribution of plants and nitrifiers to the biofiltration of ammonia (Chapter 6).

6. Establish the reconciling pH for ammonia biofiltration and cucumber yield in recirculating aquaponics (Chapter 6).

Table 2-1. Fraction of NH_3 in an ammonia solution.[z]

Temperature	pH				
(°C)	6.5	7.0	7.5	8.0	8.5
20	0.0013	0.0039	0.0124	0.0381	0.1112
21	0.0013	0.0042	0.0133	0.0408	0.1186
22	0.0015	0.0046	0.0143	0.0438	0.1264
23	0.0016	0.0049	0.0153	0.0469	0.1356
24	0.0017	0.0053	0.0164	0.0502	0.1431
25	0.0018	0.0056	0.0176	0.0537	0.1521
26	0.0019	0.0060	0.0189	0.0574	0.1614
27	0.0021	0.0065	0.0202	0.0613	0.1711
28	0.0022	0.0069	0.0216	0.0654	0.1812
29	0.0024	0.0074	0.0232	0.0697	0.1916
30	0.0025	0.0080	0.0248	0.0743	0.2025
31	0.0027	0.0085	0.0265	0.0791	0.2137
32	0.0029	0.0091	0.0283	0.0842	0.2253

[z]From Lim, C. and C.D. Webster. 2006. Tilapia: Biology, culture, and nutrition. The Food Products Press, Binghamton, N.Y.

Table 2-2. Average concentrations of mineral nutrients in plant shoot dry matter that are sufficient for adequate plant growth.[z]

Element	Abbreviation	µmol/g dry wt	mg/kg (ppm)	%	Relative number of atoms
Molybdenum	Mo	0.001	0.1	-	1
Nickel	Ni	~0.001	~0.1	-	1
Copper	Cu	0.01	6	-	100
Zinc	Zn	0.30	20	-	300
Manganese	Mn	1.0	50	-	1 000
Iron	Fe	2.0	100	-	2 000
Boron	B	2.0	20	-	2 000
Chlorine	Cl	3.0	100	-	3 000
Sulfur	S	30	-	0.1	30 000
Phosphorus	P	60	-	0.2	60 000
Magnesium	Mg	80	-	0.2	80 000
Calcium	Ca	125	-	0.5	125 000
Potassium	K	250	-	1.0	250 000
Nitrogen	N	1000	-	1.5	1 000 000

[z] From Marschner, H. 2003. Mineral nutrition of higher plants. Academic Press, Elsevier Science Ltd.

Table 2-3. Percent elemental analysis and physical properties of perlite.[z]

Typical percent elemental analysis[y]	
Silicon	33.8
Aluminum	7.2
Potassium	3.5
Sodium	3.4
Iron	0.6
Calcium	0.6
Magnesium	0.2
Trace	0.2
Bound water	3.0
Oxygen (by difference)	47.5

Typical physical properties	
Color	White
Refractive index	1.5
Free moisture, maximum	0.5%
pH (of water slurry)	6.5-8.0
Specific gravity	2.2-2.4
Bulk density (loose weight) As desired but usually 32-400 kg/m^3	
Mesh size As desired	4–8 mesh and finer

[z] From Anonymous. 2007. Basic facts about perlite. The Perlite Institute, Inc., Harrisburg, PA. Retrieved April 1, 2007, from http://www.perlite.org/

[y] All analysis are shown in elemental form even though the actual forms present are unavailable and bound in mixed glassy silicates. Free silica may be present in small amounts.

CHAPTER 3
RECONCILING WATER QUALITY PARAMETERS IMPACTING NITRIFICATION IN AQUAPONICS: THE PH LEVELS

Introduction

Aquaponics is an integrated system that links hydroponic plant production with recirculating aquaculture (Diver, 2006). 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., 2006, 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 ammonia biofiltration 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 can be written as (Hagopian and Riley, 1998):

Nitrosomonas

$NH_3 + 1.5O_2 \leftrightarrow NO_2^- + H_2O + H^+ + 84$ kcal mol^{-1} (Equation 3-1)

Nitrobacter

$NO_2^- + 0.5\ O_2 \leftrightarrow NO_3^- + 17.8$ kcal mol^{-1} (Equation 3-1)

This nitrogen transformation eliminates ammonia from the water. Nitrate is generally not toxic to fish except at very high levels (96-h LC50 > 1000mg/L NO_3-N; Colt and Tchobanoglous,

1976) and is the primary source of nitrogen for plants in hydroponic systems (Hochmuth, 2001a; Resh, 2004).

Nitrate and ammonium (NO_3^- and NH_4^+) 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_4^+ may depress uptake of the essential cations (K^+, Ca^{2-}, Mg^{2+}). The optimum nitrate to ammonium ratio for vegetables grown in hydroponics is 75:25 (Simonne et al., 1992). 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 (Prosser, 1986). Recommended pH ranges for hydroponic systems are between 5.5 and 6.5 (Hochmuth, 2001a) 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 (Antoniou et al., 1990). The causes of varying pH optima may be attributed to differences in substrate, effluent, alkalinity, 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, 2006; McMurtry et al., 1997; Rakocy et al., 2006,1997; Watten and Busch, 1984) for growing plants. 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 and pepper 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. Sodium bicarbonate and potassium hydroxide (Plant Food Systems, Zellwood, FL) 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 and sodium bicarbonate was added to increase alkalinity as needed during both trials.

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, FL) was added at 1.3 ml per tank. Ammonium chloride was added at 25 mg/L resulting in 5.0 mg/L 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 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 of nitrifying bacteria was added to each tank in an effort to speed up the nitrification process.

Total ammonia nitrogen (TAN = NH_4^+-N plus NH_3-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 26ºC and 31ºC. 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 25mg/L. "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 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 to 8.0 mg/L), nitrite (low range, 0.1 to 0.8 mg/L), chlorine, and alkalinity were measured with LaMotte Test Kits. Nitrite (high range, 0 to 150 mg/L) was measured using a Hanna Ion Specific Meter. Nitrate was measured using a Cardy Ion Specific Meter (0 to 9,900 mg/L). 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 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 were measured using a Fisher Scientific AR15 Accumet Research pH meter.

Results and Discussion

These experiments are based on typical startup characteristics for bringing a new biological filter system up to full capacity (Tetzlaff and Heidinger, 1990; Timmons et al., 2002). Relative nitrification activity is measured based on the time it takes after introduction of nitrifying

bacteria to convert ammonia to nitrate. A significant experiment by pH interaction was present in enough data sets to warrant discussion by experiment.

Total ammonia nitrogen (TAN) decreased from 5 mg/L to zero, 12 d after the introduction of nitrifying bacteria to the biofilters maintained at a target pH of 8.5 (Table 3-1). 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 an average of 44 % in 28 days during the trials at pH 5.5 but nitrite accumulation was not detected. 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. There was conservation of nitrogen through the nitrification process from ammonia to nitrate. 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 dissolved oxygen, 521 and 493 µS/cm specific conductivity, 0.25 and 0.24 ppt (parts per thousand) salinity, and 28.1 and 29.8 ºC 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. Season pH values during experiment 1 ranged from 5.2 to 5.7, 6.1 to 6.4, 6.7 to 7.5, 8.5 to 8.6, and during experiment 2 ranged from 5.5 to 5.7, 6.1 to 6.5, 7.1 to 7.7, and 8.0 to 8.6 for pH treatments 5.5, 6.5, 7.5, and 8.5, respectively. Nitrification

is an acid producing process requiring adjustment of recirculating water to maintain target pH levels. Actual pH values were within the target pH range for the treatments.

Nitrite accumulation in the trials (Table 3-1) averaged 4.9 mg/L or 98 % of the applied TAN at pH 8.5. The only source of nitrite in the aerobic biofilter system was from oxidation of ammonia by the applied *Nitrosomonas* bacteria. Therefore, TAN loss from other sources, i.e. ammonia volatilization, were minimized below 2% at pH 8.5 in the biofilter boxes and at least 98% of the observed TAN loss there was from nitrification. Since ammonia volatilization increases with increasing pH, we can assume TAN losses at the lower pH's were also primarily due to nitrification. Therefore, the loss of TAN from the biofilters was primarily the result of oxidation of ammonia by the applied nitrifying bacteria and these losses occurred at a faster rate as pH increased from 5.5 to 8.5. The lack of accumulation of nitrite at pH 5.5 was likely due to the slow rate of nitrification occurring (Alleman, 1985). This accumulation is more evident at high pH than at low pH. Nitrification is a dynamic process and as nitrite accumulates, it is simultaneously oxidized to nitrate.

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_4^+ (De Rijck and Schrevens 1999). However, as pH increases above 7.0, there is an increase in the un-ionized NH^3 form of ammonia and a decrease in the ionic NH_4^+ form. Un-ionized ammonia is the most toxic form for fish with 96-h LC50 varying by species from 0.08 mg/L NH_3-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 (Maynard and Hochmuth, 1997). However, recommended pH ranges for hydroponic nutrient solutions tend to be slightly acidic

(5.5 to 6.5–Hochmuth, 2001a; 5.8 to 6.4–Resh, 2004) 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; Simonne et al., 1992), 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

The loss of total ammonia nitrogen from perlite medium trickling biofilters increased as pH increased from 5.5 to 8.5 and these losses were primarily the result of nitrification. 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 range of 6.5 to 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 soilless 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.

Table 3-1. Changes in TAN, NO_2^--N, and NO_3^--N concentrations in perlite medium trickling biofilters as affected by water pH.

Target pH	Day 0[z]	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28	Day 32
Experiment 1				Total ammonia nitrogen (mg/L)					
8.5	5.0 a[y]	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[x]		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**
Exp x pH P-value[w]		0.01	0.09	0.14	0.21	0.01	0.11	0.83	
Experiment 1				Nitrite nitrogen (mg/L)					
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*	Q*	Q*		
Significance			Q**	Q**					
Exp x pH P-value			0.71	0.78	0.29	0.17	0.07	0.07	

Table 3-1. Continued

Experiment 1				Nitrate nitrogen (mg/L)					
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**	
Signific.	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 x pH									
P-value	0.01	0.01	0.32	0.01	0.27	0.01	0.01	0.01	

[z]Nitrifying bacteria introduced to the biofilters.
[y]Within columns, means followed by different letters are significantly different; four replicates
[x]Linear and Quadratic effects were significant at the 5% (*) or 1% (**) level.
[w]P values for experiment x pH interaction

CHAPTER 4
EFFECT OF NUTRIENT SOLUTION, NO_3^--N CONCENTRATION AND PH ON NITRIFICATION RATE IN PERLITE MEDIUM

Introduction

Aquaponics is an integrated system that links hydroponic plant production with recirculating aquaculture (Diver, 2006). The most common 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, 2006; McMurtry et al., 1997; Rakocy et al., 2006,1997; Watten and Busch, 1984) for the plant growing area integrated with a recirculating aquaculture tank system (Timmons et al., 2002). The advantages of linking plant and fish culture include fish tank waste nutrient and water removal by plants, reduced water usage and waste discharge to the environment by both systems, and increased profit potential by producing two cash crops (Rakocy et al., 2006; Rakocy, 1997; Timmons, et al., 2002). The media-filled raised bed system has potential for providing biological filtration and a root zone space for plant production. The type of media in which plants grow has been shown to significantly affect nitrifying bacteria counts (soilless potting media; Lang and Elliott, 1997) and nitrification rate (various soil types; Prosser, 1986), but no information was found on nitrification rate in perlite medium. Perlite was chosen as the medium for this trial because it was the most common root growth medium used in hydroponic plant production in Florida during 2001 (Tyson et al., 2001).

Nitrification is a biological process that maintains water quality in recirculating aquaculture systems and has been shown to transform 93%-96% of nitrogenous fish wastes (NH_3 into NO_3^-) in biofiltration units (Prinsloo et al., 1999). Un-ionized ammonia nitrogen (NH_3-N) at concentrations as low as 0.02 -0.07 mg/L reduced fish growth and cause tissue damage (Masser et al., 1999). The 96–h LC50 for un-ionized ammonia on fingerling channel catfish

(*Ictalurus punctatus*) was 3.8 mg/L (Colt and Tchobanoglous, 1976). The reactions involved in nitrification may be summarized as (Madigan et al., 2003):

Nitrosifying bacteria (primarily *Nitrosomonas*)

$NH_3 + 1½\ O_2 \rightarrow NO_2^- + H^+ + H_2O \quad \Delta G^{0'} = -275$ kJ/reaction (Equation 4-1)

Nitrifying bacteria (primarily *Nitrobacter*)

$NO_2^- + ½\ O_2 \rightarrow NO_3^- \quad \Delta G^{0'} = -74.1$ kJ/reaction (Equation 4-2)

The intermediate product of nitrification, nitrite (NO_2^-), may be toxic to both fish and plants at low levels. Nitrite at concentrations as low as 5 mg/L in nutrient solution damaged tobacco (*Nicotiana tabacum* L.) root tips (Hamilton and Lowe, 1981). Nitrite oxidation activity was suppressed by elevated pH and ammonium concentrations when urea was used in a hydroponic tobacco float system resulting in the accumulation of toxic levels (30–70 mg/L) of nitrite (Pearce et al., 1998). Gila trout (*Oncorhynchus gilae*) exposed to nitrite at 10 mg/L or more for 96 h died (Fuller et al., 2003) and the 96 h LC50 for bass (*Morone sp*.) was 12.8 mg/L (Weirich et al., 1993). Nitrate, the end product of nitrification, is not toxic to fish except at concentrations much greater than those typically used in nutrient solution for plant production (catfish 96–h LC50–6,200 mg/L NO_3^--N; Colt and Tchobanoglous, 1976) although some investigations suggest that prolonged exposure to 200 mg/L NO_3^- – N might decrease the immune response of some fish species (Hrubec et al., 1996). Nitrate is the primary source of N for plants in hydroponic nutrient solutions at concentrations from 50 to 280 mg/L NO_3^--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.

There is no single ratio of plant biomass to fish biomass that results in equilibrium concentrations of most of the essential plant nutrients (Seawright et al., 1998). The levels of

most nutrients over 2 ½ years in a commercial scale aquaponic tilapia/lettuce (*Lactuca sativa*) system remained well below the initial concentrations of nutrients in hydroponic formulations (62-779 vs. 1200-1900 mg/L total dissolved solutes), but normal lettuce growth was obtained with fertilizer supplementation of K, Ca and Fe (Rakocy et al., 1997). Levels of NO_3^--N averaged 36 mg/L during the 2.5 years, well below those recommended for hydroponic lettuce nutrient formulations (115–177 mg/L NO_3^--N; Resh, 2004). Aquaponic systems that rely solely on fish waste to supply nutrients for plants have reported low levels of phosphorus, potassium, iron and manganese (Adler et al., 1996) and phosphorus, sulfur, potassium and iron (Seawright et al., 1998) in recirculating water. It would be beneficial to supplement aquaponic water with hydroponic fertilizer to optimize nutrient levels for plants; however, research on nitrification in aquaponic systems under these conditions is lacking.

The pH is one of the most important water quality parameters that can affect the activity of nitrifying bacteria in aquaculture biofilters (Hochheimer and Wheaton, 1998; Villaverde et al., 1997). The pH recommendations for aquaculture systems range between 6.5 and 8.5 (Timmons et al., 2002), whereas, the pH tolerances of plants can range from 5.0 to 7.6 depending on the species (Maynard and Hochmuth, 1997). However, recommended pH ranges for hydroponic production systems tend to be slightly acidic (5.5 to 6.5 by Hochmuth, 2001a; 5.8 to 6.4 by Resh, 2004). Precipitation of Fe^{2+}, Mn^{2+}, PO_4^{3-}, Ca^{2+} and Mg^{2+} to insoluble and unavailable salts can occur in nutrient solution culture at water pH levels above 7. If aquaponic recirculating water pH is maintained at levels more optimum for nitrifying bacteria (7.5–9.0; Hochheimer and Wheaton, 1998), plant uptake of certain nutrients may become restricted and thus plant yield may be reduced.

Waste from fish rarely supplies plant nutrients in adequate amounts without supplementation (Adler et al., 1996; Rakocy et al., 1997; Seawright et al., 1998), thus providing a nutrient solution to optimize plant production is justifiable. However, nutrient solution effects on nitrification in aquaponic systems are untested. Testing individual plant nutrients for effect on system nitrification was deemed time and resource consuming; hence, a commercial hydroponic fertilizer blend was chosen as nutrient source for the experiment. If no adverse affect on nitrification is observed with these nutrients, then a large gap between research and application for nutrient effects could be quickly filled at once.

