POTENTIAL BIOREMEDIATION SYSTEM FOR NITRATE REMOVAL FROM PLANT NURSERY RUNOFF WATER

By

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To my deceased father Walter Joseph Mozdzen, dad, mentor and friend; and to my daughter and wife who were my principal driving force throughout.

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POTENTIAL BIOREMEDIATION SYSTEM FOR NITRATE REMOVAL FROM PLANT NURSERY RUNOFF WATER

By

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High levels of nitrate (NO_3) in ornamental plant nursery runoff water can contribute to euthrophication of water bodies, and to human health issues such as baby blue syndrome. The objectives of these studies were to characterize the range of nitrate concentrations and loadings in surface runoff water from two typical ornamental plant nurseries, and to evaluate the potential use of a common aquaculture biofiltration media for removing nitrate from nursery generated runoff water. A foliage plant nursery and a bedding plant nursery were used to evaluate NO_3-N losses. Losses of NO_3-N at the foliage nursery during a typical fertigation cycle ranged from 0.8 to 1.2 kg per acre, with concentrations ranging from 70 to 274 mg/L. Losses at the bedding plant nursery ranged from 186 to 405 g per acre with concentrations ranging from 0.7 to 26.3 mg/L. The bioremediation experiments indicated that pulsing NO_3-N considerably increased the lag phase for induction of optimal NO_3-N removal when dissolved organic carbon (DOC) was not limited. Results also indicated that pulsing DOC once optimal nitrate removal was achieved reduced NO_3-N removal rates significantly. Using sucrose as the source of carbon, optimal nitrate removal rates of 5.97 mg and 3.06 mg of NO_3-N/h were achieved after 7 and 16 days in separate studies. Performing total water exchanges did not appear to affect the rate of NO_3-N removal once the optimal rate was achieved.

CHAPTER 1
INTRODUCTION

Background

The ornamental plant production industry is among the fastest growing segments of American agriculture. Much of the plant material marketed in the United States is produced in containers in nurseries located in the Southern and Pacific coast states (Alexander 1993). These nursery operations may use large amounts of nutrients and water, which can lead to nutrient leaching and losses in runoff water. High levels of NO_3 in nursery leachate and runoff waters can contribute to environmental problems related to euthrophication of water bodies. NO_3 is also known to cause methemoglobinemia (blue baby syndrome) in infants (Ziebarth 1991).

Plant nurseries use diverse irrigation and fertilization systems. Overhead and microirrigation are the most common irrigation systems used today. The most common fertilization strategies include incorporation in the substrate, top-dressing, and application through the irrigation system. Fertilizer formulations range from controlled-release to water-soluble. Regardless of the fertilizer type, nitrate leaching and losses in surface water can occur. Even controlled-release fertilizers (CRF) have a high potential for nitrate leaching under conditions of high temperatures and excessive moisture. Also, mishandling CRF may cause cracking of the granule coatings and result in a quick release of nitrate (Dumroese 1995).

According to Mancino (1990), nitrate-nitrogen (NO_3-N) is the predominant form of nitrogen leached from plant containers containing at least 20% of an organic amendment such as sphagnum peat. The high leaching potential of NO_3 is due to its negative charge. Most substrates used for ornamental plant production have a very high cation exchange capacity and a very low anion exchange capacity, resulting in little absorptive capacity of the negatively charged NO_3 ion, and a very high absorptive capacity for the positively charged ammonium ion (NH_4). Due to

the substrates low anion holding capacity, the NO_3 molecules can move freely through the substrates, into surface waters, and/or ground waters.

Alexander (1993) reported that NO_3-N concentrations in runoff waters from plant nurseries in the Southern United States ranged from 1.6 – 55.0 mg/L. Studies have also shown NO_3-N levels in the upper 3 feet of soil under some benches and near retention ponds to exceed 2,245 kg/ha (2000 Lbs/acre), in nurseries that have been operating for over 10 years (Amos 1993).

Best management practices (BMPs) can reduce NO_3 leaching and losses in runoff, and may include the use of retention structures and water recycling. Retention structures are designed to retain a fraction of runoff water on site, allowing opportunity for natural processes to remove nutrients and sediments. Alexander (1993) reported that a lined retention structures used alone was only 20% effective in removing nutrients, while a system that included a lined retention structures, water treatment, and water recycling was 80% effective in removing nitrogen. However, once filled to capacity, retention structures may discharge surface runoff water before natural processes are able to remove NO_3. In addition, NO_3 leaching can also occur in areas with high water tables where liners for retention structures cannot be used, such as in many areas of South and Central Florida. Further more, as water is recycled, salts and some nutrients may increase with each cycle causing a significant reduction in production, and other problems associated with over fertilization, including weed and nuisance algae growth on production surfaces and in water collection structures (Alexander 1993). Treatment of recirculated water may alleviate some of these problems.

Due to the recent increase in nursery/greenhouse operations in the Southern U.S., and the high potential for NO_3 leaching and runoff, federal and state governments are evaluating NO_3

sources in nursery operations (Amos 1993). The management of nitrogen to prevent surface and ground water contamination may affect many nursery operations in the future. Many BMP's are now available that can help reduce NO_3 leaching and runoff (Yeager et al. 1997). However, NO_3 leaching and runoff losses can occur even when using appropriate BMP's. In this event, other strategies must be considered to help reduce NO_3 loads in runoff waters.

One possible strategy is the use of biofiltration systems originally developed for the aquaculture industry. These biofiltration systems have a relatively small footprint, use relatively low cost infrastructure and operating costs, and are designed to remove large quantities of NH_4-N through nitrification processes. Because these systems are designed to treat relatively large volumes of water within a short period of time, a similar system might me useful for in-situ NO_3-N removal from runoff water near production areas. However, NO_3-N and NH_4-N removal by biofiltration occurs under very different conditions. In order for NO_3-N removal to occur by biofiltration, appropriate redox conditions must be met, and a source of carbon must be provided at a minimum of a 2:1 ratio C: N (Aesoy 1998). The following studies were designed to address the following objectives:

Objectives

1. Characterize the range of NO_3-N concentrations and loadings likely in surface runoff water from ornamental plant nurseries.
2. Capture native denitrifying microflora from surface water drainage systems using biofiltration substrate.
3. Evaluate the effect of NO_3-N availability (pulsing) on NO_3-N removal potential using captured native denitrifying microflora.
4. Evaluate the effect of carbon availability (pulsing) on NO_3-N removal potential using captured native denitrifying microflora.
5. Evaluate the effect of total water exchange on NO_3-N removal potential using captured native denitrifying microflora.

CHAPTER 2
CHARACTERIZING NITRATE CONCENTRATIONS AND LOADINGS IN SURFACE RUNOFF WATERS FROM TWO ORNAMENTAL PLANT NURSERIES

Introduction

One overall objective of this program was to design a biofiltration system for use in ornamental plant production areas. In order to accomplish this, some knowledge regarding NO_3-N concentrations and loadings is needed. NO_3 is an important source of nitrogen, because it is the main form that most plants assimilate directly. NO_3 is commonly used by the ornamental plant industry, and is applied through either soluble (such as ammonium nitrate (NH_4NO_3) and calcium nitrate ($CaNO_3$)), or controlled-release fertilizers (CRF) (such as Osmocote and Nutricote). The most common soluble fertilizer-containing NO_3-N is NH_4NO_3. It is widely used by the industry because of its high solubility and ease of application through irrigation systems.

NO_3 molecules have a high potential for leaching in nursery substrates due to their negative charge, and the absence of anion holding capacity in most substrates used by the industry. High amounts of NO_3 leaching from plant nurseries can result in serious environmental problems when it enters natural aquatic ecosystems in high concentrations, resulting in euthrophication. Euthrophication often results in hypoxia, which can lead to fish kills and a rapid degradation of more nutrient-poor, aquatic ecosystems. Health concerns can also arise when infants drink ground water with high levels of NO_3 causing “Blue Baby Syndrome” (Ziebarth 1991).

Fertilizer Formulations vary in their susceptibility to leaching. The use of CRF can reduce NO_3 leaching by regulating its release (Dumroese 1995). Escobar et al. (2004) investigated nitrogen leaching from olive trees grown in plastic pots in which total nitrogen losses were higher when NH_4NO_3 and $CaNO_3$ were applied, and lower with controlled-release fertilizers. However, high temperatures and moisture can increase the release rate of nitrate from CRF’s,

making them more susceptible to leaching. Mishandling CRF's can also cause the fertilizer's granule coating to crack, resulting in a quick release of NO_3 (Mancino 1990).

The amount of NO_3 that leaches and is lost in runoff water from plant nursery operations may be influenced by several factors including irrigation and nutrition management practices. Many nursery operations use inefficient overhead irrigation systems, which can result in the delivery of up to 80% of the irrigation water to non-target areas (Alexander 1993). To compensate for the inefficiencies in water placement, growers may increase the volume of irrigation water applied, increasing the risk of NO_3 losses by leaching and runoff. Other operations use microirrigation systems with soluble fertilizers for fertigation. Improperly managed, these systems can result in leaching losses of more than 72% of the water applied per container (Dumroese 1995), and up to 95% of the NO_3 applied (Bigelow 2001). According to Mancino (1990), NO_3-N is the predominant form of nitrogen leached from plant containers containing at least 20% of an organic amendment such as sphagnum peat. Other leaching studies found similar results where substrates incorporated with inorganic and organic amendments such as sphagnum peat leached more than 95% of the NO_3 applied, while NH_4 leaching was negligible (Bigelow 2001).