The fish, the plants, and the nitrifying bacteria rely on the same recirculating water for growth hence water quality parameters have to be acceptable for all three organisms in a self-sustaining aquaponic system. The objective of this research was to determine the nitrification response in a perlite trickling biofilter (root growth medium) exposed to hydroponic nutrient solution, varying NO_3^--N concentrations, and to pH levels optimum for plants (6.5) and nitrification (8.5). These will be used to make predictions about water quality effects, nitrification / biofiltration, and plant yield in aquaponic systems.

Materials and Methods

Two similar experiments were conducted in Sanford, FL: the first (30 June–14 Aug., 2004) in a laboratory facility at the Horticultural Unit at Seminole Community College, and the second (21 Jan.–20 Feb. 2005) in a nearby garage facility due to hurricane damage at the Horticultural Unit. Aquarium heaters were installed in the biofilter boxes during experiment 2 since that facility was not heated in the winter.

Twenty liters of tap water were added to 80-L plastic biofilter boxes which were kept covered (nitrifying bacteria are adversely sensitive to UV light) during the experiments except during data collection. Air vents in the upper side 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 7 L of horticultural grade coarse perlite. Water was re-circulated through the perlite with aquarium pumps at the average rate of 1.9 L/min.

Biofilter tank setup began one week before the start of the experiments with water and nutrients added followed by addition of 1 ml per tank of "Proline Aqua-Coat" (Dechlorinator/Substrate Conditioner), and 0.5 ml per tank of "Proline Bio-Booster" solution. Perlite and recirculating pumps were added to the tanks prior to adjustment of tank water pH to treatment levels. Potassium hydroxide was added to raise pH and phosphoric acid was added to lower pH as needed during both trials (Plant Food Systems, Zellwood, FL). The pH was the last water quality adjustment made prior to inoculation of the tanks with nitrifying bacteria.

Experiments began with the addition of "Proline Freshwater Nitrifying Bacteria" (active cultures of *Nitrosomonas* and *Nitrobacter* bacteria - product number 239211) to the perlite at the rate of 150 ml per tank on 30 June, 2004 and 21 Jan., 2005 for experiments 1 and 2, respectively. The "Proline" products are proprietary blends of water conditioner, nutrients, and nitrifying bacteria recommended by the manufacturer for use when beginning new biofilter startup cycles in recirculating aquaculture (Aquatic Eco-Systems, Apopka, FL).

The pH treatments were 6.5 and 8.5 (established as described above). Hydroponic nutrient solution treatments were 1) tap water, no added nutrients, 2) tap water, 100 mg/L nitrate nitrogen plus complete hydroponic plant nutrient solution or 3) tap water, 200 mg/L nitrate nitrogen plus complete nutrient solution. The complete hydroponic nutrient solution consisted of 600 mg/L NFT Vegetable Formula (hydroponic fertilizer blend), 600mg/L calcium nitrate for the 100+ nitrate nitrogen treatments and 600 mg/L NFT Vegetable Formula, 600 mg/L calcium nitrate, and 600mg/L potassium nitrate for the 200+ nitrate nitrogen treatments. Fertilizer was obtained

from Grower's Supply Center, Lynn Haven, FL. The NFT vegetable formula as applied consisted of 18 mg/L nitrate nitrogen, 39 mg/L phosphorus, 134.5 mg/L potassium, 32 mg/L magnesium, 2.7 mg/L iron, 0.2 mg/L zinc, 0.4 mg/L manganese, 0.07 mg/L copper, 1.0 mg/L boron, and 0.07 mg/L molybdenum. The nutrient sources were derived from potassium sulfate, monopotassium phosphate, magnesium sulfate, potassium nitrate, iron EDTA, zinc EDTA, manganese EDTA, copper EDTA, sodium borate and sodium molybdate.

pH was measured using an AR15 Accumet Research pH meter (Fisher Scientific International, Inc., Hampton, NH). Total ammonia nitrogen (range 1.0 to 8.0 mg/L), nitrite (low range, 0.1 to 0.8 mg/L) and alkalinity were measured with test kits (LaMotte Company, Chestertown, MD). Nitrite (high range, 0 to 150 mg/L) was measured using an ion specific meter (Hanna Instruments USA, Woonsocket, RI). Nitrate was measured using an ion specific electrode cardy meter (Spectrum Technologies, Inc., Plainfield, IL). Dissolved oxygen, specific conductivity (EC), temperature, and salinity were measured using a YSI Model 85 meter (YSI Inc., Yellow Springs, OH).

Total ammonia nitrogen (TAN = NH_4^+-N + NH_3-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N) and pH measurements were taken on 30 June and 21 Jan., just prior to inoculation with nitrifying bacteria (for experiment 1 and 2, respectively) and every 5 d after inoculation in each experiment. Dissolved oxygen, soluble salts, salinity, and temperature water quality data were taken just prior to inoculation on 30 June and 21 Jan. and every 10 d thereafter. Ammonia oxidation rate was calculated based on the time in days after inoculation (DAI) it took for TAN concentrations to reach a measured value of 0. Nitrite oxidation rate was determined by assuming conservation of nitrogen through the reaction (8 mg/L TAN become 8 mg/L NO_2^--N) and the time interval from first measurement of NO_2^--N concentration until it was resolved to 0.

Sodium bicarbonate ($NaHCO_3$) was added when necessary to maintain recirculating water alkalinity above 50 mg/L $CaCO_3$. Alkalinity is defined as the total amount of titratable bases in water expressed as mg/L equivalent calcium carbonate. Sodium bicarbonate is 83g/eq and 1meq/L = 50 mg/L as $CaCO_3$ (Timmons et al., 2002).

A randomized block design with four replications was used in both experiments. Data were analyzed using Statistical Analysis System (SAS) software and Duncan's Multiple Range Test using a P value of <0.05.

Results and Discussion

Average water quality parameters during experiments 1 and 2, respectively, were 7.0 and 6.1 mg/L dissolved oxygen (more than adequate for the ammonia oxidation reaction to proceed in Eq. 4-1), and 29.1 and 30ºC. Electrical conductivity (EC) averaged 447 and 719 µS/cm for the no nutrient solution treatment, 1415 and 1934 µS/cm for the 100 mg/L nitrate plus complete nutrient solution, and 2241 and 2812 µS/cm for the 200 mg/L nitrate plus complete nutrient solution treatments. Salinity in parts per thousand averaged 0.23 and 0.35 for the no nutrient solution treatment, 0.71 and 0.98 for the 100 mg/L nitrate plus complete nutrient solution, and 1.14 and 1.46 for the 200 mg/L nitrate plus complete nutrient solution treatments. The differences in EC and salinity between Experiments 1 and 2 were most likely due to different public water sources for each experiment. The water source for Experiment 2 had higher initial soluble salts for the make up water. Season pH values during experiment 1 ranged from 5.9 to 7.3 and 8.2 to 8.7 and during Experiment 2 from 6.1 to 7.0 and 8.3 to 8.6, for pH treatments 6.5 and 8.5, respectively. Nitrification is an acid producing process (ammonia oxidation produces protons H^+ in Eq.1) requiring adjustment of recirculating water to maintain target pH levels. The measured pH was within the target pH range for the treatments.

This experiment was based on typical startup characteristics for bringing a new biological filter up to full capacity in aquaculture systems (Timmons et al., 2002). Relative nitrification activity was measured based on the number of days after inoculation (DAI) it takes after introduction of nitrifying bacteria to convert TAN to NO_3^--N. The end point for the oxidation reactions was the date on which NH_3-N and NO_2^--N concentrations reached 0 mg/L (Madigan et al., 2003). The complete nitrification reaction occurred in 45 days (10 sampling dates) for experiment 1 and in 25 days (6 sampling dates) for experiment 2. This was most likely the result of a more active batch of starter bacteria for experiment 2. As a result of the differences in the speed of the oxidation reactions, data will be presented separately for each experiment. In addition, no significant interaction occurred between nutrient/NO_3^--N concentration and pH for ammonia and nitrite oxidation. Therefore, data are presented as the main effects of nutrient/NO_3^--N concentration and the main effects of pH on nitrification rate.

No significant difference was observed in nitrification rate when system water contained no nutrient solution versus a complete hydroponic nutrient solution (Table 4-1). Since NO_3^--N is the end product of nitrite oxidation in the nitrification reaction (Eq. 4-2), NO_3^--N concentrations of 100 and 200 mg/L NO_3^--N were compared to test for a possible feedback inhibition effect on the reaction (Eq. 4-2), which did not occur. No significant difference in nitrification rate with NO_3^--N concentrations of 0, 100, or 200 mg/L where observed (Table 4-1). Results indicate that hydroponic plant nutrient solutions at concentrations found in plant production systems are unlikely to reduce nitrification rate in perlite medium. Thus plant nutrients in aquaponic systems may be tailored for optimum production of the plant (with consideration of the contribution by fish waste) without concern for adverse impacts on nitrifying bacteria.

Nitrification was significantly affected by system water pH (Fig. 4-1). Total ammonia nitrogen concentration was closest to 0 on 35 and 25 DAI at pH 6.5, and 20 and 15 DAI at pH 8.5, for Experiment 1 and 2, respectively. The ammonia oxidation rates were 231 and 300 μg L^{-1} d^{-1} at pH 6.5 and 400 and 540 μg L^{-1} d^{-1} at pH 8.5, in Experiments 1 and 2, respectively. The ammonia oxidation rates proceeded 1.75 times faster at pH 8.5 compared to pH 6.5. This was most likely due to the increase in substrate for oxidation because ammonia in water exists as two compounds: ionized NH_4^+ and un-ionized NH_3 ammonia. Ammonia oxidation involves the uncharged ammonia NH_3 (Prosser, 1986) and the concentration of uncharged ammonia in water increases almost 10 fold as pH increases from 7.0 to 8.0 (Masser et al., 1999).

Nitrite oxidation occurred at the rates of 231 and 375 μg L^{-1} d^{-1} for pH 6.5 and 267 and 540 μg L^{-1} d^{-1} for pH 8.5 for Experiments 1 and 2, respectively. Nitrite oxidation rates proceeded 1.2 and 1.4 times faster at pH 8.5 compared to pH 6.5, in Experiments 1 and 2, respectively. Nitrite nitrogen concentration reached a peak of 4.2 and 3.8 mg NO_2^--N /L for Experiments 1 and 2, respectively (Fig.4-1). Nitrification is a dynamic process and as nitrite nitrogen accumulates, it is simultaneously oxidized to nitrate nitrogen. However, since the minimum doubling times for ammonia oxidizers are about 7 h compared to 13 h for nitrite oxidizers (Prosser, 1986), the potential for nitrite buildup exists. In batch tests with sewages, minimal nitrite was present at pH 6, but at pH values of 8.4 and 9.2 the rate of ammonia oxidation surpassed that of nitrite oxidation causing an accumulation of nitrite (Alleman, 1985). The current investigation indicated similar results as ammonia oxidation proceeded at a higher rate compared to nitrite oxidation (1.75 vs. 1.3 times faster) at pH 8.5 compared to pH 6.5, resulting in increased nitrite accumulation (Fig. 4-1). Nitrite at concentrations as low as 5 mg/L in nutrient solution damaged tobacco root tips (Hamilton and Lowe, 1981) and Gila trout exposed to nitrite at 10 mg/L or

more for 96 h died (Fuller et al., 2003). Caution should be exercised in aquaponic system management to avoid practices that may produce surges in TAN and subsequent nitrite accumulation. Hence, nitrite should be monitored routinely.

The advantages of increasing the rate of nitrification would be to allow greater stocking density for fish and increased nutrient loads for plants thereby increasing productivity potential. The nitrification efficiency in this perlite medium biofilter startup cycle increased 19% and 26% at pH 8.5 compared to pH 6.5, for Experiments 1 and 2, respectively (Fig. 4-1). 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 rate (Villaverde et al., 1997) similar to the current study results. However, even though nitrification proceeds faster at pH 8.5 compared to pH 6.5, the potential for increased nitrite accumulation at the higher pH exists, with subsequent danger for the fish (brown blood disease; Masser et al., 1999) and plants (root damage; Hamilton and Lowe, 1981; Pearce et al., 1998). Also, increasing pH from 7.0 to 8.0 results in a near exponential increase in un-ionized ammonia concentration in system water, which can be toxic to fish (Colt and Tchobanoglous, 1976; Masser et al., 1999). In addition, nutrient availability for plant uptake at pH above 7 may be restricted due to precipitation of Fe^{2+}, Mn^{2+}, PO_4^{3-}, Ca^{2+} and Mg^{2+} to insoluble and unavailable salts (Resh, 2004). The advantages of increased nitrification efficiency at the higher pH weighed against the potential increased water quality risks to fish and plants are therefore not justified. Management of pH in aquaponic systems is currently maintained near 7 to compromise between plant and nitrifying bacteria preferences (Rakocy et al., 1997) and the current work supports this compromise.

Conclusion

Reconciling water quality parameters in sustainable aquaponic (integrated hydroponic and recirculating aquaculture) systems requires balancing nutrients and pH for the optimal growth of

3 organisms: the plant, the fish, and the nitrifying bacteria. Nitrifying bacteria convert fish waste into NO_3^--N that may be used by the plants. Fish waste rarely supplies nutrients in adequate amounts for plants without supplementation. Increasing nitrification rate and efficiency would allow greater stocking density for fish and increased nutrient loads for plants. The objective of this research was to determine the nitrification rate response in a perlite trickling biofilter (root growth medium) exposed to hydroponic nutrient solution, varying NO_3^--N concentrations, and to pH levels optimum for plants (6.5) and nitrification (8.5). The experiment used recirculating tank batch culture and was based on typical startup characteristics for bringing biological filters up to full capacity in aquaculture systems.

No significant difference (P value < 0.05) in nitrification rate was found when recirculating system water contained no nutrient solution versus a complete hydroponic nutrient solution or NO_3^--N concentrations of 0, 100, or 200 mg/L. These results indicate that hydroponic plant nutrient supplementation to concentrations found in plant production systems do not significantly affect nitrification rate in perlite medium. Nitrification was significantly impacted by water pH. Ammonia oxidation of initial total ammonia nitrogen (TAN = NH_4^+-N + NH_3-N = 8 mg/L) occurred at the rates of 231 and 300 µg/L/d at pH 6.5 and 400 and 540 µg/L/d at pH 8.5, for experiments 1 and 2, respectively. The rates proceeded 1.75 times faster at pH 8.5 than at pH 6.5. Nitrite oxidation occurred at the rates of 231 and 375 µg/L/d for pH 6.5 and 267 and 540 µg/L/d for pH 8.5 and proceeded 1.2 and 1.4 times faster, respectively. The increased ammonia oxidation rate (1.75) compared to nitrite oxidation rate (1.3) at pH 8.5 resulted in accumulation of NO_2^- – N to levels near those harmful to plants and fish (observed peaks of 4.2 and 3.8 mg/L NO_2^--N, respectively). The potential for increased levels of un-ionized ammonia which are toxic to fish and reduced plant nutrient uptake from micronutrient precipitation are additional

problems associated with pH 8.5. The advantages of increased nitrification efficiency, which averaged 23% in the current trials at the higher pH, when weighed against the potential increased water quality risks to the fish and plant, justify a compromise between pH optima for nitrification and plant production to pH 7 for aquaponic system water. A more flexible management strategy for these systems would be to supplement with plant nutrients, which would permit less reliance on the fish and nitrification to provide optimal plant nutrient levels.

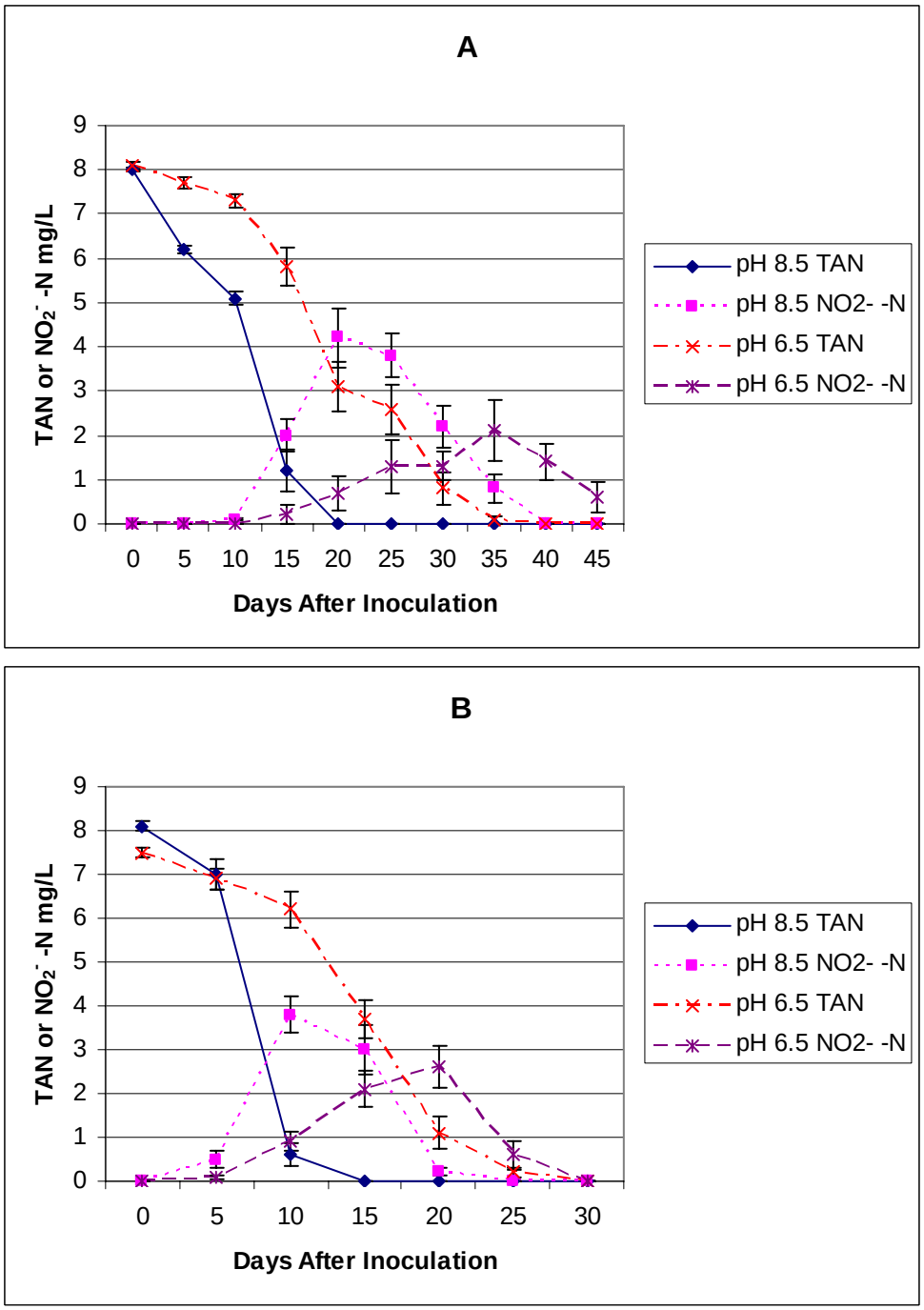
Table 4-1. Total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^--N), and nitrate nitrogen (NO_3^--N) concentrations in perlite trickling biofilter (root growth medium) when exposed to hydroponic nutrient solution.

Nutrient Solution	Days after Inoculation 0 [z]	5	10	15	20	25	30	35	40	45
Experiment 1				Total ammonia nitrogen (mg/L)						
0 [y]	8.0a [x]	7.1a	6.1a	3.1a	1.6a	1.1a	0.6a	0.0a	0.0a	0.0a
100+	8.1a	6.7b	6.0a	3.5a	1.5a	1.4a	0.0a	0.0a	0.0a	0.0a
200+	8.1a	7.0ab	6.5a	3.9a	1.6a	1.4a	0.6a	0.1a	0.0a	0.0a
Experiment 2										
0	7.5b	6.8a	3.4a	1.7a	0.1a	0.0a	0.0a			
100+	7.7b	6.8a	3.3a	1.8a	0.9a	0.1a	0.0a			
200+	8.1a	7.4a	3.4a	2.1a	0.6a	0.1a	0.0a			
Experiment 1				Nitrite nitrogen (mg/L)						
0	0.0a	0.0a	0.0a	1.6a	2.5a	1.8a	1.1b	1.6a	0.3a	0.0a
100+	0.0a	0.0a	0.0a	0.9a	2.4a	3.5a	3.0a	2.0a	0.6a	0.4a
200+	0.0a	0.0a	0.0a	0.9a	2.4a	2.4a	1.1b	0.8a	1.2a	0.6a
Experiment 2										
0	0.0a	0.1a	1.7b	2.4a	1.0a	0.6a	0.0a			
100+	0.0a	0.5a	2.4ab	2.1a	2.0a	0.0a	0.0a			
200+	0.0a	0.3a	2.9a	3.2a	1.2a	0.4a	0.0a			
Experiment 1				Nitrate nitrogen (mg/L)						
0	3c	2c	3c	2c	2c	3c	4c	5c	6c	5c
100+	104b	94b	106b	102b	102b	104b	106b	115b	119b	114b
200+	201a	180a	194a	194a	200a	204a	211a	220a	229a	221a
Experiment 2										
0	2c	2c	3c	2c	4c	5c	4c			
100+	110b	114b	119b	103b	119b	129b	116b			
200+	205a	199a	216a	190a	216a	239a	213a			

[z] Nitrifying bacteria introduced to the biofilters.

[y] Hydroponic nutrient solution 0 (tap water only), 100mg/L nitrate plus complete plant nutrient solution or 200 mg/L plus complete nutrient solution.

[x] Within columns, means followed by different letters are significantly different at the 0.05 level; four replicates.



Note: Error bars represent ± SE (n=4).

Figure 4-1. Effect of pH on ammonia and nitrite oxidation in perlite medium. A) Experiment 1 B) Experiment 2.

CHAPTER 5
EFFECT OF WATER PH ON YIELD AND NUTRITIONAL STATUS OF GREENHOUSE CUCUMBER GROWN IN RECIRCULATING HYDROPONICS

Introduction

Fresh cucumber (*Cucumis sativus*) was grown on 23,136 hectares with a market value of 235 million dollars in the United States during 2005 (National Agricultural Statistics Service, 2006). It is also an important vegetable in greenhouse production systems (Tyson et al., 2001) and has potential for production in integrated hydroponic and aquaculture systems (aquaponics) (Timmons et al., 2002). Aquaponic production requires balancing water quality and pH for the optimal growth of three groups of organisms: plants, fish, and nitrifying bacteria.

One of the most important water quality management requirements of aquaculture systems is to prevent the buildup of ammonia in system water. In water, ammonia exists in two forms, which together are called the Total Ammonium Nitrogen (Francis-Floyd and Watson, 1996) or TAN (TAN = NH_4^+- N + NH_3 – N). The equilibrium reaction is $NH_4^+ \leftrightarrow NH_3 + H^+$ (Campbell and Reese, 2002). Water temperature and pH affect which form of ammonia predominates. NH_4^+-N is predominate below pH 7.0. As pH increases from 7.0 to 8.0 there is a ten-fold increase in NH_3-N. Unionized ammonia (NH_3) is toxic to fish at concentrations above 0.05 mg/L (Francis-Floyd and Watson, 1996), producing mortality at 0.08 mg/L NH_3-N for pink salmon and 2.2 mg/L NH_3-N for common carp (Timmons et al., 2002). Thus ammonia biofiltration (nitrification) of system water by nitrifying bacteria is needed for maintenance of water quality by conversion of fish waste ammonia to NO_3^- -N which is relatively non- toxic to fish and may be used by plants.

Recommended water pH for greenhouse hydroponic production is 5.5–6.0 (Hochmuth, 2001b), 5.5–6.5 (Hochmuth, 2001a) and 5.8–6.4 (Resh, 2004). This slightly acid pH helps reduce precipitation of Fe^{2+}, Mn^{2+}, PO_4^{3-}, Ca^{2+} and Mg^{2+} into insoluble and unavailable salts

which may occur at water pH levels > 7.0. Aquaponic recirculating water pH is recommended to be maintained between 7.0 and 7.5 (Timmons et al., 2002) to balance pH for ammonia biofiltration with nutritional requirements of the plant. Aquaculture biofilter nitrification was reported to be most efficient at pH 7.5–9.0 (Hochheimer and Wheaton, 1998) and 7.0–8.0 (Masser et al., 1999). Nitrification efficiency increased 13% with each unit increase in pH from 5.0 to 9.0 (Villaverde et al., 1997) in submerged biofilters. 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).

A reconciling pH between the requirements of rapid ammonia biofiltration and the nutritional requirements of crops in hydroponic production has not been scientifically established. It may be possible to overcome nutrient deficiency and maintain crop yield under system water pH production conditions > 7.0 with the use of foliar application of micronutrients. Foliar applications of Mg, Zn, and Mn can effectively correct deficiencies in fruit and vegetable crops grown on calcareous soils with a pH 7.4 to 8.4 (Li, 2001). Overall yield increases of 33% occurred when strawberry (*Fragaria ananassa*) cultivars were sprayed once per week with Fe (1.0 kg Fe/ha) when grown in calcareous soil at pH 8.2 (Zaiter and Saad, 1993).

Fish stocking density in intensively managed recirculating aquaculture systems is directly related to the capacity of the biofilter to process and prevent the buildup of toxic ammonia since 10% of the protein in fish feed becomes ammonia nitrogen in the system water (Timmons et al., 2002). If greenhouse crops could be grown at pH 7.0–8.0 without a reduction in yield, then ammonia biofiltration rates may be improved in integrated aquaponic systems. This would allow greater fish stocking densities producing more plant nutrients from fish waste thus conserving applied fertilizer and thereby improving aquaponic system integration and sustainability.

The most common recirculating aquaponic systems employ either a media-filled raised bed, nutrient-flow technique (NFT), or floating raft system (Adler et al., 1996; Anonymous, 1997; Diver, 2006; McMurtry et al., 1997; Rakocy et al., 2006,1997; Watten and Busch, 1984) for the plant growing area. Of those systems, the media filled bed has potential for providing ammonia biofiltration and a root zone space for plant production. Using a continuous recirculating system perlite media bed with potential for use in aquaponics, the purpose of this investigation was to 1) determine the effect of pH on greenhouse cucumber yield at water pH between 5.0 and 8.0 and 2) assess the possibility of restoring nutrition and yield by foliar fertilization at pH 7.0 and 8.0.

Materials and Methods

The experiment was conducted in a passively ventilated greenhouse at the Polk Correctional Prison Farm in Sanford, FL. Six treatments were arranged in a randomized complete block design with three replications. All treatments had recirculating water maintained for a range of pH values as follows: 1) pH 5.0, 2) pH 6.0, 3) pH 7.0, 4) pH 8.0, 5) pH 7.0 with foliar applied nutrients (7-fs), and 6) pH 8.0 with foliar applied nutrients (8-fs). Plastic, 80-L rectangular tanks were filled with 40 L of tap water on 11 Aug., 2005. The plastic recirculating tanks were placed 90 cm apart in a single row parallel to the length of the house.

A complete hydroponic nutrient solution consisting of 600 mg/L NFT Vegetable Formula (hydroponic fertilizer blend - Grower's Supply Center, Lynn Haven, FL), 600 mg/L calcium nitrate ($Ca(NO_3)_2$) and 300 mg/L magnesium sulfate ($MgSO_4$) were added to each tank. The NFT vegetable formula as applied consisted of 18 mg/L nitrate nitrogen (**(**NO_3^--N), 39 mg/L phosphorus (P), 134.5 mg/L potassium (K), 32 mg/L magnesium Mg), 2.7 mg/L iron (Fe), 0.2 mg/L zinc (Zn), 0.4 mg/L manganese (Mn), 0.07 mg/L copper (Cu), 1.0 mg/L boron (B), and 0.07 mg/L molybdenum (Mo), 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. Phosphoric acid was used to lower pH and potassium hydroxide was used to raise pH during the trial as needed to maintain pH at treatment levels. Composite water samples for each treatment (75ml/tank) were collected on 15 Aug. and frozen for specific elemental analysis of starting solution (Table 5-1). Electrical conductivity (EC) was maintained between 1 and 2 ds/cm during the experiment by periodically adding the above fertilizer at the same ratios when required.

Germination trays with drainage slits in the bottom were placed above the water on plastic stools in each tank on 15 Aug. Natural burlap was double layered in the trays, horticultural grade course perlite was added, and water distribution plates were placed on top of the perlite. Water was pumped to the plates and re-circulated through the perlite with aquarium pumps (model no. SP800, Aquatic Eco-systems, Apopka, FL) at the rate of 1.9 L/min.

Two 'Millagon' (De Ruiter Seeds, Inc., Lakewood, CO) European cucumber seedlings were planted into the perlite of each plot on 16 Aug (0 DAT). This variety has powdery mildew tolerance and excellent fruit uniformity (Hochmuth et al., 1996). Two additional transplants were placed on the distribution plates of each container on 16 Aug. and their roots were bathed with constant recirculating nutrient solution for 14 d. These additional plants were included to obtain early season shoot tissue elemental content. A foliar nutrient application was made once weekly beginning 7 DAT at the rate of 20ml/L INP 3500 chelated nutritional complex (Plant Food Systems, Zellwood, FL) with 6g/L potassium nitrate to treatments 7-fs and 8-fs. Nutritional content of foliar spray was 780, 2,640, 0.4, 1.0, 0.9, 0.2, 0.2, 0.04, and 0.0008 mg/L of N, K, Mg, S, Fe, Mn, Zn, B, and Mo, respectively. Plants in the distribution plates were harvested 14 DAT and shoot tissue processed using the dry ash digestion method (Mills and

Jones, 1996). Tissue analysis and composite water sampling were analyzed by Agro Services International, Inc., Orange City, FL. using a segmented stream autoanalyzer for N, spectrophotometer colorimetry for P, atomic absorption for K, Ca, Mg, Mn, Fe, Zn, and Cu, and the curcumin method for B (Plank, 1992).

Cucumbers were trellised to overhead wires and pruned to a single leader stem. Fruit were harvested every 2–4 days between 34 to 55 DAT. Fruits showing poor tip fill and angled fruit approaching 45% were considered non-marketable and were pruned from the plants as soon as defects were detected. Those with marketable potential were allowed to grow to commercial size. Early yield was calculated from fruits in the first three harvests and total yield from all ten harvests.

Nitrate nitrogen concentrations in petiole sap were measured using ion specific electrode meters (Cardy Spectrum Technologies, Inc., Plainfield, IL) on 22 and 45 DAT. The cardy meter was also used to measure NO_3^--N and K concentrations in the nutrient solution during the experiment. Water pH and EC were measured using an Accumet Research pH meter (model no. AR15, Fisher Scientific International, Inc., Hampton, NH) and a YSI Model 85 meter (YSI Inc., Yellow Springs, OH), respectively.

Data were analyzed using ANOVA (SAS, 2001) and Duncan's Multiple Range Test using a P value of ≤ 0.05. Data were analyzed for significant linear and quadratic trends over the non-repeating equal distant pH units.

Results and Discussion

Season pH values ranged from 3.8 to 5.9, 5.4 to 6.9, 6.4 to 7.6, 7.2 to 8.5, 6.3 to 7.7, and 7.2 to 8.5, respectively, for treatments pH 5.0, 6.0, 7.0, 8.0, 7-fs, and 8-fs. The measured pH was within the target pH ranges of the treatments for the trial. Composite samples from the nutrient solutions of treatments were analyzed for selected nutrient concentrations after fertilizer addition

and pH adjustment (four days after tank setup) to determine potential availability for uptake by the plant (Table 5-1). The concentrations of Ca, P, Fe, and Mn in the nutrient solution declined as pH increased, but magnesium was unaffected by pH. These nutrients were identified as potentially restricted at high pH due to precipitation to insoluble salts (Resh, 2004).