- **Hypothesis:** Nitrate loss occurs in surface runoff water from container ornamental plant nurseries.
- **Objective:** To characterize nitrate losses in surface runoff water from two container nurseries.

Materials and Methods

Site Description

Two nurseries were selected for investigation of NO_3 losses in surface runoff/drainage water. Both nurseries were located in South Florida (St. Lucie and Martin Counties). The nursery located in St. Lucie County produces high quality foliage plants using fertigation through

a drip irrigation system. This nursery will be referred to in this study as the “Foliage Nursery” (FN). This nursery employs a water recycling system, and the water used to irrigate/fertigate is pumped from a non-lined water reservoir. The total area of the drained production area studied at this nursery was 1 hectare (ha) (2.5 acres or 108,900 ft^2). This area was drained through three discrete discharge pipes.

The nursery located in Martin County produces high quality bedding plants using an overhead irrigation/fertigation micro-sprinkler system, coupled with the addition of controlled-release fertilizers in the container substrate. This nursery will be referred to in this study as the “Bedding Nursery” (BN). This nursery does not recycle water. Water used to irrigate/fertigate is pumped from an onsite well. The nursery collects its runoff water in a small non-lined pond. During storm events runoff water leaves the nursery site once the pond reaches capacity. The total nursery area studied at this location was 0.112 ha (0.28 acres or 12,000 ft^2), and was drained through two discrete drainage pipes.

Discharge Measurements

V-notch weirs were constructed and used at both nursery sites to estimate the instantaneous and cumulative flow volumes of runoff water during monitored events. The respective weirs were constructed of PVC sewer pipe by cutting a v-notch into the pipe at angles varying from 30 to 60º. Plexiglass panels with a thickness of 5 mm were marked at 1-cm intervals, and glued with clear silicon to each side of the v-notch. The constructed v-notch weirs were attached to 90º elbows, which were attached to the downstream end of each discharge pipe evaluated.

Three 25.4 cm (10-in) discharge pipes drained the FN study area, while a 10 and 15 cm (4 and 6-in) discharge pipe drained the BN study area. In order to estimate discharge volumes during monitored events, a flow rate versus depth of flow (through each weir) relationship was derived by regression analysis. Each weir was manually calibrated at 1 cm depth increments

throughout the useable range. Regression equations used for each nursery are shown in Table 2.1.

Water depth readings were taken every 5 min, and the flow (L/min) was calculated using the regression equation determined during each weir's calibration. The weir calibrations were checked during each irrigation/fertigation event to confirm accuracy. The total amount of water discharged through each pipe during the irrigation/fertigation event was calculated by averaging the flows every 5 min. The average flow was then multiplied by the time (5 min) to determine the water volume discharged during each 5-min interval. Water discharge volumes for each 5-min interval during the entire duration of the runoff event were summed to estimate total discharge per drainage pipe. Total discharge from the production area was estimated by summing the total volume for each pipe.

Sampling

To determine the amount of water and NO_3 loads applied during an irrigation/fertigation event, sampling containers were randomly placed throughout the different irrigation zones within the production area monitored. For the FN, five gallon buckets were used as sampling containers to collect irrigation/fertigation water. Two buckets were randomly placed inside each zone within the area being studied, and one microirrigation emitter was placed inside each bucket. There were a total of twelve zones in the production area with two buckets per zone for a total of 24 buckets. At the end of the irrigation/fertigation event the total volume of water per bucket was measured, the average water volume applied per emitter within each zone was determined, and then multiplied by the total number of functional emitters present in each respective zone during the irrigation/fertigation event. The total water volume applied per zone was summed to determine the total volume of water applied during the event at the production area being evaluated.

For the BN, plastic cups were used as sampling containers to collect irrigation/fertigation water. Two cups were randomly placed inside each zone within the area being studied to directly collect the water applied by the overhead micro sprinkler irrigation system. There were a total of six (6) zones in the area with two (2) cups per zone for a total of 12 cups. The total volume of water applied for all zones during the irrigation/fertigation event was determined using a flow meter at the well. Flow measurements were taken every 5 min, and the flow was multiplied by the time or duration the pumping occurred in order to determine the total water volume applied.

To determine the NO_3-N loads applied during the irrigation/fertigation event, a water sample was collected from each bucket or cup. Samples were preserved by adding 2 drops of 11 N sulfuric acid to lower the pH below 2, and were immediately cooled (4ºC) with ice. Samples were refrigerated until analysis. The average of the NO_3-N concentrations per zone was determined, and then multiplied by the total volume of water applied per zone to determine the total load applied per zone. The total NO_3-N load applied during an irrigation/fertigation event was determined by adding the loads of all zones within the production area.

The total NO_3-N load discharged during the irrigation/fertigation event was estimated by sampling for NO_3-N at 10-min intervals from the water discharged at the v-notch weirs. The total water volume in liters discharged at the weir during each 10-min interval was multiplied by the NO_3-N concentration (mg/L) sampled at each particular interval to estimate the total load in milligrams of NO_3-N. Finally, the total NO_3-N load lost during the irrigation/fertigation event was calculated by adding all of the 10-min interval loads for the entire runoff event.

Analysis

NO_3-N samples collected from the nurseries runoff events were centrifuged for 5 min at 3000 revolutions per minute (rpm) to remove any suspended solids. Then, 100 µL of 1 N hydrochloric acid (HCl) was added to 5 ml of each centrifuged sample prior to

spectrophotometric analysis using a Cary model 300 Bio UV-visible spectrophotometer at 220 nanometers (nm) (Walnut Creek, CA). All NO_3-N samples were analyzed using the NO_3-N ultraviolet spectrophotometric screening method described under Standard Methods for the Examination of Water and Wastewater (Clesceri et al. 1998). In order to account for possible interference from dissolved organic materials, light absorbance was also measured at 275 nm. The corrected UV-light absorbance (Abs) of NO_3-N in the sample was calculated using the following equation:

Abs (corr) = Abs (220) – 2 x Abs (275)

Results from NO_3-N screening method were confirmed using a Westco auto-analyzer (model: Smartchem) and USEPA method 353.1 for colorimetric determination of NO_3-N. Results were also confirmed using a Dionex ion chromatograph model: ICS-1000 (Dionex Corporation Sunnyvale, CA) and operating under conditions outlined in USEPA method 300.6.

Staged surface runoff events: foliage plant production nursery

Runoff sampling events were staged on two separate occasions at the FN. Irrigation/fertigation was applied through emitters at a rate of 3.875 L/h Each irrigation/fertigation cycle was 0.5 h in duration. During the summer event, two cycles were performed for each of the 12 zones of the production area, resulting in a total application of 3.8 L (1gal) per emitter. The total water volume applied during the summer 2004 irrigation/fertigation event was 57,853 L (15,285 gal) (appendix A). During the fall monitoring event, irrigation/fertigation was applied at a rate of 3.875 L/h (1 gal/h), but for only one (1) 0.5 h cycle. The total water volume applied during the fall 2004 event was 27,735 L (7,328 gal) (appendix B).

Staged surface runoff events: bedding plant production nursery

Irrigation/fertigation was applied through overhead micro-sprinklers. Irrigation cycles were generally 20 min per zone, while fertigation cycles were 10 min per zone. The irrigation well pump was programmed to supply water at a rate of approximately 190 L/min (50 gal /min) to the production area evaluated. During the spring 2005 event, an irrigation cycle was monitored at a 0.112 ha (12,000 ft²) production area with a total of six irrigation zones. Each zone was irrigated for about 20 min, and the well pump supplied irrigation water at an average rate of 51.6 gal/min for a total of 120 min. The total water volume applied during the irrigation event was 23,435 L (6,192 gal.) (Appendix E). During the summer 2005 irrigation event, each zone was irrigated for 15 to 20 min, and the well pump supplied water at an average rate of 49.3 gal/min for a total of 90 min. During this event, zone 5 was not irrigated. The total water volume applied during the irrigation event was 15,894 L (4,199 gal) (Appendix E).

Results & Discussion

Foliage Plant Production Nursery

A total of 20,935 L (5,403 gal) of water drained from the monitored area during the summer study. This runoff accounted for 36% of the total volume of water applied during the irrigation/fertigation event. The runoff volume was highest at pipe 1 followed by pipes 2 and 3, which were nearly equal (Figure 2-1). During the fall event, 13,668 L (3,611 gal) of water drained from the application site. This total volume accounted for 49% of the water applied during the event. In this event the runoff volume was also higher at pipe 1 followed by pipes 2 and 3 (Figure 2-1).

The total NO_3-N load applied during the summer 2004 event was 4.862 Kg, while the total NO_3-N load applied during the fall 2004 irrigation/fertigation event was 2.976 Kg (appendix 3). The NO_3-N load during the fall was approximately 40% lower than the load observed during the

summer event since only one fertigation cycle was applied. NO_3-N was injected into the irrigation water as NH_4NO_3 and $CaNO_3$ soluble fertilizers. NO_3-N concentrations in the irrigation water ranged from 23 mg/L to 182.4 mg/L during the summer event and 23 mg/L to 160.5 mg/L during the fall event (appendix C). As expected NO_3-N concentrations in the runoff water varied depending on which zones were being fertigated. During the summer event, higher NO_3-N concentrations in the runoff water, ranging from 45.3 to 274.0 mg/L, were observed at pipes 1 and 2; compared to 21.0 to 65.8 mg/L at pipe 3 (appendix 4). Likewise, during the fall event NO_3-N concentrations ranging from 74.2 to 252.7 mg/L were again highest in pipes 1 and 2 followed by pipe 3 (70.5 to 121.4 mg/L) (appendix 4). Pipes 1 and 2 collected leachate from an area containing heavily fertigated *Rhaphis exelsa* palm trees, thus higher NO_3-N concentrations were expected at these pipes.

The total NO_3-N load discharged from the production area during the summer event was 3.02 Kg (Figure 2-2 and appendix G). This load comprised 62% of the NO_3-N that was applied during the irrigation/fertigation event, indicating that more then half of the NO_3-N applied to the plants through micro-irrigation may have leached through the containers and left the production area (appendix G). A portion of this load may have also been comprised of residual NO_3-N from previous applications. Only 38% of the NO_3-N applied likely remained in the container, and was possibly available for plant uptake. The total NO_3-N load leaving the production area during the fall event was 1.99 kg. This load comprised 67% of the NO_3-N that was applied during the event (appendix G).

Bedding Plant Production Nursery

The cumulative runoff estimates at this site are only representative of loses during the monitored portion of discharge events since some water was flowing before the start of monitoring. This particular site had drainage problems that resulted in ponding of water

throughout the production area. A total of 7,523 L (1,988 gal) of irrigation water was discharged during the spring 2005 study (appendix G). Cumulative runoff volume was higher at pipe 1 than pipe 2 (Figure 2-3). Flow rates for pipes 1 and 2 ranged from 6.7 to 59.2 L/min and 0.05 to 19 L/min, respectively, during this event. The total runoff water volume was 4,994 L for pipe 1 and 2,529 L for pipe 2.

During the summer 2005 irrigation event, a total of 8,190 L (2,164 gal) of irrigation water was discharged during the 4.2 h period monitored. Likewise, discharge through pipe 1 was higher than pipe 2 (Figure 2-3). Discharge from pipes 1 and 2 occurred for >24 h and 4.2 h, respectively, with discharge rates ranging from 2.6 – 13.7 L/min at pipe 1 and 0.004 – 14.7 L/min at pipe 2.

The total NO_3-N load applied to the crops during the spring irrigation and summer irrigation events were estimated to be 17,053 and 37,131 mg, respectively (appendix E). Ground water pumped from a well was used for irrigation. NO_3-N was present in the well water at concentrations ranging from 0.47 to 1.24 mg/L (spring irrigation) and 1.45 - 3.69 mg/L (summer irrigation). NO_3-N concentrations in discharge water for the spring and summer irrigation events ranged from 1.6 – 26.3 and 0.7 – 10 mg/L, respectively (appendix F). A total of 111,417 mg NO_3-N left the production site in runoff water during the monitored period of the spring irrigation event, and 51,200 mg during the summer irrigation event (appendix G). Cumulative NO_3-N runoff loads for each discharge pipe for both spring and summer events are shown in Figure 2-4. Total NO_3-N losses from the Bedding Nursery production area were likely much higher than reported in this study, because the area continued to drain from pipe 1 after the 6.6 h monitored. Continuous monitoring beyond 6.6 hr was not possible, due to nursery operating hours. Pipe 1 continued to drain into the next day at a flow rate of 4.3 L/min.

Conclusions

These results indicate that significant amounts of NO_3-N can leave the production sites in normal irrigation runoff drainage water associated with both micro and overhead irrigation / fertigation practices. Likewise, it can be assumed that rain fall events causing drainage through containers and surface runoff from the production areas may result in similar losses. NO_3-N concentrations in the majority of the samples collected during the runoff events exceeded the 10-mg/L drinking water limit set by the U.S. EPA. These high levels indicate a need for remedial action if the drainage water interacts with drinking water sources. In addition, remedial action is also needed to prevent adverse effects to natural water bodies. With regard to the overall project objective of developing a biofiltration system for removing NO_3-N from plant nurseries surface drainage water, this project provided valuable information regarding expected, realistic loadings and flow rates that must be considered.

Table 2-1. Exponential equations describing the discharge at the foliage nursery.

Pipe	Equation	R^2
1	Y=0.1261X2.7123	0.999
2	Y=0.4636X2.0732	0.997
3	Y=0.1681X2.4596	0.990

Note: Flow depth relationships. Y = Flow (L/min); X = Height (cm).

Table 2-2. Exponential equations describing the discharge at the bedding nursery.

Pipe	Equation	R^2
1	Y=0.1854X2.8615	0.997
2	Y=0.6924X2.2017	0.992

Note: Flow depth relationships. Y = Flow (L/min); X = Height (cm).

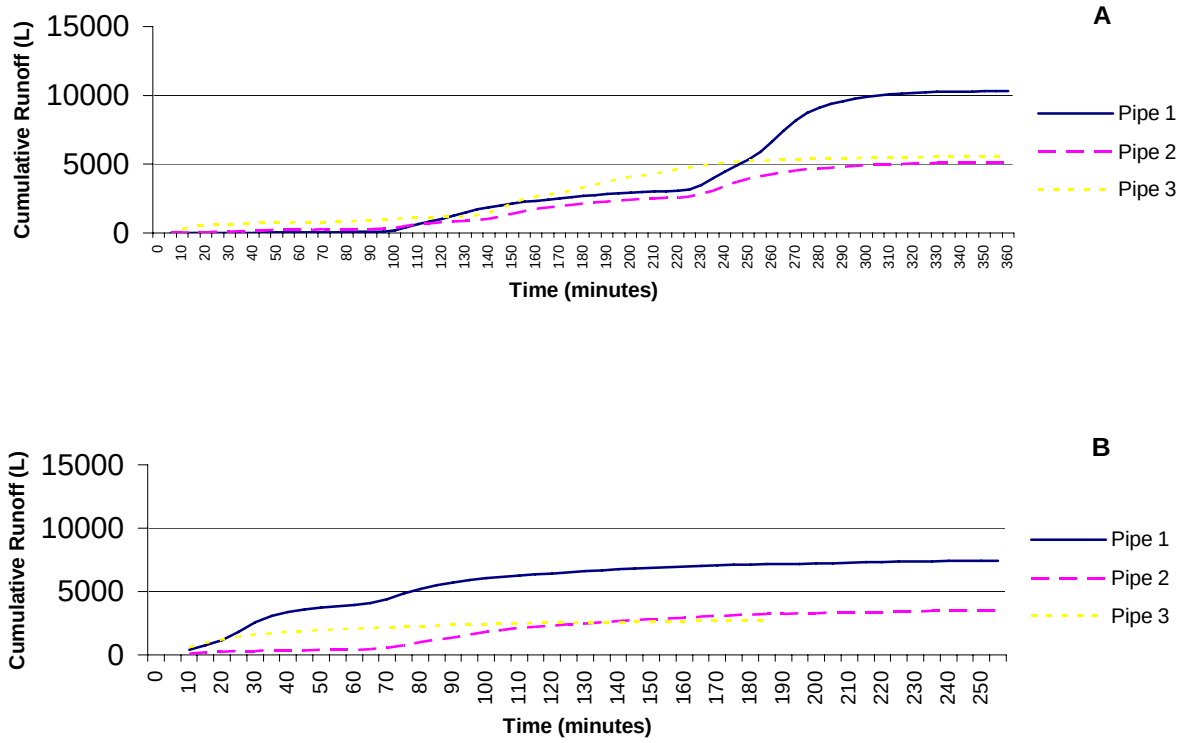


Figure 2-1. Fertigation event cumulative water volume runoff for the flow studies at the foliage nursery. A) Summer. B) Fall.