Shoot fresh and dry weight, and length of young cucumber plants on14 DAT were similar among pH 5.0, 6.0, or 7.0 treatments, but were significantly reduced in the pH 8 treatment (Table 5-2). Shoot fresh and dry weight and length declined linearly as the pH increased from 5.0 to 8.0. Differences between pH 7.0 and 7.0-fs and 8.0 and 8.0-fs were not significantly different. Foliar spray at one week after planting had no effect on early growth. This is most significant at pH 8.0 since plants where already smaller at this stage of growth compared to the other treatments (Table 5-2). This difference in early biomass production most likely contributed to the significant difference in early marketable yield observed between pH 5.0 and 8.0 (Table 5-6).

Results of shoot tissue analysis from the samples above indicate that Mg content increased as pH increased from 6.0 to 8.0 (Table 5-3). This was most likely due to the competition for binding and transport sites on the plasma membrane (Marshner, 2003) between cations Mn^{2+} and Mg^{2+} and the declining concentration of Mn^{2+} in the nutrient solution as pH increased (Table 5-1). Nitrogen and phosphorus content were significantly reduced at pH 8.0 compared to 5.0, 6.0, or 7.0. This may be a result of reduced P in the nutrient solution as pH increased (Table 5-1) but not for N since NO_3^--N increased as pH increased (Table 5-6). However, these results suggest that (1) lower concentrations in recirculating solutions may be adequate and (2) reduced P in solution is affected primarily by water only at pH 8.0. Although foliar spray samples were not washed prior to analysis, no significant increase in N, K, Ca, Mg, Cu, or B were observed compared to unsprayed treatments (pH 7.0 versus 7-fs and 8.0 and 8-fs). Consequently, the

increase in Fe, Mn, and Zn, were most likely actual tissue content increases and not residual spray on the leaves (Table 5-4). This agrees with earlier work (Li, 2001; Zaiter and Saad, 1993) for overcoming nutrient deficiency at high pH for Fe, Mn, and Zn.

Visual observation of cucumber foliage on 24 DAT showed pH 5.0 with dark green leaf color, pH 6.0 with medium green leaf color, pH 7.0 with slightly mottled yellow leaf color, pH 8.0 with pronounced mottled yellow leaves, pH 7-fs with noticeably less mottled leaf appearance compared to pH 7, and pH 8-fs with slightly less mottled leaf color than pH 8.0. At this point in the experiment three foliar sprays had been made and indicate that visual symptoms of nutrient deficiency were less pronounced with foliar nutritional sprays compared to unsprayed treatments.

The concentrations of NO_3^--N in petiole sap were unaffected by pH (Table 5-5). Sap leaf petiole NO_3^--N levels were higher than recommended for cucumber production (800-1000 mg/L NO_3^--N first flower stage, 400-600 mg/L first harvest; Hochmuth, 2003), thus nutrient solution concentrations during the trial were kept below recommended levels of 113-275 mg/L N (Chaverria et al., 2005; Hochmuth, 2001a; Schon and Compton, 1997b) and averaged between 52 and 84 mg/L (Table 5-5). Tissue concentrations of all nutrients tested were within adequate to high ranges (Table 5-3 and 5-4) with the exception of manganese, which was low < 30 mg/kg at pH 8.0 (Olson et al., 2006). However, manganese was restored to an adequate level by foliar spray. Even though Fe dropped to the same level (Table 5-1) as the source water at pH 8 (0.03 mg/L), the plant was able to accumulate 70 mg/kg Fe in the shoot tissue, well within an adequate range for the plant (Table5- 4). This indicates that the analyzed nutritional content of the cucumber plants appeared adequate at all pH levels (except manganese at pH 8.0) even though certain nutrient levels were lower than recommended (Hochmuth, 2001a;Olson, et al., 2006) in the nutrient solution for non-circulating systems (Table 5-1 and 5-5). This may be due to the

continuous recirculation of the nutrient solution through the media, bathing the roots of the plant, resulting in no depletion of nutrients in the root zone despite low nutrient concentrations in the solution.

Two studies (Olson, 1950; Rakocy et al., 1997) indicate that optimum plant yields may be maintained at lower nutrient solution concentrations if roots are constantly exposed to the solution rather than receiving it intermittently. In soils, nutrients move to the surface of roots by diffusion and bulk flow of the soil solution resulting from transpiration (Taiz and Zeiger, 2002). Concentration gradients can form in the soil solution as nutrients are taken up by the roots and the concentration of nutrients at the root surface is lowered compared to the surrounding area. This can result in a nutrient depletion zone near the root surface. The capacity for continuous growth by roots however, extends this region of nutrient uptake beyond the depletion zone. Thus, optimum nutrient acquisition by plants depends on the capacity of their root systems not only to absorb nutrients, but also, to grow into fresh soil. In hydroponic production, the media volume is finite and nutrient depletion can be recovered only in the next irrigation event. N depletion can occur at lower N (90-175 mg/L) nutrient solution concentrations (Schon and Compton (1997b) under commercially recognized intermittent fertigation of cucumber in hydroponic media. Irrigation frequencies adequate to prevent water stress are not necessarily adequate to prevent nutrient depletion except at high N (225-275 mg/L) nutrient solution concentrations. Therefore, it seems logical to propose that more frequent flushing of the media with lower concentrations of N would obviate N depletion between irrigation events. If this flushing was continuous, there would be no appreciable depletion of nutrients in the root zone. This reasoning could apply to all nutrients in the solution. Thus precipitation of certain nutrients at pH 8.0, suggested in Table 5-1, may not limit the overall nutritional status of the plant

provided continuous recirculation through the root/media zone occurs to eliminate nutrient depletion.

Early marketable cucumber fruit yield was significantly higher at pH 5.0 compared to pH 8.0 (Table 5-6). Foliar sprays during the growing season did not increase yields compared to unsprayed treatments. Total marketable and cull cucumber fruit yields were not significantly different among treatments. Early marketable yield response to pH was significantly linear at the 5.2% level. Results indicate an early yield advantage to keeping nutrient solution pH between 5.0 and 7.0 but not an advantage for total yield. This would indicate that maintaining the recirculating pH at 8.0 to accommodate nitrifying bacteria activity would be detrimental from an economic standpoint if the grower were producing for an early market but would not adversely affect the overall production during middle to late portions of a multiple cropped season.

Conclusion

Shoot fresh, dry weight, and length of cucumber plants harvested on 14 DAT decreased linearly as pH increased from 5.0 to 8.0. Early marketable cucumber fruit yield was higher at pH 5 compared to pH 8 but total yield was unaffected by pH treatment. Foliar nutritional sprays during the season reduced visual deficiency symptoms at pH 7.0 and 8.0, but did not significantly increase yield. Foliar spray rescue treatments did not work. However, results suggest that nutrient concentrations in recirculating systems can be maintained lower than in non-circulating systems overcoming potential limits to production which may occur from selected nutrients prone to precipitation at pH > 7.0. This study indicates that aquaponic systems utilizing cucumber in recirculating aquaponic culture may be maintained at pH levels more optimum for nitrifying bacteria 7.5–8.0 with no reduction in total yield except during production for early season markets where pH 7.0 would be recommended. Increased system ammonia biofiltration through nitrification will allow higher fish stocking densities producing more plant

nutrients from fish waste thus conserving applied fertilizer and thereby improving aquaponic system integration and sustainability.

Table 5-1. Initial water analysis for pH and selected nutrients.

Composite Sample	Actual pH	Solution elemental concentration (mg/L) Ca	Mg	P	Fe	Mn
H_2O	7.6	31.2	7.6	0.3	0.03	0.03
pH 5.0	5.4	102.8	45.0	66.4	0.16	0.24
pH 6.0	6.1	87.3	40.4	34.4	0.06	0.16
pH 7.0	7.0	80.1	43.8	15.7	0.08	0.07
pH 8.0	8.3	73.5	42.9	2.8	0.03	0.04
Recom.[z] level	5.5-6.5	130.0	50.0	62.0	2.5	0.62

[z]Recommended nutrient solution level for non-circulating hydroponic cucumbers (Hochmuth, 2001b)

Table 5-2. Cucumber shoot fresh and dry weight and plant length on 14 DAT stage of growth as influenced by system water pH and foliar spray.

Target pH	Shoot fresh wt.	Shoot dry wt.	Shoot length
	g	g	cm
5.0	70.5 a[z]	10.2 a	57 a
6.0	68.2 a	10.0 a	56 a
7.0	68.5 a	9.3 a	55 a
8.0	42.0 b	6.5 b	40 b
Contrast	L*[y]	L**	L*
7.0-fs[x]	65.2 a	8.8 a	50 ab
8.0-fs	41.3 b	6.3 b	40 b

[z]Within columns, means followed by different letters are significantly different; three replicates.
[y]Linear contrast were significant at the 5% (*) or 1% (**) level.
[x]fs = Foliar nutritional spray once per week.

Table 5-3. Cucumber shoot nutrient content (% DM) 14 DAT as influenced by solution pH and foliar spray.

Target pH	N	P	K	Ca	Mg
	%	%	%	%	%
5.0	5.2 a	0.78 a	4.4 ab	2.9 ab	0.83 c
6.0	5.2 a	0.74 a	5.4 a	2.2 c	0.80 c
7.0	5.1 a	0.71 a	4.7 ab	2.5 bc	1.10 b
8.0	4.5 b	0.39 b	4.5 ab	3.1 a	1.43 a
Contrast	L** Q*	L** Q*		Q*	L**
7.0-fs[x]	5.3 a	0.69 a	5.1 ab	2.3 bc	1.17 b
8.0-fs	4.7 b	0.36 b	4.2 b	2.5 bc	1.17 b
Sufficiency Range[w]	2.5-5.0	0.25-0.6	1.6-3.0	1.0-3.5	0.3-0.6

[z]Within columns, means followed by different letters are significantly different; three replicates.
[y]Linear and quadratic contrasts were significant at the 5% (*) or 1% (**) level.
[x]fs = Foliar nutritional spray once per week.
[w]Plant tissue analysis at early bloom stage for cucumber, dry weight basis.
(Olson et al., 2006)

Table 5-4. Cucumber shoot nutrient content (mg/kg) 14 DAT as influenced by solution pH and foliar spray.

Target pH	Shoot nutrient concentration				
	Fe	Mn	Zn	Cu	B
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
5.0	120 b[z]	143 a	71 b	10 a	96 a
6.0	84 b	57 bc	61 b	7 b	84 a
7.0	82 b	31 c	63 b	10 ab	87 a
8.0	70 b	24 c	66 b	11 a	91 a
Contrast	L** Q**[y]	L** Q*			
7.0-fs[x]	367 a	68 b	109 a	10 ab	87 a
8.0-fs	363 a	79 b	120 a	11 a	98 a
Sufficiency Range[w]	40-100	30-100	20-50	5-10	20-60

[z]Within columns, means followed by different letters are significantly different; three replicates.
[y]Linear and quadratic effects were significant at the 5% (*) or 1% (**) level.
[x]fs = Foliar nutritional spray once per week.
[w]Plant tissue analysis at early bloom stage for cucumber, dry weight basis (Olson et al., 2006).

Table 5-5. Concentration of NO_3 –N and K in cucumber petiole sap and average season nutrient solution NO_3 –N and K levels.

Target pH	Petiole sap First flower NO_3 –N	Petiole sap Sixth harvest NO_3 –N	Avg. season nutrient solution concentration NO_3 –N	Avg. season nutrient solution concentration K
	mg/L		mg/L	
5.0	1150 a[z]	1233 a	52 c	71 ab
6.0	1100 a	1053 a	61 bc	54 b
7.0	1123 a	1163 a	72 ab	60 b
8.0	1133 a	1083 a	78 a	84 a
Contrast			L**[y]	Q*
7.0-fs [x]	1167 a	983 a	73 ab	56 b
8.0-fs	1233 a	1267 a	84 a	67 ab
Recom. Levels[w]	800-1000	400-600[v]	133	150

[z]Within columns, means followed by different letters are significantly different; three replicates.
[y]Linear and quadratic effects were significant at the 5% (*) or 1% (**) level.
[x]fs = Foliar nutritional spray once per week.
[w] Recommended for petiole sap (Olson et al., 2006) and N and K in non-circulating hydroponics (Hochmuth, 2001b).
[v] First harvest recommendation.

Table 5-6. Cucumber fruit yield as influenced by nutrient solution pH and foliar spray.

Target pH	Early marketable[z] kg/plant	Early marketable[z] no/plant	Total marketable kg/plant	Total marketable no/plant	Total cull kg/plant	Total cull no/plant
5.0	1.38 a[y]	4.2 a	2.81 a	6.83 a	0.24 a	0.7 a
6.0	1.04 ab	2.8 ab	3.18 a	7.17 a	0.61 a	0.7 a
7.0	0.73 ab	2.3 ab	2.93 a	7.00 a	0.42 a	0.5 a
8.0	0.62 b	2.0 b	2.83 a	7.17 a	0.03 a	0.2 a
Contrast	0.052[x]					
7.0-fs[w]	1.08 ab	3.2 ab	3.16 a	7.83 a	0.13 a	0.2 a
8.0-fs	0.64 b	2.2 b	2.58 a	6.67 a	0.25 a	0.3 a

[z]Early = first three harvests, Total = all ten harvests, Marketable = 34-42 cm in length, less than 45° fruit angle, few blemishes, Cull = greater than 45° fruit angle, poor tip fill, frequent blemishes.
[y]Within columns, means followed by different letters are significantly different; three replicates.
[x]Significant linear contrast trend between pH 5.0 through 8.0 (p = 0.052) level for early marketable fruit.
[w]fs = Foliar nutritional spray once per week.

CHAPTER 6
WATER QUALITY INLUENCES AMMONIA BIOFILTRATION AND CUCUMBER YIELD IN RECIRCULATING AQUAPONICS

Introduction

Aquaponics is the integrated production 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 Farm Bill, Title XVI, Subtitle A, Sec. 1603. Aquaponics is "an integrated system of plant and animal production practices" using vegetables with aquaculture species, "having a site-specific application" in greenhouse production units. It will "over the long term satisfy human food needs" and "enhance environmental quality" by producing crops using practices that minimize water and nutrient waste discharges to the environment. Aquaponics will "make the most use of nonrenewable resources" by conserving fertilizer nitrogen derived from fossil fuels and reducing water use. It will "integrate natural biological cycles" by using nitrifying bacteria in the process of nitrification to convert harmful ammonia fish waste to usable, nitrate nitrogen for plants. Aquaponics will "sustain the economic viability of farm operations" and "enhance the quality of life for farmers…and society as a whole" by producing food in a sustainable bio-rational manner without wasteful discharge to the environment. However, with all its promise, there is no information in the literature on how aquaponic system water quality impacts nitrification in a

perlite biofilter/root growth medium, little information on the plant root/nitrifying bacteria interaction affects on ammonia biofiltration, and how this interaction affects plant yield and nitrifying bacteria activity.

Aquaponic production requires balancing nutrient concentrations and pH for the optimal growth of 3 organisms: plants, fish, and nitrifying bacteria. Recommended pH for aquaculture is 6.5–8.5 (Timmons et al., 2002) and for greenhouse cucumber is 5.5–6.0 (Hochmuth, 2001a). Aquaculture biofilter nitrification was reported to be most efficient at 7.5–9.0 (Hochheimer and Wheaton, 1998). The reactions involved in nitrification may be summarized as (Madigan et al., 2003):

Nitrosifying bacteria (primarily *Nitrosomonas*)

$NH_3 + 1½\ O_2 \rightarrow NO_2^- + H^+ + H_2O \quad \Delta G^{0'} = -275$ kJ/reaction (Equation 6-1)

Nitrifying bacteria (primarily *Nitrobacter*)

$NO_2^- + ½\ O_2 \rightarrow NO_3^- \quad \Delta G^{0'} = -74.1$ kJ/reaction (Equation 6-2)

Unionized ammonia is the substrate ion used for the nitrification reaction (Prosser, 1986). In water, ammonia exists in two forms, which together are called the Total Ammonium Nitrogen (Francis-Floyd and Watson, 1996) or TAN (TAN = NH_4^+- N + NH_3 – N). The equilibrium reaction is as follows (Campbell and Reese, 2002): $NH_4^+ \leftrightarrow NH_3 + H^+$. Water temperature and pH will affect which form of ammonia is predominate. For example, at 22ºC the unionized ammonia fraction of TAN is 0.46% and 4.4% for pH 7.0 and 8.0, respectively (Francis-Floyd and Watson, 1996). This represents nearly a ten fold increase unionized ammonia substrate for the nitrification reaction. A concentration versus activity plot of a biofilter (Hagopian and Riley, 1998) will often demonstrate a first order response (activity increases with increase in substrate concentration). 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. These reported effects and pH range differences require reconciling water quality parameters affecting ammonia biofiltration and cucumber in aquaponics to improve systems integration and sustainability. Recommended "consensus" pH levels have not been scientifically established in aquaponics.