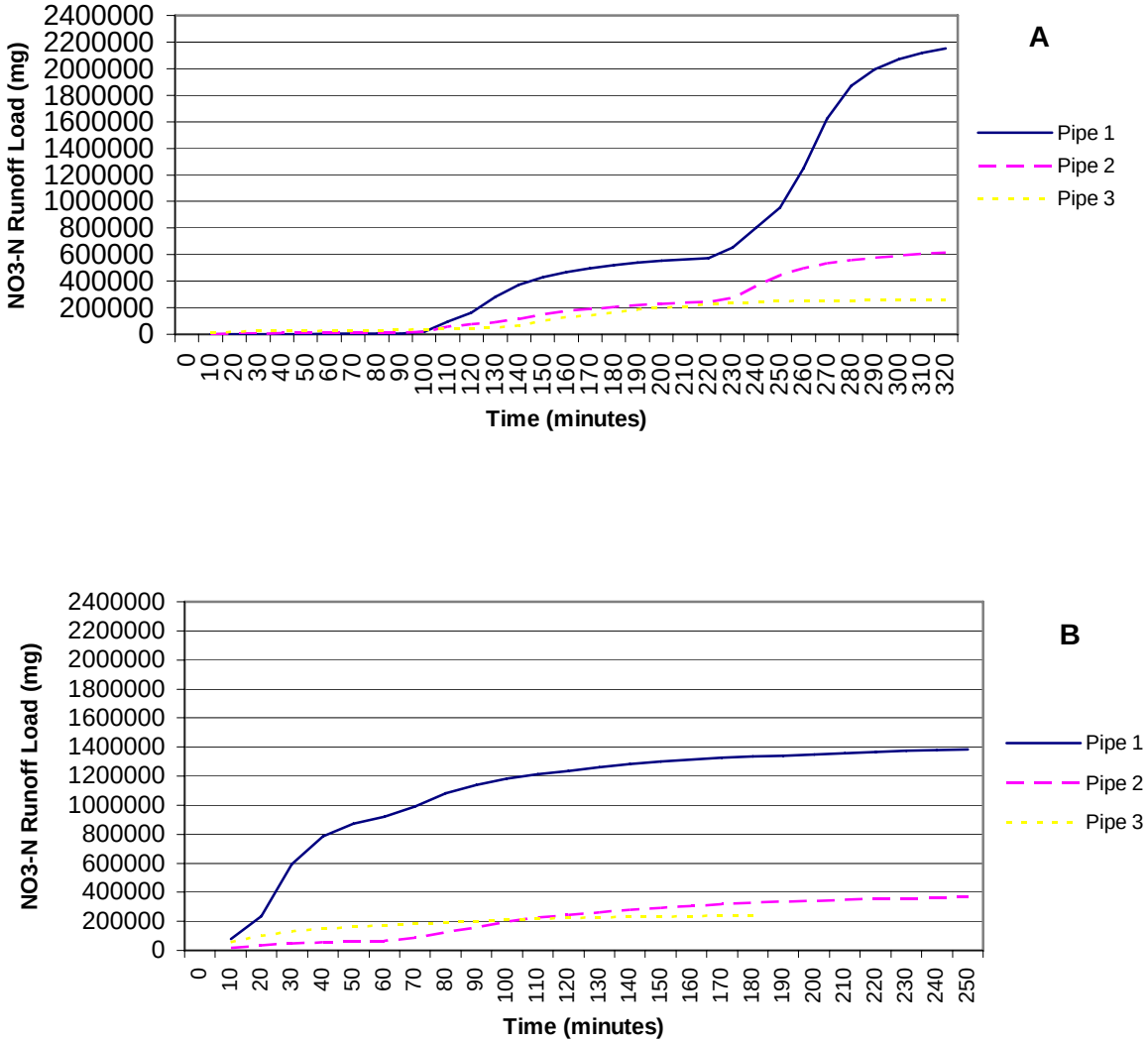


Figure 2-2. Cumulative NO_3-N load for each discharge pipe during the flow studies at the foliage nursery. A) Summer. B) Fall.

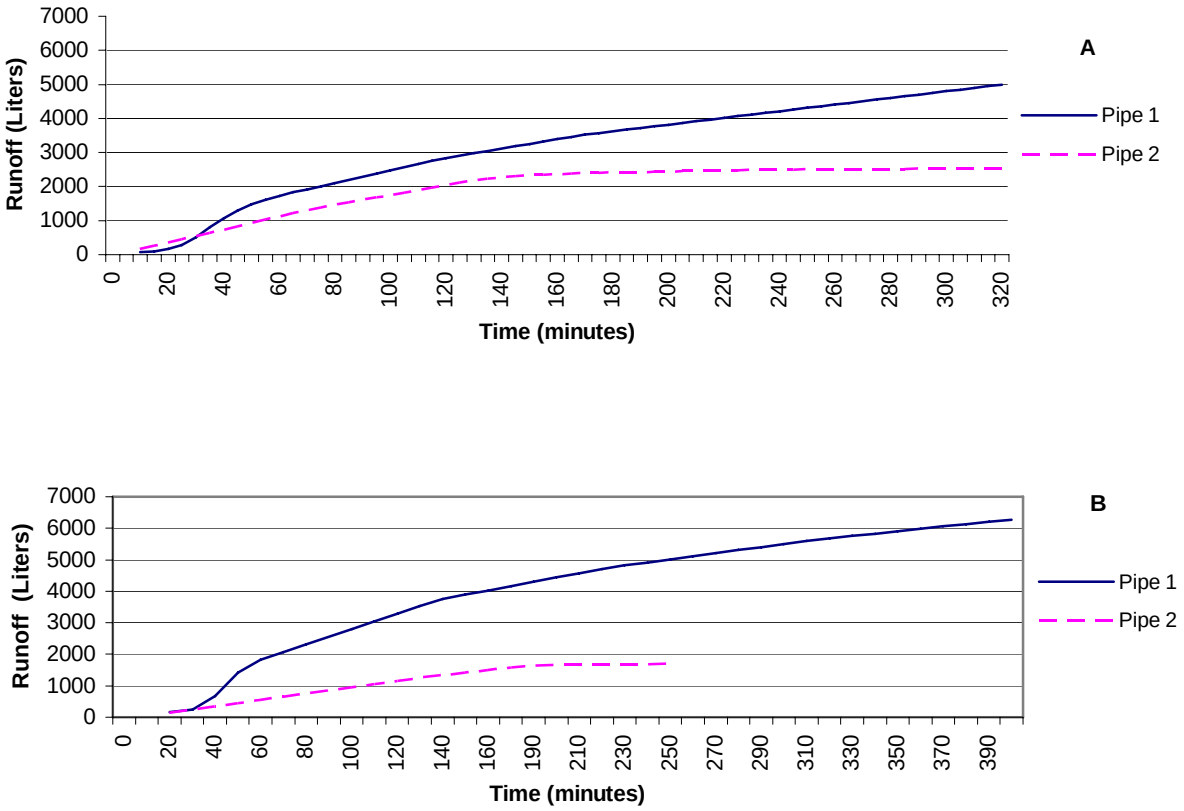


Figure 2-3. Cumulative runoff volume for each discharge pipe during the flow studies at the bedding nursery. A) Spring. B) Summer.

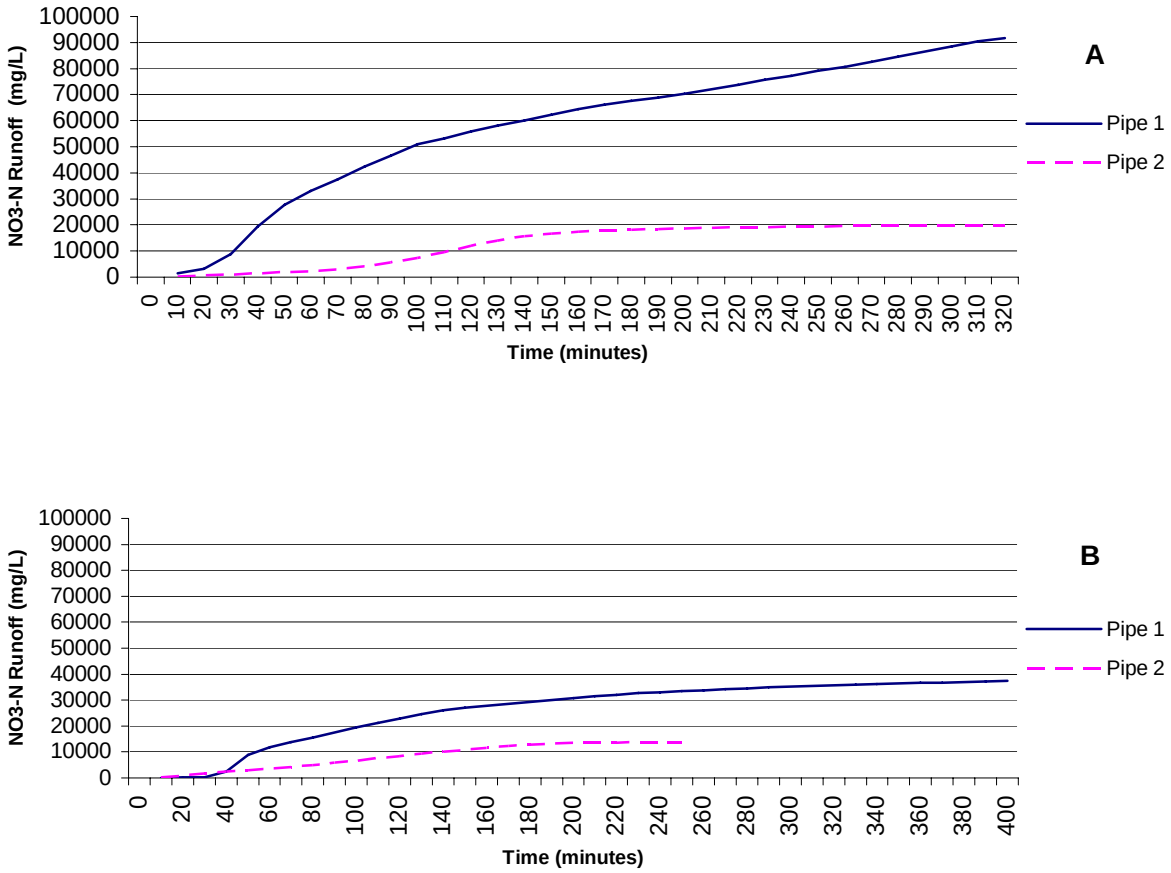


Figure 2-4. Cumulative NO_3-N loads for each discharge pipe during the flow studies at the bedding nursery. A) Spring. B) Summer.

CHAPTER 3
CHARACTERIZATION OF THE POTENTIAL BIOFILTRATION SYSTEM

Introduction

These studies were conducted in an effort to develop a bioremediation system for removing NO_3 from surface runoff water associated with ornamental plant production. Most research on NO_3 removal by biological and/or chemical processes has largely concentrated on city and industrial wastewater treatment technology, which requires high infrastructure costs and energy inputs. These models are not feasible for use in most nursery operations. Very little research has addressed the use of biological and chemical processes to remove NO_3 from plant nursery drainage water. Much of the research reported focuses on the use of constructed wetlands and reed beds. However, constructed wetlands require extensive land areas, a relatively long time period for establishment, and relatively long hydraulic retention times for nutrient removal (Hume et al. 2000). These restrictions reduce the feasibility of constructed wetlands for many small to medium-sized nurseries in regions such as South Florida were land value is high and its availability is limited. Reed beds require less land area than constructed wetlands, around 200 m^2 for each hectare of nursery area, and require a 2-day hydraulic retention time for efficient nutrient removal (Headly et al. 2001). Besides relatively long hydraulic retention times (Stephens 2003), reed beds may discharge water during heavy rainstorm events and require excessive maintenance.

Other research studies have focused on biological removal of NO_3 by microbial mediated denitrification. In order for denitrification to occur, a source of available carbon must be present. Many studies have investigated different carbon sources such as methanol, corn silage, yeast, whey, and spent sulfite liquor (Skrinde 1982). Aesoy (1998) investigated sludge and solid organic waste as possible carbon sources, reporting that they were comparable to ethanol and

acetic acid. Constantin (1997) determined that acetic acid was more efficient for denitrification than ethanol, because of its more directly assimilable structure. Hamersley (2002) reported that increasing particulate organic carbon reduced the lag-phase for induction of denitrification and increased denitrification rates. Menasveta et al. (2000) conducted a study on a closed recirculating system using both nitrification and denitrification processes. NO_3-N concentrations were reduced with a hydraulic retention time (HRT) of 1-2 h from 165 mg/L to 25 mg/L using methanol as the carbon source and crushed oyster shells as the substrate. Initially, aquaculture designed plastic balls were used as substrate, but nitrate reduction did not occur at appreciable levels. Other studies have focused on specific organisms that mediate denitrification such as *Pseudomonas denitrificans* (NBIMCC 1625) (Beschkov 2002), and *Pseudomonas* sp. ASM-2-3 isolated from the Ariake Sea tideland (Kariminiaae-Hamedaani et al. 2003). Hamid et al. (2003) compared the denitrification efficiency of the bacterium *Pseudomonas* sp. ASM-2-3 relative to simple structured, low molecular weight carbon sources such as succinate, acetate, citrate, ethanol, and glucose. They reported that succinate, acetate, and citrate stimulated the removal of nearly 25 mg/L NO_3-N reduction in 20 to 24 h by denitrification.

Several factors directly related to the denitrification environment must be addressed when considering bioremediation. These include: available carbon sources, appropriate physical conditions (pH, redox, temperature etc.), and appropriate substrates. One objective of this study was to develop a biofiltration model that can efficiently reduce the NO_3-N present in plant nursery runoff water. Within the context of ornamental plant production nurseries, a desirable bioremediation system must be inexpensive, easy to maintain, simple to use, require very little technical knowledge, and most importantly, it must function under the environmental conditions present in a typical ornamental plant nursery. From an operational perspective, the system must

have capacity for expected NO_3 loads, water flow volume and rates, and it must meet the needs of the denitrifying microflora. The primary purpose of this research was not to perform an in-depth study of denitrification processes, nor to perform a microbiological study and characterization of the denitrification process. Instead, it was to use the well-understood denitrification process to develop a simple and low cost tool that plant nursery managers can use to reduce NO_3 pollution in their runoff water.

Objectives

- Capture native denitrifying microflora.
- Evaluate the effect of NO_3-N availability (pulsing) on NO_3-N removal potential using captured native denitrifying microflora.
- Evaluate the effect of carbon availability (pulsing) on NO_3-N removal potential using captured native denitrifying microflora.
- Evaluate the effect of total water exchanges on NO_3-N removal potential using captured native denitrifying microflora.

Materials and Methods

Capture of Native Denitrifying Microflora

Native denitrifying microflora were captured from an irrigation/drainage ditch located in the UF/IFAS-IRREC research farm, in Fort Pierce, Florida. Water within the ditch originated from the King's Highway canal. Kaldness media served as the substrate for the attachment of the microflora. This biofiltration media, known as "biofilm carrier elements", is designed and commonly used for the purpose of removing toxic ammonia waste in aquaculture recirculating biofiltration systems. This media is constructed of polyethylene, has a large surface area (259 ft^2/ft^3), is light, slightly positively buoyant, and self-cleaning. The self-cleaning action allows for exfoliation of the older, less active bacterial layers, and eliminates the need for backwashing. These characteristics should reduce clogging of the media and facilitate maintenance. The

elements are 7-mm long and 10-mm in diameter. The size and porosity of the media make it an ideal candidate for developing a flow-through biofiltration system for treating discharge waters from ornamental nursery production areas.

For initial inoculation, 189 L (50 gal) of media were placed in each of the 18-230 L (60 gal) polyethylene containers connected as shown in Figure 3-8. These containers were located next to the IRREC irrigation/drainage ditch. Three (3) large submersible pumps (Big Versa Pump, model VP3900) capable of pumping 13,815 L/h at a head of 1.5 meters were placed inside a screened floating cage, and submerged in the ditch. The water was pumped into a large circular 1,665 L holding tank, which was used to settle any suspended solids before feeding water to the biofiltration media. Inside the holding tank, six submersible pumps (Big Versa Pump, model VP1225) capable of pumping 3,974 L/h at a head of 1.5 m supplied each of the 6 sets of containers with water at a rate of 37.9 L/min. Prior to the NO_3 removal studies, surface water in the ditch was pumped through the media for several weeks. Once conditioned and inoculated with native microflora, the media were mixed, and aliquots were used for the assays.

Experimental Units

Lab-scale biofilters were created using 18.9 L (5 gal) polyethylene containers with lids. Ten liters of biomedia and 10 liters of water were taken from the microflora harvesting apparatus, and placed inside each container. To provide water circulation and improve water contact with the biomedia, a submersible water pump (Resun, model SP-800) capable of pumping 250 L/h was placed on the bottom of each container. Ten percent of the H_2O volume in each container was exchanged every other day with water from the microflora harvesting apparatus in order to replenish necessary elements possibly needed by the microorganisms.

All NO_3 removal studies were conducted inside of a glass greenhouse. The greenhouse allowed better control of climatic conditions such as temperature, rain, sunlight, and provided

electric power for the recirculation pumps. A shade cloth (60% shade) was placed over the study area to reduce sunlight penetration and heat that could create excessive water loss due to evaporation inside the experimental units.

Nitrate and Carbon Analysis

NO_3-N samples were analyzed colorimetricaly using a Cary 300 Bio UV-visible spectrophotometer (Walnut Creek, CA) and the NO_3-N ultraviolet spectrophotometric screening method described in Standard Methods for the Examination of Water and Wastewater (Clesceri et al., 1998). Before each determination, the samples were centrifuged for 5 min at 3000 rpm to remove any suspended solids. One hundred µL of 1 N HCl was added to 5 ml of sample. The light absorbance was read against nanopure water at 220 nm. In order to correct for interference caused by organic matter, the sample absorbance (Abs) was measured at 275 nm. The corrected UV-light abs of NO_3-N in the sample was calculated using the equation: Abs (corr) = Abs (220) – 2 x Abs (275). NO_3-N concentrations were confirmed using a Dionex ion chromatograph model: ICS-1000 (Dionex Corporation, Sunnyvale, CA) and USEPA method 300.6 protocols.

Dissolved Organic Carbon (DOC) was analyzed colorimetricaly using a Hach DR/4000 UV-visible spectrophotometer (Hach Co., Loveland, CO) and the Hach Direct Method 10173, (mid range; 15 - 150 mg/L C). Every sample was first centrifuged for 5 min at 3000 rpm to remove suspended solids. Ten ml of sample was placed in a 50 ml Erlenmeyer flask containing a stir bar, followed by the addition of 0.4 ml of buffer solution (pH 2.0) and stirring for 10 min. While the samples were stirring, a persulfate powder pillow was added to each acid digestion vial and properly labeled. One ml of each sample was then added to each digestion vial and swirled gently. Blue ampules were rinsed with deionized water, wiped with a soft, lint-free wipe, and opened before lowering into the vial contents. The digestion vials were capped, and allowed to digest in a heating block for 2 h at a temperature ranging from 103-105 ºC. Following

digestion, the vials were allowed to cool for 1 h, and then read colorimetricaly at multiple wavelengths of 598 and 430 nm using the spectrophotometer.

Dissolved organic carbon (DOC) consumption was determined by performing a linear regression from the day carbon was dosed to the day before carbon was re-dosed. The DOC consumption per day was calculated by dividing the difference between day-today DOC loads by the number of days included in the regression. Finally, the carbon to nitrogen ratio (C:N) was calculated by the DOC consumption per day over the amount of NO_3-N reduced daily.

Physical/Chemical Measurements

The pH and redox potential (Eh) were monitored using a portable Accumet model AP 63 meter (Fisher Scientific, Singapore). Dissolved oxygen (DO) was monitored using a portable YSI model 95 meter (Yellow Springs Instruments, Yellow Springs, Ohio). Greenhouse air temperature and the water temperatures inside the experimental units were monitored at 15-min intervals during NO_3 removal studies using two Hobo model H8 data loggers.