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., 2002). 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 (Feray et al., 1999; Smorczewski and Schmidt, 1991), and terrestrial soils (Prosser, 1986; Papen and Berg, 1998). 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 effect on bacterial reproduction provided initial cell inoculation numbers are the same among treatments.

Maintaining aquaponic system water above pH 7.0 should increase system ammonia biofiltration by nitrification, thereby allowing higher fish stocking densities, producing more plant nutrients from fish waste. This will conserve applied fertilizer and thereby improve aquaponic 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 aquaponic production, 2) make predictions about 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 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. The 5 treatments were 1) aquaponic pH 6.0, 2) aquaponic pH 7.0, 3) aquaponic pH 8.0, 4) hydroponic control pH 6.0, and 5) aquaculture control pH 7.0, with four replicates. Aquaponic treatments consisted of cucumber, tilapia, and nitrifying bacteria. Hydroponic system contained cucumber plants only and aquaculture control system contained fish and nitrifying bacteria. 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

Phosphoric acid was used to lower pH and potassium hydroxide was used to raise pH to treatment levels (target 6.0, 7.0, and 8.0) during the experiment. Sodium bicarbonate ($NaHCO_3$) and $CaCO_3$ were added to tank water during the trial to maintain alkalinity (a measure of the capacity of water to neutralize acids, also known as the buffering capacity, due primarily to the presence of available bicarbonate, carbonate, and hydroxide ions). Alkalinity during the experiment averaged 35, 42, 84, 39 and 41 mg/L, respectively, for treatments described above. Biofilters consisting of rectangular plastic milk carrying cases lined with natural burlap and filled with 20 L of horticultural-grade perlite were placed on top of the recirculating tanks on 14 July. One aquarium pump (model HX2500, Aquatic Eco-systems, Apopka, FL), was placed in the bottom of each tank and water was re-circulated to distribution plates on top of the perlite in the biofilter and allowed to trickle down through the perlite back to the tanks at the average rate of 100 L/hr. An additional pump (model SP800, Aquatic Eco-systems, Apopka, FL) was added on 30 Aug. to each tank to increase water turnover rates through the biofilter from 1 to 3 turnovers of tank water per hour.

An ammonia surge test was conducted to determine ammonia volatilization rates from tanks at pH 6.0, 7.0 and 8.0 prior to inoculation of bacteria and introduction of fish. Ammonium chloride (2.5 g/tank) was added on 12 July to create a TAN concentration of 6 mg/L. TAN measurements were taken on 13 July (all tanks reading 6 mg/L TAN) and 20 July. 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 NFT Vegetable Formula (hydroponic fertilizer blend, Grower's Supply Center, Lynn Haven, FL), was added to each tank on 21 July. The NFT vegetable formula consisted of 6 mg/L nitrate nitrogen, 13 mg/L phosphorus, 45 mg/L potassium, 11 mg/L magnesium, 0.9 mg/L iron, 0.07 mg/L zinc, 0.1 mg/L manganese, 0.02 mg/L copper, 0.3 mg/L boron, and 0.02 mg/L 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 Vegetable Formula, other nutrients [$Ca(NO_3)_2$, KNO_3, and $MgSO_4$] were added during the season based on water analysis and visual observations, to maintain plant nutrition and similar soluble salt concentrations among treatments. Plant nutritional status was monitored by measuring leaf petiole sap NO_3^--N and K levels beginning on 26 Aug. and every two weeks thereafter. One leaf petiole per plot from the most recently fully expanded leaf (from the 6^{th} to 8^{th} leaf below the growing point) was measured. Leaf petiole sap NO_3^--N and K measurements were made using ion specific electrode meters (Cardy Spectrum Technologies, Inc., Plainfield, IL). In addition, NO_3^--N, NO_2^--N, NH_4^+-N, and K^+ levels in recirculating tank water were measured weekly. Tank water total ammonia nitrogen (low range 1.0 to 8.0 mg/L), nitrite nitrogen (low range, 0.1 to 0.8 mg/L) and alkalinity were measured with test kits (LaMotte

Company, Chestertown, MD). TAN (high range 1.0 to 50.0 mg/L), nitrite nitrogen (high range, 0 to 150 mg/L) were measured using an ion specific meter (Hanna Instruments USA, Woonsocket, RI). Nitrate nitrogen was measured using an ion specific electrode (Cardy meter, range 0 to 9,900 mg/L, Spectrum Technologies, Inc., Plainfield, IL).

Ammonium chloride (2 g/tank, 23 July and 0.5 g/tank, 30 July) was added to provide adequate ammonia for the nitrification reaction (Eq. 6-1). 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 6.7×10^4 cells per ml according the supplier (Aquatic Eco-systems, Apopka, FL). Another 50 ml per tank of Proline Freshwater Nitrifying Bacteria was added on 14, 15, and 16 Aug. to control an ammonia spike after introduction of fish.

Fish (Nile tilapia, *Oreochromis niloticus* from Harbor Branch Oceanographic Institution, Fort Pierce, FL) were stocked into treatment tanks (except hydroponic control) on 1-2 Aug. at similar density of 15 fish averaging 32.4 g/fish per tank). Care was taken to insure that an equal proportion of large and small fish were in each tank. The fish population size was variable ranging from a low of 8 g to a high of 122 g at initial stocking. At the end of the trial it was noticed that several fish had spawned with live fry in their mouths indicating a mixed population of male and female fish.

Based on previous greenhouse trials (Hochmuth et al., 1996) 'Fitness' (Asgrow Seed Company, St. Louis, MO) European cucumber seeds were planted into the perlite of the biofilter of each plot except the aquaculture control treatment on 4 Aug, four seeds on each side of the distribution plate. Plant germination was complete in all plots on 7 Aug. (= 0 DAG). Starter fertilizer (400 ml of 2g/L veg mix) was added to each tank biofilter around the young seedlings

to stimulate growth on 2 DAG. Plants were thinned to 4 plants per biofilter on 7 DAG. Cucumbers vines were trellised to overhead wires and pruned to a single leader stem. Fruit were harvested 36 DAG and each week thereafter for four weeks.

An 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 due to presence of residual TAN from applied fertilizer) was added at 11:30 am on 10 Oct. TAN measurements were taken at noon on 10 Oct. and every 6 hrs. thereafter for 24 hrs. Ammonia biofiltration rates in mg/L/d 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 hour TAN measurement and multiplying by 4.

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 5ºC. Nitrifier culture medium for *Nitrosomonas sp*. was prepared and autoclaved as described (ATCC, 2006) on 17 Oct. Ninety grow-out and ten inoculation test tubes were prepared for each of the 5 treatments. The ATCC liquid medium in the test tubes measured 0 mg/L for NO_2^--N and 5 mg/L for NO_3^--N prior to inoculation with nitrifying bacteria core samples. Grow-out test tubes were replicated three times with 30 test tubes per replicate per treatment. On 21 Oct., 200 ml sub-samples of perlite from each treatment were mixed with 200 ml D.I. water and blended in a Hamilton Beech blender for 30 s. One ml of this blended solution was used to inoculate MPN test tubes. Tubes were shaken 8 hrs. per day for 30 days and then measured for nitrifier activity. A measurement $\geq$ 0.1 mg/L

NO_2^--N or ≥ 10 mg/L NO_3^--N was considered positive for *Nitrosomonas* bacteria activity (Eq. 6-1 and Eq. 6-2). NO_2^--N (low range, 0.1 to 0.8 mg/L) was measured with a test kit (LaMotte Company, Chestertown, MD) and NO_3^--N was measured using an ion specific electrode (Cardy meter, range 0 to 9,900 mg/L). One measurement for each replicate (3 measurements per treatment) were recorded based on statistical tables (Feng, 2001) and used to determine data significance by ANOVA (SAS, 2001) and Duncan's Multiple Range Test at the 0.05 level.

Water pH was measured 2 to 6 times per week (avg. of 4 measurements) using pH meter model WD-35624-86 (Oakton Instruments, Vernon Hills, IL). Water dissolved oxygen, specific conductivity (EC), temperature, and salinity were measured once every two weeks using a YSI Model 85 meter (YSI Inc., Yellow Springs, OH).

The experimental design was a randomized complete block design with 4 replications. Data were analyzed using ANOVA (SAS, 2001) and Duncan's Multiple Range Test at the 0.05 level. Significant linear and quadratic trends over the non-repeating equal interval pH units (excluding the hydroponic and aquaculture control treatments) were analyzed using the method of orthogonal polynomials (Gomez and Gomez, 1984).

Results and Discussion

Average high and low greenhouse air temperatures were 35.3 and 24.4, 38.1 and 24.1, 31.7 and 23.5, and 29.4 and 19.4ºC for the months of July, August, September, and October, respectively. Average tank water temperatures were 27.0, 29.3, 25.9, and 23.3ºC and oxygen levels were 5.5, 4.6, 3.9, and 4.4 mg/L for the same four months. Average season tank water TAN was 2.1, 0.7, 0.3, 3.0, and 0.7 and NO_2^--N was 0.2, 1.8, 1.4, 0, and 2.1, respectively, for the five treatments as listed above. Season pH values ranged from 5.5 to 6.7, 6.5 to 7.5, 7.1 to 8.3, 5.5 to 6.8, and 6.5 to 7.4, respectively, for treatments 1) aquaponic pH 6.0, 2) aquaponic pH 7.0, 3) aquaponic pH 8.0, 4) hydroponic control pH 6.0, and 5) aquaculture control pH 7.0.

Nitrification is an acid producing process (ammonia oxidation produces protons H^+ in Eq.6-1) requiring adjustment of recirculating water to maintain target pH levels. Actual pH values were close to the target pH ranges for the treatments.

Visual nutrient deficiency symptoms (mottled yellow middle to lower leaves) were most notable early in the growing season on pH 8.0 plants. The plants grown at pH 7.0 showed light mottled yellow leaf symptoms. These symptoms were significantly reduced at pH 8.0 and disappeared at pH 7.0 when fertilizer applications were increased prior to fruit set. Early marketable cucumber fruit yield (weight and number) decreased linearly as pH increased from 6.0 to 8.0 (Table 6-1). Total marketable and cull cucumber fruit yields were not significantly different among treatments. Results indicate an early yield advantage to keeping nutrient solution pH between 6.0 and 7.0, but not an advantage for total yield. This would indicate that keeping the recirculating pH at 7.5–8.0 to accommodate nitrifying bacteria activity would be detrimental from an economic standpoint only if the grower were producing for an early market window but would not adversely affect the overall production for the year during normal multiple cropped middle and late season periods. Early market windows can occur when seasonal crop production shifts from one region to another and are important in order to target temporary spikes in price from supply interruption or to extend seasonal product availability from the incoming startup production region.

Nitrifying activity is commonly determined by measuring ammonia oxidation, intermediate and end product production such as nitrite or nitrate accumulation, and/or oxygen uptake (Hagopian and Riley, 1998; Prosser, 1986). Ammonia biofiltration rate of the perlite trickling biofilters in aquaponic production was determined by measuring ammonia loss during a 24–hour period after introduction of ammonia to the system water (Table 6-2). This ammonia

surge test was conducted after the last cucumber fruit harvest. Ammonia loss from the system increased linearly as the pH increased from 6.0 to 8.0. The linear increase in ammonia loss could be the result of an increased unionized ammonia (NH_3 - Eq. 6-1) concentration in the system water due to increasing pH (Francis-Floyd and Watson, 1996). Increased biofilter activity occurs with an increase in substrate (NH_3) concentration (Hagopian and Riley, 1998). Linear trends in nitrite buildup (6 h and 12 h samples, Table 6-2), confirm that ammonia oxidation increased at the 0.05 level as pH increased from 6.0 to 8.0. Nitrite accumulation in a steady state biofilter is low compared to a biofilter in startup cycle due to mature populations of nitrifiers able to process nitrite even though inhibition of *Nitrobacter sp.* (Eq. 6-2) at high pH slows the conversion of NO_2^- -N to NO_3^- -N somewhat (Prosser, 1986).

For the system as designed (20 L of trickling perlite biofilter medium for 100 L of recirculating water – 1:5 ratio), ammonia loss from system water was 3.8, 6.1, 16, 1.3, and 5.9 mg/L/day (Table 6-3), for treatments pH 6.0-aqpon, 7.0-aqpon, and 8.0-aqpon, 6.0-hc, and 7.0-ac, respectively. 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 ammonia from these tanks was 0.39, 0.53, and 0.63 mg/L/d for pH 6.0, 7.0, and 8.0, respectively. Thus the difference between these numbers is the minimum ammonia biofiltration occurring by the process of nitrification (7.0-ac, aquaculture control), plant uptake of ammonia (6.0-hc, hydroponic control) and nitrification and plant uptake (6.0-aqpon, 7.0-aqpon, and 8.0-aqpon). Since average greenhouse temperatures were 6ºC 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 ammonia loss in Table 6-2 for each treatment. Considering TAN loss volumetrically, as a function of biofilter volume, 16 mg/L ammonia loss

from 100 L of tank water per day using a 20 L perlite trickling biofilter would be equivalent to 80g TAN removal /m^3 of biofilter/d, which is near the amount recommended for trickling biofilters in aquaculture production–90g TAN/m^3/d (Losordo, et al., 1999). For all treatments, the volumetric (TAN removal per biofilter media volume) results were 19, 31, 80, 6, and 29g TAN /m^3/d, respectively, for the above treatments (Figure 6-3). Comparing 6 g/m^3/d (TAN removal with cucumber plants only), and 19 g/m^3/d (with plants and nitrifying bacteria) at pH 6.0, indicates that nitrifiers were 3.2 times more efficient as plants in removing ammonia from aquaponic system water under the conditions of this experiment. More work should be done to test the nitrification/plant biofiltration relationship during very active stages of plant growth, since the current test was done after the last harvest when plants were 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 for natural air aerated systems (Masser et al., 1999; Megan Davis, personal communication). If tilapia grown at maximum carrying capacity were fed at 1.5% of body weight per day with 30% protein in the feed (10% of protein in feed becomes the ammonia generation – Timmons et al., 2002) then ammonia generation from feed would be producing 13.5 and 27 mg/L/day of ammonia nitrogen for the naturally aerated and oxygen injected systems, respectively. The ammonia removal rate of the designed biofilter in this experiment (perlite volume to tank water volume) 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 system biofilter volume relative to tank water volume would have to increase to match the ammonia generation rate based on the stocking/feeding scenarios for water held at pH 7.0 or 6.0. Thus biofilter volumes need to be

adjusted to compensate for differences in ammonia removal rates caused by production pH. Recirculating systems with high densities of fish may need to be managed with water quality parameters closer to those favoring nitrification (7.5–8.0) in order to efficiently convert waste ammonia.

Results of Most Probable Number (MPN) bacterial cell counts from biofilter core sampling indicate that the aquaculture control (pH 7.0-aqpon) with no plants in the biofilter had a significantly higher (0.01% level) number of *Nitrosomonas sp*. bacteria compared to treatments containing plants in the biofilter, which were not significantly different among themselves (Figure 6-1). In other work, numbers of nitrifying bacteria were reduced 200-fold in the presence of plants than without them as roots were more competitive for ammonium than *Nitrosomonas europaea* (Verhagen et al., 1994). However, in the current trial, aquaponic (plants, fish and bacteria) pH 8.0 had the best ammonia biofiltration rate of all treatments (Table 6-2). MPN bacteria counts (Figure 6-1) were not a good indicator of biofilter performance (Figure 6-2). This indicates that pH of system water is a more important factor in determining biofilter activity than bacterial population and is most likely due to pH induced increases in unionized ammonia available for the nitrification reaction (Eq. 1) as the pH increases. The hydroponic control was not inoculated with nitrifying bacteria but the MPN test indicated a low level of bacteria—2 cells/ml of perlite. Therefore, some unintentional or low level natural inoculation occurred during the growing season.

Two hundred g of fish feed (41% protein with 10% of the protein becoming the ammonia generation amount (Timmons et al., 2002) were used during the trial resulting in 8.2 g of ammonia nitrogen being released into the system water from the fish feed (Table 6-4). Fertilizer was added to tank water during the season to maintain similar overall soluble salt levels in all

treatments. Since there was no plant uptake, the aquaculture control received much less NO_3^--N. Makeup water from the source well had a NO_3^--N concentration of 4 mg/L. Perlite contains 0.06% N and a dry weight of 117 g/L. Since this fraction of the filter could not be separated from the ending filter measurements containing roots and bacteria it was included in the input section. The burlap used to line the biofilter containers was made of jute and hemp plants which together had an N content of 1.6%. The burlap was not recovered separately from the biofilter because of disintegration by the end of the season. The estimate of ammonia and nitrate nitrogen mass balance in the aquaponic treatments (6-,7-,8-aqpon) indicate an average of 51 g total nitrogen input to the system with 16 g being withdrawn for the plant stem biomass and 16 grams for the fruit biomass. Of this nitrogen input total, 38 g consisted of fertilizer nitrogen and 8.2 g from fish feed. The ammonia and nitrite spike in system water which occurred one week after stocking the tanks with fish resulted in large water changes which would normally not have to be made under steady state conditions. Thus the losses of nitrogen from the system observed in the discharge water (Table 6-4) is most likely higher than would be expected. Slightly acidic soils (pH 5.5–6.5) generally favors root growth (Taiz and Zeiger, 2002). The roots growing out of the bottom of the biofilters were recovered. Dry weight and subsequent nitrogen recovered decreased as pH increased. The difference between the input and output nitrogen which averaged 3 g per treatment could be attributed to ammonia volatilization from the system water which was not recoverable.

Although no differences in tilapia growth were expected among treatments due to their adaptation to wide ranges of water quality conditions (Chapman, 2000; Lim and Webster, 2006; Watanabe et al., 1997) there were significant differences in initial feeding activity and fish mortality by treatment (Table 6-5). Initial feeding activity (average data from 5 days), or the

response of fish when feed is first thrown into the re-circulating 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) and results imply that fish were healthier, under less environmental stress, and more likely to survive as pH increased from 6.0 to 8.0.

There are several possible reasons for this difference among treatments that could have occurred in combination. First, there was an ammonia and nitrite spike in the system water seven days after stocking which resulted in gill damage. This damage was diagnosed at the Fish Health Lab at the University of Florida's Fisheries and Aquatic Sciences Department and it was suggested that the fish could recover within three to four weeks provided water ammonia was kept low and adequate aeration maintained. Consequently, water changes were made and aeration added with tank water recirculation rates increased from one to three revolutions through the biofilter per hour. A second possible reason to explain the difference is that the fish population size was variable ranging from a low of 8 g to a high of 122 g at initial stocking. Care was taken at initial stocking and during the season so that the same relative proportion of small and large fish were in each tank to ensure biomass consistency among tanks. However, these size differences contribute to a social hierarchy with more aggressive (male or larger fish) affecting the feeding activity of subordinate fish (Lim and Webster, 2006) especially at low densities, ie, < 100 fish per m^3. At the end of the trial it was noticed that several fish had spawned with live fry in their mouths indicating a mixed population of male and female fish. Third, the available fish population was limited so that fish were moved between tanks (no more than one pH difference) during the experiment to maintain a similar density among treatment tanks. This handling probably also increased stress on the fish. Another reference (Chen et al.,

2001) found an interaction between lethal dissolved oxygen levels and pH. Lethal DO for *O. mossambica* tilapia was 7.14, 4.02, 3.36, 0.84 and 3.20 mg/L at pH 4.0, 5.0, 6.0, 8.3, and 9.6, respectively.

Conclusion

Biofilter removal of total ammonia nitrogen (TAN) increased linearly in a perlite trickling biofilter/root growth medium and occurred at the rate of 3.8, 6.1, and 16 mg/L/d of system water for aquaponic treatments pH 6.0, 7.0, and 8.0, respectively. Maximum volumetric ammonia biofiltration rate for the biofilters was 80 $g/m^3/d$ for aquaponic production at pH 8.0. MPN analysis of *Nitrosomonas sp.* bacteria populations indicated a significantly higher population of bacteria in biofilters without plant roots. However, the highest ammonia biofiltration rate in the trial occurred in aquaponic plots produced at pH 8.0. Thus pH appeared more important than bacteria population in removing ammonia from biofilters. Fish vigor increased as pH increased from 6.0 to 8.0. Early marketable cucumber fruit yield in re-circulating integrated hydroponic and aquaculture (aquaponic) production decreased linearly as pH increased from 6.0 to 8.0. However, there were no differences in total marketable yield among treatments. Results indicate that given the importance of pH in biofilter activity and that total cucumber yields are unaffected by pH in the range of 6.0 to 8.0, then aquaponic systems may be maintained at pH levels more optimum for nitrifying bacteria (7.5–8.0) except during production for early season markets where pH 7.0 would be recommended.

Table 6-1. 'Fitness' cucumber fruit yield response to pH and production system.

Target pH & prod. method	Early marketable[z] kg/plant	Early marketable[z] no/plant	Total marketable kg/plant	Total marketable no/plant	Total cull kg/plant	Total cull no/plant
6.0-aqpon[y]	1.52 a[x]	3.3 a	3.64 a	8.3 a	0.44 a	3.3 a
7.0-aqpon	1.32 a	2.9 a	4.12 a	9.7 a	0.33 a	3.0 a
8.0-aqpon	0.67 b	1.8 b	3.54 a	8.8 a	0.33 a	3.0 a
Contrast[w]	L**	L*				
6.0-hc	1.57 a	3.3 a	3.63 a	8.4 a	0.53 a	3.9 a

[z]Early = first harvest - 36 DAG, Total = four harvests – 36, 43, 50, 58 DAG, Marketable = 34-42 cm in length, less than 45° fruit angle, few blemishes, Cull = greater than 45° fruit angle, poor tip fill, frequent blemishes. Average of 4 plants per plot; 4 reps.

[y]6.0-aqpon, 7.0-aqpon, 8.0-aqon = pH with plants, fish and nitrifying bacteria, 6.0-hc = pH hydroponic control.

[x]Within columns, means followed by different letters are significantly different at the 0.05 level; four replicates.

[w]Linear contrasts were significant at the 5% (*) or 1% (**) level.

Table 6-2. Twenty-four hour total ammonia nitrogen (TAN) and nitrite nitrogen ($N0_2$ -N) concentrations in a perlite trickling biofilter after introduction of ammonium chloride.

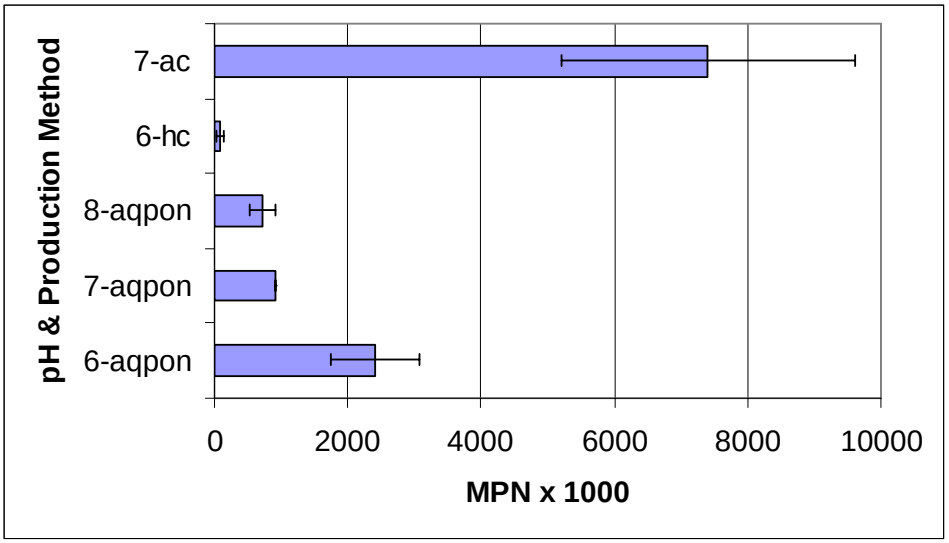
Target pH & prod. method	0[z]	Hours after introduction of ammonia 6	12	18	24
		Total ammonia nitrogen (mg/L)			
6.0-aqpon[y]	6.1 ab[x]	4.9 a	4.0 a	2.9 b	2.4 b
7.0-aqpon	6.4 a	3.4 b	2.1 b	1.3 c	0.3 c
8.0-aqpon	6.0 ab	2.0 c	0.0 c	0.0 d	0.0 c
Contrast[w]		L**	L**	L**	L**Q**
6.0-hc	5.4 b	4.8 ab	4.5 a	4.4 a	4.4 a
7.0-ac	6.5 a	4.5 ab	2.1 b	1.4 c	0.6 c
		Nitrite nitrogen (mg/L)			
6.0-aqpon	0.0 a	0.0 b	0.0 c	0.0 a	0.0 a
7.0-aqpon	0.0 a	0.3 b	0.3 cb	0.1 a	0.1 a
8.0-aqpon	0.0 a	1.4 a	1.1 a	0.2 a	0.0 a
Contrast		L**	L**		
6.0-hc	0.0 a	0.0 b	0.0 c	0.0 a	0.0 a
7.0-ac	0.0 a	0.3 b	0.4 b	0.0 a	0.1 a

[z]Ammonium chloride introduced into the biofilters.
[y]6.0-aqpon, 7.0-aqpon, 8.0-aqon = 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.
[x]Within columns, means followed by different letters are significantly different at the 0.05 level; four replicates.
[w]Linear and quadratic effects were significant at the 5% (*) or 1% (**) level.

Table 6-3. Twenty-four hour TAN loss from recirculating system tank water and perlite trickling biofilter after introduction of ammonium chloride.

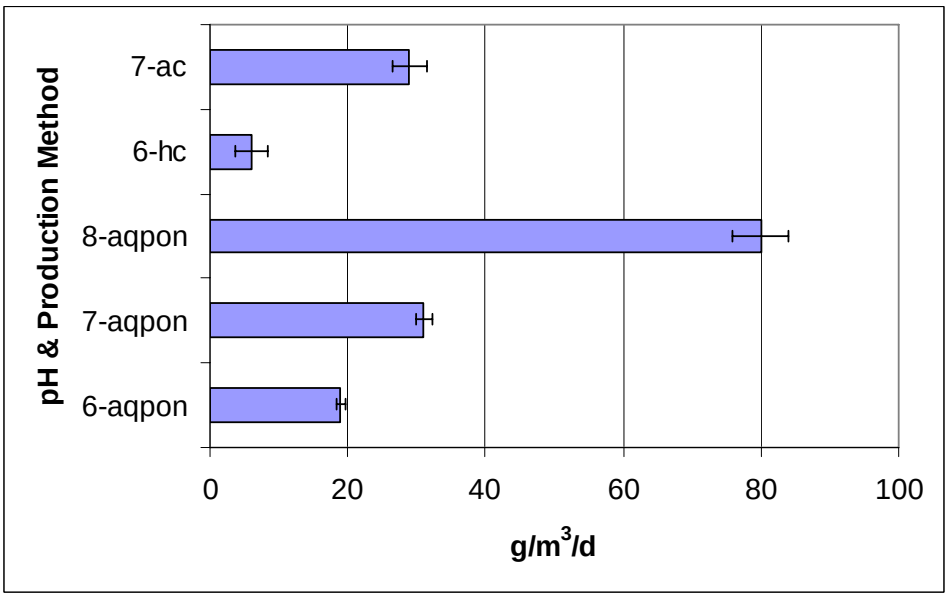
Target pH & Prod. Method	TAN loss[z]	TAN loss[y]
	mg/L/d	$g/m^3/d$
6-aqpon[x]	3.8 c[w]	19 c
7-aqpon	6.1 b	31 b
8-aqpon	16.0 a	80 a
Contrast[v]	L**Q**	L**Q**
6-hc	1.3 d	6 d
7-ac	5.9 b	29 b

[z]Loss of TAN from recirculating tank water.
[y]Loss of TAN converted to biofilter volume.
[x]6.0-aqpon, 7.0-aqpon, 8.0-aqon = 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.
[w]Within columns, means followed by different letters are significantly different at the 0.05 level; four replicates.
[v]Linear effects were significant at 1% (**) level.



Note: 6.0-aqpon, 7.0-aqpon, 8.0-aqon = 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 ± SE (n=3).

Figure 6-1. Most probable number (MPN) of *Nitrosomonas sp*. bacteria in perlite trickling biofilters as influenced by pH and production method.



Note: 6.0-aqpon, 7.0-aqpon, 8.0-aqon = 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 ± SE (n=4).

Figure 6-2. Perlite trickling biofilter 24-hour TAN loss as influenced by pH and production method.

Table 6-4. Estimate of ammonia and nitrate nitrogen mass balance in an aquaponic system with a raised bed perlite media trickling biofilter.

Nitrogen Source	pH and production method[z] 6-aqpon	7-aqpon	8-aqpon	6-hc	7-ac
			Nitrogen input – g/tank		
Fish:					
Stocking	12.3	12.0	13.0	-	12.5
Feed - NH_4^+-N	8.2	8.2	8.2	-	8.2
Fertilizer:					
NO_3^--N	35.6	35.6	35.6	34.9	7.2
NH_4^+-N	2.2	2.2	2.3	3.0	2.2
Makeup water:[y]					
NO_3^--N	2.9	3.0	2.9	2.1	2.2
Biofilter:					
Perlite	1.4	1.4	1.4	1.4	1.4
Burlap	0.5	0.5	0.5	0.5	0.5
Total nitrogen	63.1	62.9	63.9	41.9	34.2
			Nitrogen output – g/tank		
Plant:					
Shoot	16.7	15.7	15.7	15.7	-
Root	0.12	0.02	0.01	0.30	-
Fruit	15.1	16.6	16.8	15.0	-
Fish:					
Net loss	8.4	6.2	4.4	-	4.6
Harvest	5.7	6.2	6.1	-	5.5
Filters:					
Perlite	4.4	5.4	4.3	2.9	4.4
Solids	0.24	0.17	0.24	0.01	0.11
Discharge water:					
NO_3^--N	8.8	9.0	12.4	4.1	15.0
NO_2^--N	0.1	1.0	0.8	0.0	1.1
NH_4^+-N	1.8	0.04	0.0	1.3	0.04
Total nitrogen	61.4	60.3	60.7	39.3	30.7

[z]6.0-aqpon, 7.0-aqpon, 8.0-aqon = 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.
[y]Well water contained 4 mg/L NO_3^--N.

Table 6-5. Tilapia initial feeding activity and overall mortality as influenced by system water pH and production method.

Target pH & prod. Method	Initial feeding[z] activity	Fish[y] mortality
6.0-aqpon[x]	1.6 b[w]	18.5 a
7.0-aqpon	2.3 ab	8.0 b
8.0-aqpon	4.0 a	3.4 b
Contrast[v]	L**	L**
7.0-ac	3.0 ab	8.8 b

[z]Initial feeding activity rating: 1 = don't come to feed, 3 = half strike feed, and 5 = all strike feed.
[y]Avg. no. per tank dead during growing season.
[x]6.0-aqpon, 7.0-aqpon, 8.0-aqon = 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.
[w]Within columns, means followed by different letters are significantly different at the 0.05 level; four replicates.
[v]Linear effects were significant at 1% (**) level.

CHAPTER 7
CONCLUSIONS

Combining hydroponic plant and aquaculture fish production systems (aquaponics) requires reconciling water quality parameters for the survival and growth of plants, fish, and nitrifying bacteria. Aquaponics has the potential to be a sustainable minimum discharge or zero agricultural discharge system since the waste by-products of aquaculture can be used by plants in hydroponic systems. However, with all its promise as a sustainable alternative to conventional food production, many unanswered questions must be resolved regarding optimum water quality parameters when the organisms present in aquaponics are grown together.