Experimental Design

Effects of NO_3-N availability (pulsing) on NO_3-N removal potential

The effect of NO_3-N availability on the achievement of optimal NO_3-N removal rates was investigated in the summer of 2004 from July 14 to July 30. A total of 36 small-scale bioreactors were assembled to evaluate three NO_3-dosing scenarios: 1) daily dosing, 2) dosing every 3rd day, and 3) dosing every 5 days (Fig. 3-1). A total of 12 replicate experimental units were used for each treatment. All experimental units were assembled as previously described. On the day of initial treatment, each unit was dosed with 1,515 mg of calcium nitrate ($CaNO_3$) to achieve a NO_3-N concentration of 25 mg/L in 10 L of water. The carbon source used for these studies was laboratory-grade sucrose. Initially, 10 g of sucrose was added to each bioreactor to achieve a

concentration of 420 mg/L of dissolved organic carbon (DOC). DOC was maintained at a minimum of 2:1 ratio (C:N) during the entire study.

NO_3 dosings were performed at 8 AM for the duration of the experiment. Following dosing, media and H_2O within each experimental unit was mixed using a clean plastic pipe. After mixing, a sample was collected for the analysis of NO_3-N to confirm the initial concentration. DOC was analyzed daily to provide a baseline concentration.

Following the initial dose and sampling (referred to as time "0"), a 10 ml sample was collected from three randomly selected units/treatments, and then after 1, 2, 3, 4, 8, and 24 h to determine the rate of NO_3-N removal. It was neither practical nor feasible to collect samples from all units at each interval. However, an equal number of samples were collected from each unit by the end of the study. After the 24 h sampling, each replicate was dosed again with $CaNO_3$ on its corresponding day (i.e. daily or every 3 or 5 days). Measurements for redox potential (Eh), dissolved oxygen (D.O.), pH, and temperature were taken during the experiment on a daily basis using the previously described equipment.

A ten percent water exchange was performed every-other-day on all replicates to replenish nutrients and minerals possibly needed by the microorganisms. The fresh water added to each bioreactor came from the same surface water source where the microbes were harvested. This investigation continued for a total of 16 days; after which the same replicates were used to conduct an investigation into the effects of pulsing carbon on optimal NO_3-N removal rates.

Effects of carbon availability (pulsing) on NO_3-N removal potential

Once optimal denitrification rates were established under saturated carbon conditions, this investigation assessed the impact of carbon depletion on NO_3-N removal potential. During this investigation DOC was allowed to fall below a 2:1 carbon:nitrogen ratio for a period of 5 days before adding more carbon. This investigation was conducted in the summer of 2004 from

August 1st through the 18th. The same replicates used in the previously described study were used. In this study, half of the replicates (6 experimental units) within each of the NO_3-dosing treatments continued to receive a carbon source above the 2:1 carbon:nitrogen ratio, while the other half (6 experimental units) were allowed to fall below the 2:1 carbon:nitrogen ratio for a total of 4 consecutive days before re-dosing with 420 mg/L of DOC as sucrose on the 5th Day. A 10% water exchange was performed every other day on all replicates as previously described. In this study, samples for NO_3-N analysis were collected at the same intervals previously described. In this case, three samples were randomly collected for each DOC treatment (saturated vs. pulsed) within each of the three NO_3-N pulsing treatments.

Confirmation of NO_3-N removal rates under optimal NO_3-N and DOC dosing scenarios

This investigation was conducted in the spring of 2005 (March 15 through April 1st). The experimental bioreactor setup was similar to that previously described except that an aquarium heater was added to each bioreactor to stabilize temperatures. These heaters were necessary because of hurricane (Francis and Jeanne) damage that prevented proper functioning of the glasshouse temperature controls. The aquarium heaters were set to maintain optimal water temperatures ranging between 29- 30 ºC inside the experimental units.

During this investigation NO_3 was added on a daily basis at 25 mg/L NO_3-N and DOC was maintained at saturated (2:1 C:N ratio) concentrations by addition of sucrose at 1000 mg/L (420 mg/L DOC). Samples were collected at the time of dosing, and then at 4, 8, and 24 h after daily dosing in order to determine the rate of NO_3-N removal. Fifteen replicate bioreactors were used for this study. Samples for carbon analysis were collected each day before dosing.

Effect of total water exchange on NO_3-N removal potential

The purpose of this study was to evaluate the effect a total water exchange would have on optimal NO_3-N removal potential. Results would provide information on the biofilter's capability

in a plug-flow system; the most ideal system to treat NO_3 polluted discharge waters from plant nursery operations that irrigate or fertigate on a daily basis.

After the maximum NO_3-N removal rate was achieved, all of the replicates received a 100% water exchange with fresh surface water, followed by addition of 1000 mg/L of sucrose, and the initial 25 mg/L of NO_3-N. Samples were collected at the previously described time intervals 0, 4, 8 and 24 h to determine NO_3-N concentrations and removal rates. This water exchange evaluation was conducted for 2 days (Days 16 and 17).

Data Analysis

Least squares linear regression analysis was used to estimate NO_3-N removal rates based on the log-transformed NO_3-N concentration data. This analysis was performed for each day of sampling, and was based on the respective sampling intervals. Once the linear regression model was determined, the time required to remove 25, 50, 75, and 90% of the NO_3-N load in the experimental bioreactors was estimated.

Results and Discussion

Effects of NO_3-N Availability (Pulsing) on NO_3-N Removal Potential

Results indicate that optimal NO_3-N removal rates were achieved only with treatment one (1) where NO_3-N was dosed daily into the bioreactors. With this treatment, maximum NO_3 removal rates were achieved by Day 16; where 98% of the NO_3-N dosed was removed within 8 h. In comparison, only 54% of the nitrate load was removed for the 3rd day pulse treatment at Day 15, and 10.9% for the 5th day pulsed treatment at Day 15 (Figure 3-3).

Comparable NO_3-N removal rates were not achieved for the treatments where NO_3-N was dosed every 3 or 5 days. This is likely a response to the lower total loads of NO_3-N in each respective treatment limiting denitrifying microflora establishment. Interestingly, after 9 days of pulsing nitrate every 3rd day, over 90% of the NO_3-N was removed within 24 h of dosing,

indicating that denitrifying microflora populations were increasing relative to the initial day of study. Likewise, over 90% of the NO_3-N added every 5th day was removed within 24 h after 15 days (Figure 3-3).

A summary of the estimated NO_3-N removal rates (linear regression slope) for the daily NO_3-dose treatment is shown in Table 3-1. Linear regression slopes were only possible for the daily NO_3-dosed treatment. For this treatment, removal rates were similar from Day 1 though Day 11; generally more than 15 h was needed to remove 90% of the nitrate load added. After Day 11 a significant decrease was apparent. On Days 12-16 only 4.7 – 6.7 h was needed to remove 90% of the NO_3 load. These results indicate a long lag phase of 17 days after day of treatment is needed to achieve the maximum NO_3-N reduction rate. During the lag phase, NO_3-N reduction improves on a 24 h basis as the denitrifying microbial populations increase.

Physical Conditions

A summary of the dissolved oxygen (D.O.) concentrations, redox potentials (Eh), and pH is shown in Figure 3-4. D.O. concentrations dropped from 1.4 mg/L to < 0.4 mg/L (anoxic) within the first 24 h for all treatments. Eh dropped from +1 dV to < -4 dV within the first 24 h for all treatments, and remained negative during the duration of the study with the exception of the 5th day treatment which rose to positive levels near the end of the study. Negative Eh values indicated constant activity by the microbial populations as electron acceptors were reduced.

The average pH for each treatment appeared to vary. The NO_3 daily dosed treatment pH was consistently higher than the 3rd and 5th day dosing treatments. Mean pH for each treatment over 17 days was 7.0, 5.9, and 5.7 for the daily, 3rd, and 5th day NO_3-dosing treatments, respectively. Because the initial pH of all treatments was approximately 7.5, some type of buffering capacity reducing activities were apparent in the 3rd and 5th day dosing treatments. The optimal pH for denitrification is approximately 7.0 (Aesoy 1998). The higher pH observed

in the daily-dosed treatment is likely because denitrification results in the recovery of alkalinity (calcium carbonate), and denitrification rates were much higher in the daily-dosed treatment followed by 3rd and 5th day treatments (Jeyanayagam 2000). The water temperatures within each treatment were similar, averaging 29ºC.

Effect of Carbon Availability (Pulsing) on NO_3-N Removal Potential

Because optimal NO_3-N removal rates were not achieved with the treatments where NO_3-N was dosed every 3rd or 5th day, those treatments were not evaluated in this study. For the replicates maintained with saturated carbon, NO_3-N removal continued on Days 17-34. However, NO_3-N removal rates began to significantly decrease after Day 21 of the study (Day 5 of the DOC evaluation). This decrease in NO_3-N removal rates may be due to a variety of factors, including limited nutrients and co-factors not replenished by the partial water changes. The estimated time required to remove 25, 50, 75, and 90% of the NO_3-N load for the replicates maintained with saturated carbon during the pulsed carbon study is shown in Table 3-2. The reduction in nitrate removal rate is reflected in the increases in the time needed to remove each respective portion of the NO_3-N load.

NO_3-N removal was significantly reduced when carbon became limited. Because of this reduction, a slope analysis was not possible. This is expected since carbon is a necessary ingredient for the denitrification process. Carbon is the energy source for the denitrifying bacteria (Hamersley 2002). In addition, NO_3-N removal rates remained low even after restoration of saturated carbon conditions. This is important because in the field, steps will have to be taken to ensure that carbon levels do not become limited. Otherwise, the efficiency of the biofilter may be compromised.

Figures 3-5 and 3-6 compare the amount of NO_3-N reduced during the pulsed carbon study. Results depict how the saturated carbon treatment reduced significantly higher amounts

of NO_3-N (Figure 3-5) than the pulsed carbon treatment (Figure 3-6). These results confirm that carbon amounts of at least a 2:1 ratio with nitrogen must be maintained for optimal NO_3-N removal to occur.

Confirmation of NO_3 Removal Rates under Optimal NO_3 and DOC Dosing Scenarios

A summary of the time required to remove 90% of NO_3-N (25 mg/L) on a daily basis is shown in Figure 3-7. A lag phase of 6 days was observed during this study before achieving maximal NO_3-N removal rates after 7 days. These maximal NO_3-N removal rates persisted throughout the study (from 7-17 Days). These rates were also nearly twice as fast as those observed during the summer 2004 studies. The optimal removal rate from 7-17 Days ranged from 5.7 to 6.2 mg NO_3-N/h. At these rates, the estimated time required to remove 90% of the nitrate load ranged from 3.6-3.8 h (Table 3-3).

Linear regressions for carbon consumption were also calculated during this study. On average a C:N ratio of 1.84:1 was needed during the spring 2005 confirmation study (Table 3-4). A C:N ratio of 7.8 was needed during the total water exchange treatment from Day 16 to Day 17. The high C: N ratio seen during this last treatment may have been caused by the possible reduction of other electron acceptors such as iron, manganese, and sulfate, which may have been present in high quantities when the fresh ditch water was added to the system. A strong hydrogen sulfide smell was apparent from Day 16 to 17, indicating high reduction activity of sulfate (SO_4) into hydrogen sulfide gas (H_2S).

Effect of Total Water Exchange on NO_3-N Removal Potential

Results demonstrated that performing 100% water exchanges did not adversely affect NO_3-N removal potential during the 2-day evaluation. Total water exchanges appeared to slightly improve NO_3-N removal rates.

Conclusions

Pulsing NO_3-N negatively affected the lag phase for induction of denitrification and the NO_3-N removal rates in the bioreactors. Denitrifying microorganisms are facultative anaerobes meaning that they can respire in both aerobic and anaerobic conditions by using both O_2 and NO_3-N as electron acceptors (Jones et al. 2000). When both NO_3-N and O_2 are absent, denitrifying microorganisms cannot continue their metabolic activities, because of the lack of a terminal electron acceptor needed by their electron transport chain during energy generation.

Pulsing DOC in the bioreactors appeared to halt denitrification processes, due to the absence of carbon. Carbon is used by denitrifying bacteria for both an energy and carbon source. Without carbon, denitrification metabolic activities cannot function. Pulsing DOC prolonged the lag phase. In this case, the denitrifyers are either killed or could not continue to proliferate. The results in this study suggest that a carbon source cannot be absent from the biofilter system for NO_3-N removal to occur. Maintaining constant optimal water temperatures of 29ºC to 30ºC inside the bioreactors may have significantly increased the rate of NO_3-N removal, and reduced the lag phase for denitrification induction. This indicates that regions with lower temperatures, and a high degree of temperature fluctuations may have prolonged lag phases, and reduced NO_3-N removal rates. Performing water exchanges of the total water volume of the bioreactors does not appear to adversely affect the denitrifying microbial populations. This opens the possibility of using the bioreactors as a plug - flow system, in which they may be used to treat nursery runoff water at nurseries that irrigate or fertigate on a daily basis.

The DOC consumption after maximum NO3-N removal rates have been achieved indicated that sucrose was a highly efficient carbon source. Sucrose may be a very practical carbon source for NO3-N removal at plant nursery operations due to its relatively low cost as compared to more costly sources such as ethanol, acetic acid, glucose, and others (Aesoy 1998).

Table 3-1. Predicted time intervals (h) to remove 25%, 50%, 75% and 90% of the NO_3-N load for the daily NO3-dosing.

Day	T25	T50	T75	T90	Slope	Intercept
DOT	5.2	5.6	14.4	25.9	-0.03	1.90
1	3.8	6.6	11.3	17.6	-0.06	2.51
2	5.2	10.4	19.2	30.9	-0.03	2.41
3	4.1	6.5	10.7	16.2	-0.07	2.57
4	1.8	5.0	10.6	17.8	-0.05	2.37
5	3.8	7.1	12.7	20.1	-0.05	2.43
6	2.2	4.8	9.3	15.2	-0.07	2.44
7	2.9	5.5	9.9	15.7	-0.07	2.45
8	2.1	4.7	9.0	14.8	-0.07	2.40
9	3.4	7.2	13.6	22.0	-0.05	2.43
10	2.4	5.7	11.5	19.1	-0.05	2.44
11	2.6	6.3	12.7	21.1	-0.05	2.42
12	1.2	2.3	4.2	6.7	-0.16	2.57
15	1.5	2.7	4.8	7.6	-0.14	2.57
16	0.6	1.4	2.8	4.7	-0.21	2.37

Note: Slope and intercept apply to the linear regression models using the log-transformed NO3-N concentration data.

Table 3-2. Predicted time intervals (h) to remove 25%, 50%, 75% and 90% of the NO_3-N load for the daily NO_3-dosing during the pulsed carbon study.

Day	T25	T50	T75	T90	Slope	Intercept
17	0.3	0.9	2.0	3.4	-0.28	2.31
18	1.1	2.0	3.6	5.6	-0.19	2.40
19	1.2	2.1	3.6	5.7	-0.19	2.57
20	0.8	1.6	3.1	5.0	-0.21	2.45
21	0.8	2.1	4.4	7.5	-0.13	2.40
22	0.5	1.8	4.2	7.3	-0.13	2.35
25	1.0	2.2	4.2	6.9	-0.15	2.19
33	1.0	2.8	5.8	9.8	-0.10	2.44
34	1.2	3.0	6.2	10.4	-0.10	2.42

Note: Slope and intercept apply to the linear regression models using the log-transformed NO3-N concentration data.

Table 3-3. Predicted time intervals (h) for removing 25%, 50%, 75%, and 90% of the NO_3-N load for the daily NO_3-dosing treatment for the spring 2005 study.

Day	T25	T50	T75	T90	Slope	R2 (transf)
1	1.479	3.750	7.414	12.769	-0.078	0.997
2	1.063	3.003	6.135	10.710	-0.091	0.982
3	1.063	3.003	6.135	10.710	-0.091	0.982
4	1.201	3.116	6.207	10.723	-0.092	0.995
5	0.950	2.432	4.822	8.316	-0.119	0.998
6	0.969	1.944	3.517	5.817	-0.181	0.986
7	1.047	2.094	3.141	3.769	-5.970	1.000
8	1.036	2.073	3.109	3.731	-6.030	1.000
9	1.036	2.073	3.109	3.731	-6.030	1.000
10	1.028	2.056	3.084	3.701	-6.080	1.000
11	1.054	2.108	3.162	3.794	-5.930	1.000
12	1.096	2.193	3.289	3.947	-5.700	1.000
13	1.025	2.049	3.074	3.689	-6.100	1.000
14	1.054	2.108	3.162	3.794	-5.930	1.000
15	1.054	2.108	3.162	3.794	-5.930	1.000
16	1.008	2.016	3.024	3.629	-6.200	1.000
17	1.003	2.006	3.010	3.612	-6.230	1.000

Note: Slope and intercept apply to the linear regression models using the log-transformed NO3-N concentration data.

Table 3-4. Linear regression coefficients, DOC consumption, and C:N ratios during optimal NO_3-N removal period (Day 7 to 17).

Days	r2	Slope	TOC consumption (mg/L)	TOC consumption / day	C:N ratio
7 - 9	0.87	-77	77	38.5	1.54
10 - 14	0.85	-80	80	20.0	0.80
15 - 16	1.00	-79	79	79.0	3.20
Averages	0.91	-79	79	46.0	1.84

Notes: Carbon consumption for the total water exchange treatment (Day 16 to 17) was 196 mg/L, C:N ratio of 7.8.

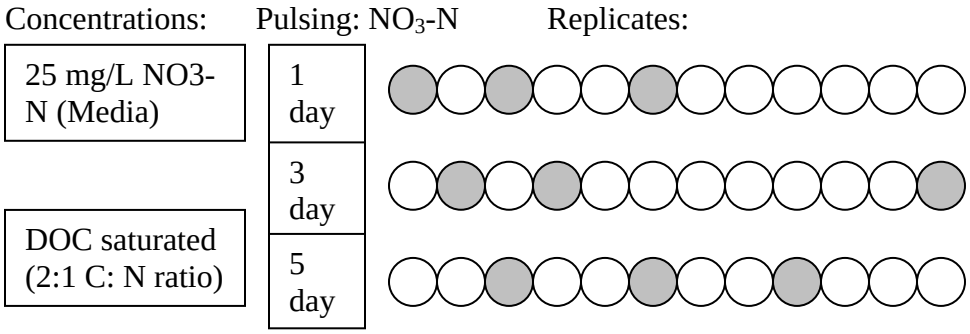


Figure 3-1. NO_3-pulsing experimental design.