Aquaculture production water pH is recommended to be between 6.5 and 8.5. However, there is a dichotomy between the optimum pH for plant nutrient availability in hydroponics (pH 5.5–6.5; Hochmuth, 2001a) and pH maintained at levels more optimum for nitrifying bacteria activity (7.5–9.0; Hochheimer and Wheaton, 1998). There is no information in the literature on how aquaponic system water quality impacts nitrification when perlite growth medium is used as the biofilter medium, little information on the plant/nitrification interactions in root growth media biofilters, and how this interaction affects ammonia biofiltration and plant yield. In addition, systems management would be improved with the addition of fertilizer nutrients to aquaponic system water to optimize plant nutrient levels but science based information is needed before recommendations can be made. The reconciling pH in aquaponic systems for plant production and nitrification will be affected by their relative importance as biological filters and the pH effects on plant yields and nitrification. In order to improve the integration of sustainable aquaponic systems, a series of trials were conducted to 1) determine the optimum pH for nitrification and evaluate perlite as an aquaponic biofilter/root growth medium 2) determine the affect of hydroponic nutrients on nitrification, 3) make predictions about the relative contribution

of plants and nitrifiers to the biofiltration of ammonia, and 4) establish a reconciling pH for ammonia biofiltration and cucumber yield in an aquaponic production system.

Nitrification activity in a perlite medium trickling biofilter, as evidenced by ammonia loss and nitrite accumulation, increased linearly as pH increased from 6.5 to 8.5. Biofilter removal of TAN was 19, 31, and 80 $g/m^3/d$ for aquaponic perlite biofilters operating at pH 6.0, 7.0, and 8.0, respectively, at average tank water temperatures of 22.2ºC. Optimum performance for tricking biofilters in aquaculture have been reported as 90 $g/m^3/d$. Thus perlite, in addition to being a common medium for plant growth, provides adequate TAN removal rates when used as a biofilter/root zone media in aquaponic production at pH 8.0.

No difference in nitrification rate was found when recirculating system water contained no nutrient solution versus a complete hydroponic nutrient solution at nitrate nitrogen concentrations of 100 or 200 mg/L. Thus fertilizer nutrients, at levels commonly used in hydroponics, may be added to aquaponic systems if needed to provide optimum plant nutrition with no significant adverse impact on nitrifying bacteria. The concentration of certain elements (Ca, Fe, and Mn) in the nutrient solution declined as pH increased from 5.0 to 8.0. Nutrient depletion of the root zone can occur with low nutrient solution levels and intermittent irrigation applications. However, since the nutrient solution was continuously recirculating, cucumber shoot uptake was within or near the sufficiency range. Ammonia biofiltration was 3.7 times higher for aquaponic treatments (plants, fish and nitrifying bacteria) at pH 6.0 compared to a hydroponic control (plants only) at pH 6.0 indicating that nitrification activity contributes significantly more to TAN loss from system water than plant removal of ammonia. The presence of roots in the perlite biofilter reduces the most probable number (MPN) cell count of *Nitrosomonas sp.* bacteria compared to biofilters without plants. However, pH was a more

significant factor affecting ammonia biofiltration than bacteria population with removal rates maximized at aquaponic treatment pH 8.0. Even though early marketable cucumber fruit yield decreased linearly as pH increased from 5.0 to 8.0 and 6.0 to 8.0, total marketable yield was unaffected.

For the recirculating aquaponic system under study—perlite trickling biofilter / root growth medium with cucumber and tilapia—the greatest ammonia biofiltration (nitrification and plant removal of TAN) was 80 $g/m^3/d$ and occurred at pH 8.0. This was four times the amount of TAN removal at pH 6.0. Keeping pH at more optimum levels for nitrifying bacteria activity will allow increased fish stocking density. This will provide additional plant nutrients from the waste stream and reduced need for fertilizer supplementation, thus increasing systems integration and sustainability. In addition, systems management may be improved by the addition of fertilizer to system water when needed without harm to nitrifying bacteria. The reconciling pH for ammonia biofiltration and cucumber yield in this recirculating aquaponic system should be pH 7.5–8.0 given the importance of pH to the ammonia biofiltration rate and given that no difference in total cucumber fruit yield among treatments was found.

REFERENCES

Adler, P. 2001. Overview of economic evaluation of phosphorus removal by plants. Aquaponics J., 5:15–18.

Adler, P.R., J.K. Harper, F. Takeda, E.D. Wade and S.T. Summerfelt. 2000. Economic evaluation of hydroponics and other treatment options for phosphorus removal in aquaculture effluent. HortScience, 35(6):993–999.

Adler, P.R., F. Takeda, D.M. Glenn and S.T. Summerfelt. 1996. Utilizing byproducts to enhance aquaculture sustainability. World Aquaculture, 27(2):24–26.

Alleman, J.E. 1985. Elevated nitrate occurrence in biological waste-water treatment systems. Water Sci. Technol. 17:407–419.

Anonymous. 2007. Basic facts about perlite. The Perlite Institute, Inc., Harrisburg, PA. Retrieved April 1, 2007, from http://www.perlite.org/

Anonymous. 1998. Linking hydroponics to an 880 gallon recycle fish rearing system. The Conservation Fund's Freshwater Institute, Shepherdstown, WV, 17 pgs.

Anonymous. 1997. The Freshwater Institute natural gas powered aquaponic system design manual. The Conservation Fund Freshwater Institute, Shepherdstown, WV, 37 pgs.

Antoniou, P., J. Hamilton, B. Koopman, R. Jain, B. Holloway, G. Lyberatos and S.A. Svoronos. 1990. Effect of temperature and ph on the effective maximum specific growth rate of nitrifying bacteria. Water Research, 24(1):97–101.

ATCC. 2006. ATCC 221 Nitrosomonas, American Type Culture Collection, Manassas, VA. Retrieved October 2, 2006 from http://www.bibliographics.com/LAB/PCR/NITRIFIER%20CULTURE%20MEDIA.htm

Bernardo, L.M., R.B. Clark and J.W. Maranville. 1984. Nitrate/ammonium ratio effects on nutrient solution pH, dry matter yield, and nitrogen uptake of sorghum. J. Plant Nut. 7(10):1389–1400.

Brentlinger, D. 1999. Status of the commercial hydroponic industry in the United States. Proc. Intl. Symp. Growing Media Hydroponics. Acta Hort. 481:731–734.

Bronson, C.H. 2005. Florida Agricultural Statistical Directory 2005. Florida Department of Agriculture and Consumer Services, Florida Agriculture Statistics Services, Tallahassee, FL.

Brunty, J.L. 1995. Biological filtration for ornamental fish production and factors affecting total ammonia nitrogen and nitrite removal rates. University of Florida, Dept. of Agriculture & Biological Engineering, M.S. Thesis:143 pgs.

Campbell N.A. and J.B. Reese. 2002. Biology. 6th Ed., Pearson Education Inc. (Benjamin Cummings) San Francisco, CA.

Cantliffe, D.J. and J.J. VanSickle. 2000. European greenhouse industry: Growing practices and competitiveness to U.S. markets. Proc. Fla. Tomato Institute, C.S. Vavrina (ed.), University of Florida, Gainesville, 517:6–9.

Chapman, F.A. 2000. Culture of hybrid tilapia: a reference profile. Circular 1051. Gainesville: Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Retrieved April 5, 2007 from http://edis.ifas.ufl.edu/FA012

Chen, Su-Mei, Chen, Ping-Chun, Hsiao, I-Chuan and Jiann-Chu Chen. 2001. Effects of pH on the nitrogenous excretion and lethal do of tilapia *Oreochromis mossambica*. 6th Asian Fisheries Forum Book of Abstracts:51.

Chaverria, C. J., G. Hochmuth, R. Hochmuth, and S. Sargent. 2005. Fruit yield, size, and color responses of two greenhouse cucumber types to nitrogen fertilization in perlite soilless culture. HortTechnology 15:565–571.

Cockx, E. and E.H. Simonne. 2003. Reduction of the impact of fertilization and irrigation on processes in the nitrogen cycle in vegetable fields with BMPs. HS948. Gainesville. Horticultural Sciences Department, Florida Cooperative Extension Service, University of Florida. Retrieved April 5, 2007 from http://edis.ifas.ufl.edu/HS201

Colt, J.E. and G. Tchobanoglous. 1976. Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus*. Aquaculture 8:209–224.

Debusk, W.F. 1999. Wastewater treatment wetlands: contaminant removal processes. University of Florida Soil and Water Science Fact Sheet SL 155:4 pgs.

De Rijck, G. and E. Schrevens. 1999. Anionic speciation in nutrient solutions as a function of pH. J. Plant Nut. 22:269–279.

Diver, S. 2006. Aquaponics – integration of hydroponics and aquaculture. Apropriate Technology Transfer for Rural Areas: Horticulture Systems Guide, Fayetteville, AR:21 pgs. www.attra.ncat.org

EPA Nonpoint Source News-Notes. 2003. Phosphorus reduction in the Everglades. IN71. U.S. Environmental Protection Agency. Retrieved April 5, 2007 from http://notes.tetratech-ffx.com/newsnotes.nsf/606a2768c7ff5f63852565ff0061ae0d/be2994b893f9129d85256d0100618bbf?OpenDocument

Epstein, E. and A.J. Bloom. 2005. Mineral nutrition of plants: principles and perspectives, 2nd Ed. Sinauer Associates, Inc., Publishers, Sunderland, MA:400pgs.

Feng, P. 2001. Most probable number from serial dilutions. Bacterial Analytical Manual Online, U.S. Food and Drug Administration. , Final Revision: 2001-Jan-25. Retrieved January 2, 2007 from http://www.cfsan.fda.gov/~ebam/bam-a2.html

Feray, C. B. Volat, V. Derange, A. Clays-Josserand and B. Montuelle. 1999. Assessment of three methods for detection and quantification of nitrite-oxidizing bacteria and *Nitrobacter* in freshwater sediments (MPN-PCR, MPN-Griess, Immunofluorescence). Microb. Ecol. 37:208–217.

Fitzimmons, K. 2003. Tilapia aquaculture in recirculating systems. Aquaculture 29 (2):73–76.

Flanagan, W.P., W.A. Apel, J.M. Barnes, B.D. Lee. 2002. Development of gas phase bioreactors for the removal of nitrogen oxides from synthetic flue gas systems. Fuel, 18 (5):1953–1961.

Fowler, P., D. Baird, R. Bucklin, S. Yerlan, C. Watson, and F. Chapman. 1994. Microcontrollers in recirculating aquaculture systems. University of Florida Energy Extension Service EES–326:7 pgs.

Francis-Floyd, R. and C. Watson. 1996. Ammonia. FA-16. Gainesville. Department of Fisheries and Aquatic Sciences. Florida Cooperative Extension Service, University of Florida. Retrieved April 5, 2007 from http://edis.ifas.ufl.edu/FA031

Fuller, S.A., J.P Henne, G.J. Carmichael and J.R. Tomasso. 2003. Toxicity of ammonia and nitrite to the Gila Trout. N. American J. Aquaculture 65(2):162–164.

Good, A.G., Shrawat, A.K. and D.G. Muench. 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? Trends in Plant Science 9:597–605.

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research. John Wiley & Sons, New York, N.Y.: 680 pgs.

Grossman, A. and H. Takahashi. 2001. Macronutrient utilization by photosynthetic eukaryotes and the fabric of interactions. A. R. of Plant Physiol. Plant Molecular Biology 52:163–210.

Grotz, N., and M.L. Guerinot. 2002. Limiting nutrients: an old problem with new solutions. Current Opinion in Plant Biology 5:158–163.

Gutierrez-Wing, M. and R. F. Malone. 2006. Biological filters in aquaculture: trends and research directions for freshwater and marine applications. Aquacultural Engineering 34:163–171.

Hagopian, D.S. and J.G. Riley. 1998. A closer look at the bacteriology of nitrification. Aquacultural Engineering 18:223–244.

Hamilton, J.L. and R. H. Lowe. 1981. Organic matter and N effects on soil nitrite accumulation and resultant nitrite toxicity to tobacco transplants. Agron. J. 73:1981.

Harmon, T., 2001. A look at filtration in aquaponic systems: bead filters. Aquaponics J., 3:16–19.

Hassall & Associates. 2001. Hydroponics as an agricultural production system. Rural Industries Research and Development Corporation, government of Australia, Kingston, ACT, project no HAS-9A, publ. no 01/141.

Heimlich, R.E. and W.D. Anderson. 2001. Development at the urban fringe: impacts on agriculture and rural lands. Economic Research Service, U.S. Department of Agriculture. Agricultural Economic Report No. 803.

Hochheimer, J.N. and F. Wheaton. 1998. Biological filters: trickling and RBC design. Proc. Second International Conference of Recirculating Aquaculture:291–318.

Hochmuth, G. 2003. Plant petiole sap-testing for vegetable crops. CIR1144. Gainesville. Horticultural Sciences Department, Florida Cooperative Extension Service, University of Florida. Retrieved April 5, 2007 from http://edis.ifas.ufl.edu/CV004

Hochmuth, G. J. 2001a. Fertilizer management for greenhouse vegetables, Florida greenhouse vegetable production handbook, volume 3. HS787. Gainesville. Horticultural Sciences Department, Florida Cooperative Extension Service, University of Florida. Retrieved April 5, 2007 from http://edis.ifas.ufl.edu/CV265

Hochmuth, R.C. 2001b. Greenhouse Cucumber Production - Florida Greenhouse Vegetable Production Handbook, Vol 3. HS790. Gainesville. Horticultural Sciences Department, Florida Cooperative Extension Service, University of Florida. Retrieved April 5, 2007 from http://edis.ufl.wdu/CV268

Hochmuth, G. and R. Hochmuth. 2003. Keys to successful tomato and cucumber production in perlite media. HS927. Gainesville. Horticultural Sciences Department, Florida Cooperative Extension Service, University of Florida. Retrieved April 5, 2007 from http://edis.ifas.ufl.edu/HS169

Hochmuth, G.J., Chaverria, C. J., R.C. Hochmuth, S.C. Stapleton, S.A. Sargent, E.M. Lamb, and M.A. Wade. 2002. Field soilless culture as an alternative to soil methyl bromide for tomato and pepper. Proc. Fla. State Hort. Soc. 115:197–199.

Hochmuth, R.C., L.L. Davis and G.J. Hochmuth. 1996. Evaluation of twelve greenhouse cucumber cultivars and two training systems over two seasons in Florida. North Florida Research and Education Center, Suwannee Valley, Research Report 96–10. Retrieved April 5, 2007 from http://nfrec-sv.ifas.ufl.edu/Reports%20HTML/96-10_report.htm

Hochmuth, G.J. and E.A. Hanlon. 1995. IFAS standard fertilization recommendations for vegetable crops. Circular1152. Horticultural Sciences Department, Florida Cooperative Extension Service, University of Florida, Gainesville, FL.

Hochmuth, B. and E. Belibasis. 1991. Costs of production for greenhouse tomatoes in North Florida. North Florida Research & Education Center, Suwannee Valley Report 91–20:9pgs.

Hrubrec, T.C., S.A. Smith and J.L. Robertson. 1996. Nitrate toxicity: a potential problem of recirculating systems. Successes and failures in commercial recirculating aquaculture; Aquacultural Engineering Society Proceedings II, Eds. G.S. Libey and MB Timmons, NARES Vol I:41–48.

Jensen, M.H. 1997. Hydroponics. HortScience, 32(6):1018–1021.

Johnson, H., Hochmuth, G.J., and D. N. Maynard. 1985. Soilless culture of greenhouse vegetables. Bulletin 218. Horticultural Sciences Department, Florida Cooperative Extension Service, University of Florida, Gainesville, Fl.

Jones, S. 2001. The birth of an aquaponic facility. Aquaponics J. 5(1):8–13.

Joshi, J.A., J.A. Hogan, R.M. Cowan, P.F. Strom, M.S. Finstein. 2000. Biological removal of gaseous ammonia in biofilters: Space travel and earth-based applications. J. Air and Waste Management Assoc. 50 (9):1647–1654.

Kovach, S. P. 1984. Determination of water requirements for Florida vegetable crops. Circular 607. Horticultural Sciences Department, Florida Cooperative Extension Service, University of Florida, Gainesville, FL..

Lang. H.J. and G.S. Elliott. 1997. Enumeration and inoculation of nitrifying bacteria in soilless potting media. J. Amer. Soc. Hort. Sci. 122(5):709–714.

Li, Y. 2001. Calcareous soils in Miami-Dade county. Fact Sheet SL 183. Gainesville. Soil and Water Science Department, Florida Cooperative Extension Service, University of Florida. Retrieved April 5, 2007 from http://edis.ifas.ufl.edu/TR004

Lim, C. and C.D. Webster. 2006. Tilapia: Biology, culture, and nutrition. The Food Products Press, Binghamton, N.Y.:678 pgs.

Lin, Y.F., S.R. Jing, D.Y.Lee, and T.W. Wang. 2002. Nutrient removal from aquaculture wastewater using a constructed wetlands system. Aquaculture, 209:169–184.

Lorsordo, T.M., M.P. Masser and J.E. Rakocy. 1999. Recirculating aquaculture tank production systems: a review of component options. S. Region Aquaculture Center Publication No. 453:12 pgs.

Losordo, T.M., M.P. Masser and J. Rakocy. 1998. Recirculating aquaculture tank production systems: an overview of critical considerations. S. Region Aquaculture Center Publication No. 451:6 pgs.

Madigan, M.T., J.M. Martinko and J. Parker. 2003 Brock Biology of Microorganisms. Pearson Education, Inc., Upper Saddle River, NJ, 10th Ed.:1019pgs.

Marschner, H. 2003. Mineral nutrition of higher plants. Academic Press, Elsevier Science Ltd.:889pgs.

Marr, C.W. 1995. Greenhouse cucumbers. Kansas State University Ag. Exp. Station Coop. Extension Service Commercial Greenhouse Production Publication MF-2075:4pgs.

Masser, M.P., J. Rakocy, and T.M. Losordo. 1999. Recirculating aquaculture tank production systems: management of recirculating systems. S. Regional Aquaculture Center Publication No. 452:12 pgs.

Maynard, D.N. and G.J. Hochmuth. 1997. Knott's Handbook for Vegetable Growers, 4th Ed. John Wiley and Sons, Inc. New York, N.Y.

McGee, M., and C. Cichra. 2000. Principles of water recirculation and filtration in aquaculture. FA-12. Gainesville. Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, University of Florida. Retrieved April 5, 2007 from http://edis.ifas.ufl.edu/FA050

McMurtry, M.R., D.C. Sanders, J.D. Cure, R.G. Hodson. 1997. Effects of biofilter/culture tank volume ratios on productivity of a recirculating fish/vegetable co-culture system. J. Applied Aquaculture 7(4):33–51.

McMurtry, M.R., P.V. Nelson, D.C. Sanders and L. Hodges. 1990. Sand culture of vegetables using recirculated aquacultural effluents. Applied Agricultural Res. 5:280–284.

Mills, H. and J.B. Jones. 1996. Plant Analysis Handbook I. Micromacro Publishing, Inc., Athens, GA:422pgs.

Mitsch, W.J. and J.G. Gosselink. 2000. Wetlands, 3rd Edition. John Wiley & Sons, Inc., New York, NY: 920 pgs.

Myers, R. 2003. The Basics of Chemistry. Greenwood Press, Westport, CT.

National Agricultural Statistics Service. 2006. National statistics: cucumbers. http://www.nass.usda.gov/

Neal, C.A., R.V. Tyson, E.A. Hanlon, J.M. White and S. Cox. 1996. Reducing nutrient applications for vegetable production in the Lake Apopka basin. Proc. Fla. State Hort. Soc. 109:156–159.

Olson, C. 1950. The significance of concentration on the rate of ion absorption by higher plants in water culture. Physiol. Plant. 3:152–164.

Olson, S.M., E. H. Simonne, W.M. Stall, P.D. Roberts, S.E. Webb, T.G. Taylor and S.A. Smith. 2006. Cucurbit production in Florida. HS725. Gainesville. Horticultural Sciences Department, Florida Cooperative Extension Service, University of Florida. Retrieved April 5, 2007 from http://edis.ifas.ufl.edu/CV123

Olson, S.M., W.M. Stall, M.T. Momol, S.E. Webb, T.G. Taylor, S.A. Smith, E.H. Simonne and E. McAvoy. 2006. Tomato production in Florida. HS739. Gainesville. Horticultural Sciences Department, Florida Cooperative Extension Service, University of Florida. Retrieved April 5, 2007 from http://edis.ifas.ufl.edu/CV137

Paul, M.J. and T.K. Pellny. 2002. Carbon metabolite feedback regulation of leaf photosynthesis and development. J. Exp. Bot. 54:539–547.

Pearce, R.C., Y. Zhan and M.S. Coyne. 1998. Nitrogen transformations in the tobacco float tobacco system. Tobacco Science 42:82–88.

Papen, H. and R. von Berg. 1998. A Most Probable Number method (MPN) for the estimation of cell numbers of heterotrophic nitrifying bacteria in soil. Plant & Soil 199:123–130.

Peng, Y.-Z., J.-F. Gao, S.-Y. Wang and M.-H. Sui. 2003. Use of pH as fuzzy control parameter for nitrification under different alkalinity in SBR process. Water Science Technology 47-11:77–84.

Plank, C.O. 1992. Plant analysis reference procedures for the southern region of the United States. Southern Cooperative Series Bulletin #368, Georgia Agricultural Experiment Station, University of Georgia, Athens, GA.

Prinsloo, J.F., W. Roets, J. Theron, L.C. Hoffman, and H.J. Schoonbee. 1999. Changes in some water quality conditions in recycling water using three types of biofiltration systems during the production of the sharptooth catfish Clarias gariepinus (Burchell). Part1. Relative efficiency in the breakdown of nitrogenous wastes by the different biofiltration units. Water SA 25(2):239–252.

Prosser, J.I. 1986. Nitrification. Society for Microbiology, Special Publication Volume 20:217 pgs.

Rakocy, J.E. 1999. Aquaculture engineering – the status of aquaponics, part 1. Aquaculture Magazine 25 (4):83–88.

Rakocy, J.E. 1997. Integrating tilapia culture with vegetable hydroponics in recirculating systems. World Aquaculture Society, Tilapia Aquaculture in the Americas 1:163–184.

Rakocy, J.E., T.M. Losordo, and M.P. Masser. 2006. Recirculating aquaculture tank production systems: Aquaponics - integrating fish and plant culture. S. Region Aquaculture Center Publication No. 454:16 pgs.

Rakocy, J.E., D.S. Bailey, K.A. Shultz, and W.M. Cole. 1997. Evaluation of a commercial-scale aquaponic unit for the production of tilapia and lettuce. Fourth International Symposium on Tilapia in Aquaculture 1:357–372.

Redner, B.D. and R.R. Stickney. 1979. Acclimation to ammonia by *Tilapia aurea*. Transactions of the American Fisheries Society 108:383–388.

Reed, D.W. 1996. Water, media, and nutrition for greenhouse crops. Ball Publishing, Batavia, IL: 314 pgs.

Resh, H.M. 2004. Hydroponic Food Production, 6th ed. New Concept Press Publishing Co., Mahwah, NJ.

Rhodes, T.L. 2001. Florida Agricultural Statistics: vegetable summary, 1999-2000. Florida Department of Agriculture and Consumer Services, Tallahassee, FL.

Rijn, J.V. and G. Rivera. 1990. Aerobic and anaerobic biofiltration in an aquaculture unit – nitrite accumulation as a result of nitrification and denitrification. Aquacultural Engineering 9: 217–234.

Schon, M.K. and M.P. Compton. 1997a. Comparison of cucumbers grown in rockwool or perlite at two leaching fractions. HortTechnology 7:30–33.

Schon, M.K. and M.P. Compton. 1997b. Nitrogen and phosphorus requirements for rockwool grown cucumbers trained with a double-stem method. HortTechnology 7:33–38.

Schwarz, D., S. Ruppel and R. Kuchenbuch. 1999. Nitrogen cycle and microorganisms in a hydroponic system as influenced by the amount of nitrogen applied. Proc. Int. Sym. Growing Media and Hydroponics, Acta Hort. 481: 371–377.

Seawright, D.E., R.R. Stickney and R.B. Walker. 1998. Nutrient dynamics in integrated aquaculture-hydroponics systems. Aquaculture 160:215–237.

Shaw, N.L., D.J. Cantliffe, J.C. Rodriguez, S. Taylor, and D.M. Spencer. 2000. Beit Alpha cucumber – an exciting new greenhouse crop. Proc. Fla State Hort. Soc.113:247–253.

Shnel, N., Y. Barak, T. Ezer, Z. Dafni and J.van Rijn. 2002. Design and performance of a zero-discharge tilapia recirculating system. Aquacultural Engineering 26:191–203.

Simonne, E.H., H.A. Mills and D.A. Smittle. 1992. Ammonium reduces growth, fruit yield and fruit quality of watermelon. J. Plant Nut. 15:2727–2741.

Smith, J., T. Hewitt, R. Hochmuth and G. Hochmuth. 2003. A profitability and cash flow analysis of typical greenhouse production in north Florida using tomato as an example. North Florida Research and Education Center - Suwannee Valley, Live Oak, Fl:11 pgs.

Smither-Kopperl, M.L. and D.J. Cantliffe. 2004. Protected agriculture as a methyl bromide alternative? Current reality and future promise. Proc. Fla. State Hort. Soc. 117:21–27.

Smorczewski, W.T. and E.L. Schmidt. 1991. Numbers, activities, and diversity of autotrophic ammonia-oxidizing bacteria in a freshwater, eutrophic lake sediment. Can. J. Microbiol. 37:828–833.

Spillane, M. 2001. Fresh greens from Quebec. The Growing Edge, Vol. 12, No. 6:52–59.

Ste-Marie, C. and D. Pare, 1999. Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. Soil Biol. Bioch. 31:1579–1589.

Steta, M. 2004. Mexico as a new player in the vegetable greenhouse industry. International Society for Horticultural Science, Acta Hort. 659:31–36.

Taiz, L. and E. Zeiger. 2002. Plant Physiology, 3rd Edition. Sinauer Associates, Inc., Publishers, Sunderland, MA:690 pgs.

Tetzlaff, B.L., and R.C. Heidinger. 1990. Basic principles of biofiltration and system design. SIUC Fisheries and Illinois Aquaculture Center, Bulletin 9:17 pgs.

Tillman, D. 1999. Global environmental impacts of agricultural expansion: The need for sustainable and efficient practices. Proceedings of the National Academy of Sciences of the United States of America 96:5995–6000.

Timmons, M.B., J.M. Ebeling, F.W. Wheaton, S.T. Summerfelt, and B.J. Vinci. 2002. Recirculating aquaculture systems, 2nd Edition. Northeast Regional Aquaculture Center Publication No. 01–002:769 pgs.

Tyson, R. 2004. Water and nitrogen budgets for aquaponic systems. University of Florida Horticultural Sciences Dept., Vegetarian Newsletter 04–09:7.

Tyson, R. 2002. Cropping systems for greenhouse vegetables in Florida. Proc. Fla. Agricultural Conference and Tradeshow:17–18.

Tyson, R.V., R.C. Hochmuth, E.M. Lamb, G.J. Hochmuth, and M.S. Sweat. 2001. A decade of change in Florida's greenhouse vegetable industry: 1991-2001. Proc. Fla State Hort. Soc. 114:280–283.

Tyson, R.V., J.M. White, and K.W. King, 1999. Outdoor floating hydroponic systems for leafy salad crop and herb production. Proc. Fla State Hort. Soc. 112:313–315.

Tyson, R.V., C.A. Neal, L.I. Morrell. 1996. Buyout plan for Zellwood area vegetable farms. Citrus and Vegetable Magazine, Vance Publishing, Tampa, FL, August/96:45.

Van Gorder, S.D. 2000. Small scale aquaculture. Alternative Agriculture Association, Breinigsville, PA: 190 pgs.

Verhagen,F.J.M., P.E.J. Hageman, J.W. Woldendorp and H.J. Laanbroek. 1994. Competition for ammonium between nitrifying bacteria and plant roots in soil pots; effects of grazing flagellates and fertilization. Soil Biol. Bioch. 26:89–96.

Villaverde, S., P.A. Garcia-Encina and F. Fdz-Polanco, 1997. Influence of pH over nitrifying biofilm activity in submerged biofilters. Water Res. 31:1180–1186.

Wallace A. and G.A. Wallace. 1993. Limiting factors, high yields, and law of the maximum. Hort. Reviews 15:409–448.

Wallihan, E.F., R.G. Sharpless and W. L. Printy. 1977. Effect of pH on yield and leaf composition of hydroponic tomato. HortScience 12(4):316–317.

Watanabe, W.O., B.L. Olla, R.I. Wicklund and W.D. Head. 1997. Saltwater culture of Florida red tilapia and other saline-tolerant tilapias: a review. World Aquaculture Society, Tilapia Aquaculture in the Americas 1:54–141.

Watten, B.J. and R.L. Busch. 1984. Tropical production of tilapia (*Sarotherodon aurea*) and tomatoes (*Lycopersicon esculentum*) in a small-scale recirculation water system. Aquaculture 41:271–283.

Weirich, C.R., J.R. Tomasso and T.I.J. Smith. 1993. Toxicity of ammonia and nitrite to sunshine bass in selected environments. J. of Aquatic Health 5–1:64–72.

Wheaton, F.W. 1993. Aquacultural engineering. Krieger Publishing Company, Malabar, FL: 708 pgs.

Wright, P.C., and J.A. Raper. 1998. Investigation into the viability of a liquid-film three-phase spouted bed biofilter. J. Chemical Technology and Biotechnology, 73 (3):281-291.

Zaiter, H.Z. and I. Saad. 1993. Yield of iron-sprayed and non-sprayed strawberry cultivars grown on high pH calcareous soil. J. Plant Nut. 16:281-296.

BIOGRAPHICAL SKETCH

Richard V. Tyson was born on November 30, 1950 in West Palm Beach, Florida. The youngest of three children, he grew up in Pahokee, Florida, graduating from Pahokee High School in 1968. Richard attended Florida State University during the 1968–69 school year and played offensive guard on the freshman football team. During 1969–70, he attended Palm Beach Community College and then served in the United States Army Medical Corp from 1970–1973 as a Clinical Specialist.

During the next several years, Richard worked with a landscape company, was co-owner of a retail plant store, and worked for the United States Sugar Corporation as a clarifier operator and juice chemist. He earned his Associate of Arts degree from Palm Beach Community College in 1977 and entered the University of Florida Horticultural Sciences Department in 1978. He received his Bachelor of Science degree in 1980, majoring in plant science, and a Certificate in Tropical Agriculture the same year. He entered graduate school in the same department and received a Master of Science in 1983 with a vegetable crop emphasis.

Richard began serving as a commercial vegetable Extension Agent in Dade County, Florida in the fall of 1982 where he served for five years. He then took a position with Collier Farms, Immokalee, FL, a subsidiary of Collier Enterprises, as tomato division manager from 1987 to 1994. During that time, he managed the production and harvesting of an average of 1,000 acres of tomatoes each year with double cropped production of watermelons, sweet corn, cucumbers, yellow squash, and zucchini.

Richard became a science instructor at Immokalee High School in 1994. He taught classes in marine biology, honors physics, and physical sciences. In 1995, he moved to central Florida to take the position of multicounty commercial vegetable Extension Agent in five Florida counties: Seminole, Volusia, Orange, Lake, and Sumter. He was housed with the Seminole

County Extension Service in Sanford, FL. In 1999, the position changed to include 50% commercial turfgrass in three counties and landscape maintenance responsibility in one county, in addition to the commercial vegetable duties.

Richard received several awards as an Extension Agent including the Sadler Distinguished Extension Professional and Enhancement Award and the Marshall and Mildred Watkins Professional Improvement Award. He was also a national winner in the National Association of County Agricultural Agents, Search for Excellence in Crop Production program.

On a personal level, Richard was married to Gladys Moreno in June of 1981. Their daughter Natalie Lissette was born in October of 1984 and their son Alexander James was born in August of 1987. He has served in various leadership roles in the United Methodist Church in the communities he has lived including chairman of the Leadership Council of the First United Methodist Church in Sanford for three years. Richard and Gladys celebrated their 25th wedding anniversary in 2006.

Richard entered the University of Florida's Employee Education Program in 2002 to pursue the PhD degree. He passed the qualifying exam and was admitted to candidacy in the spring of 2005, with a successful defense of the dissertation in March of 2007. He received his PhD degree in Horticultural Science in May of 2007.