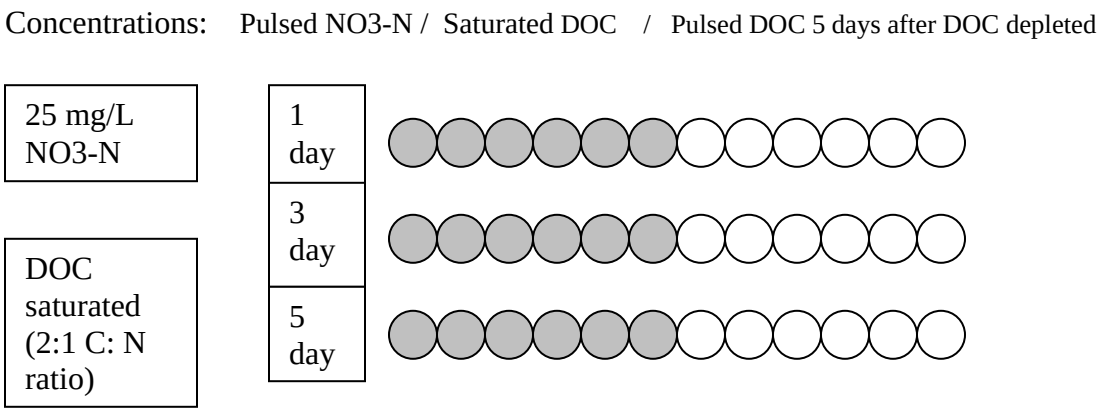


Figure 3-2. DOC-pulsing experimental design.

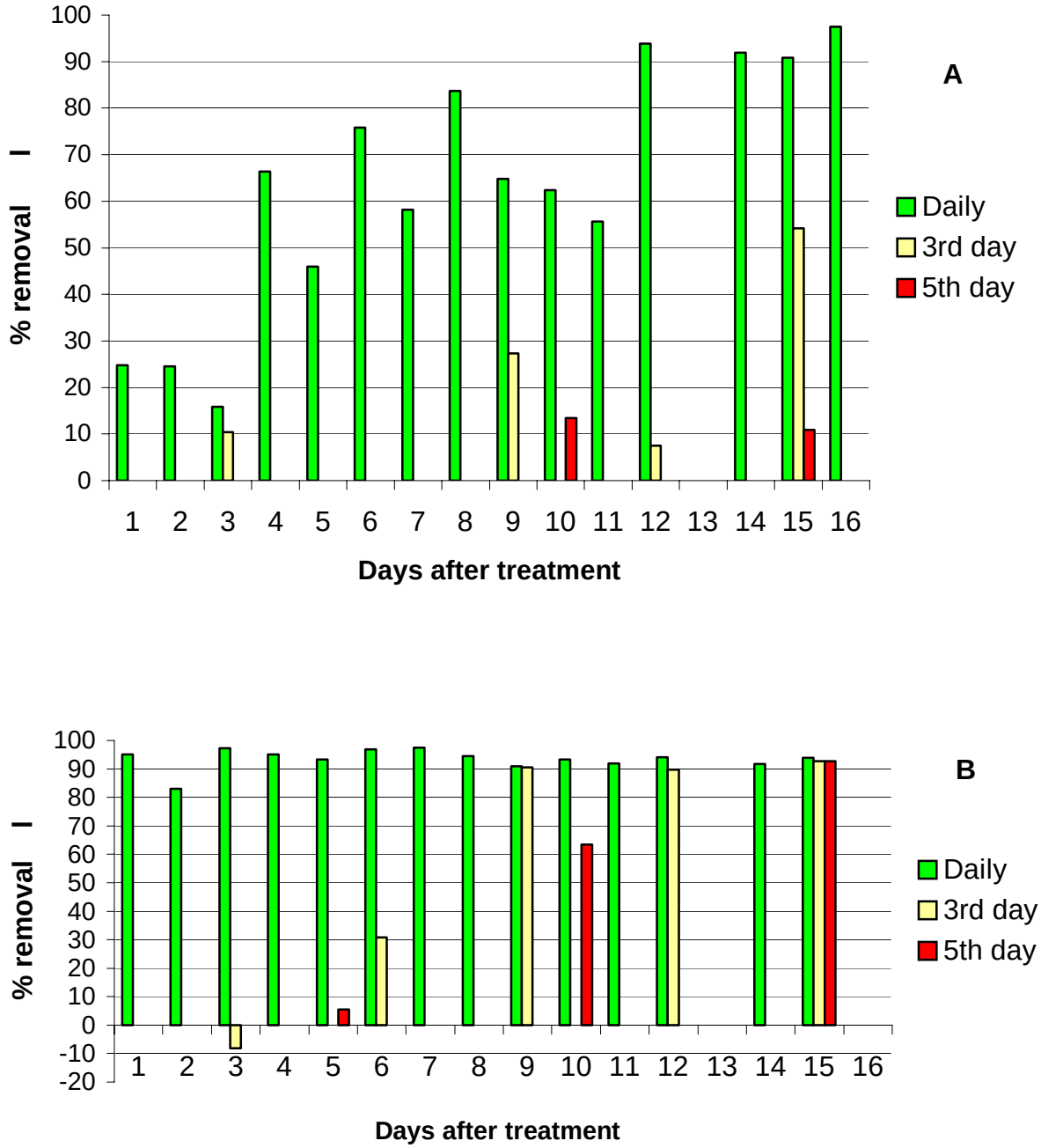


Figure 3-3. Percentage of NO_3 removal during the summer 2004 studies. A) 8 h and B) 24 h after dosing with 25 mg/L of NO_3-N and DOC constantly saturated.

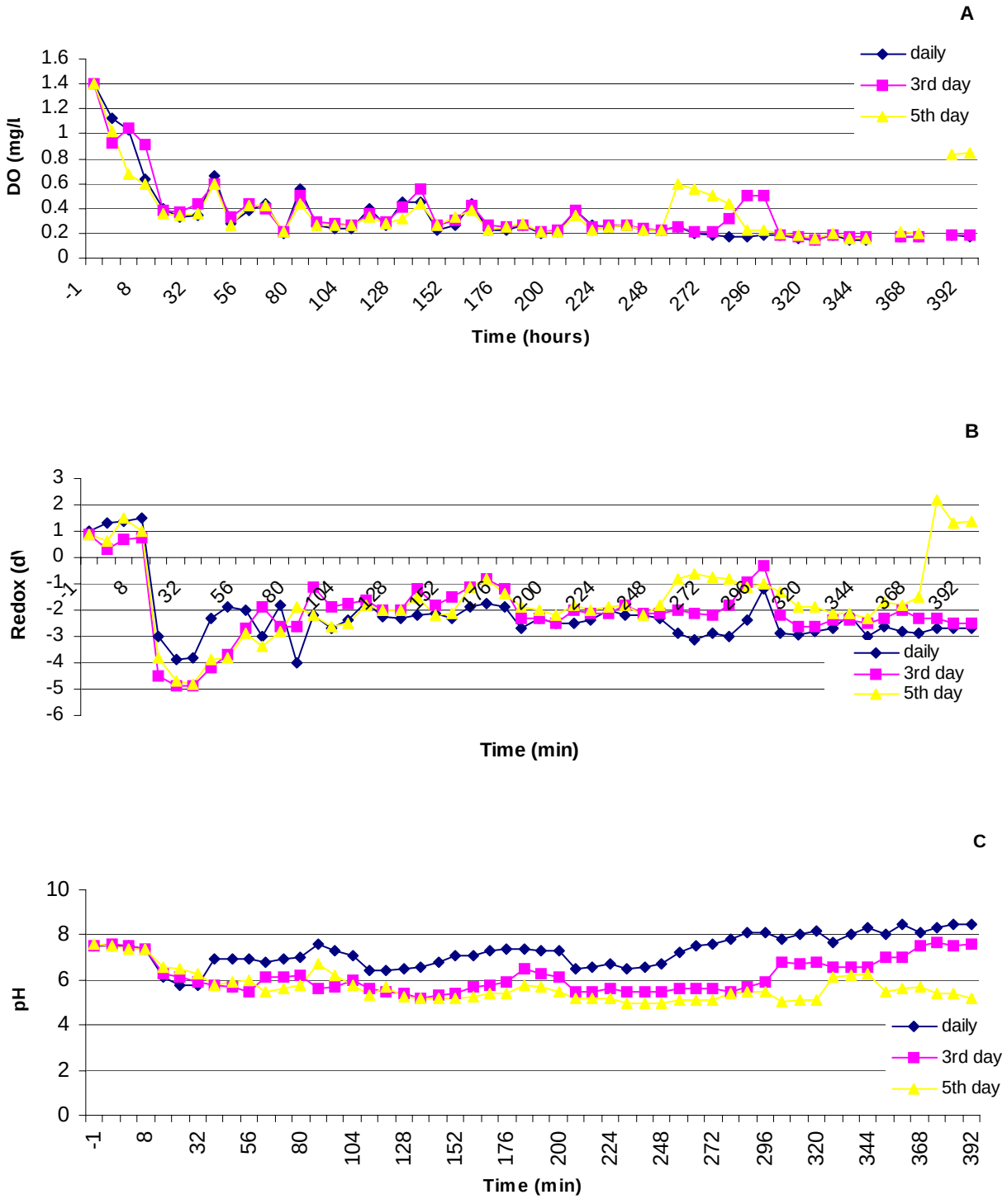


Figure 3-4. Summary of A) dissolved oxygen concentrations. B) Redox potential. C) pH measurements during the summer 2004 studies.

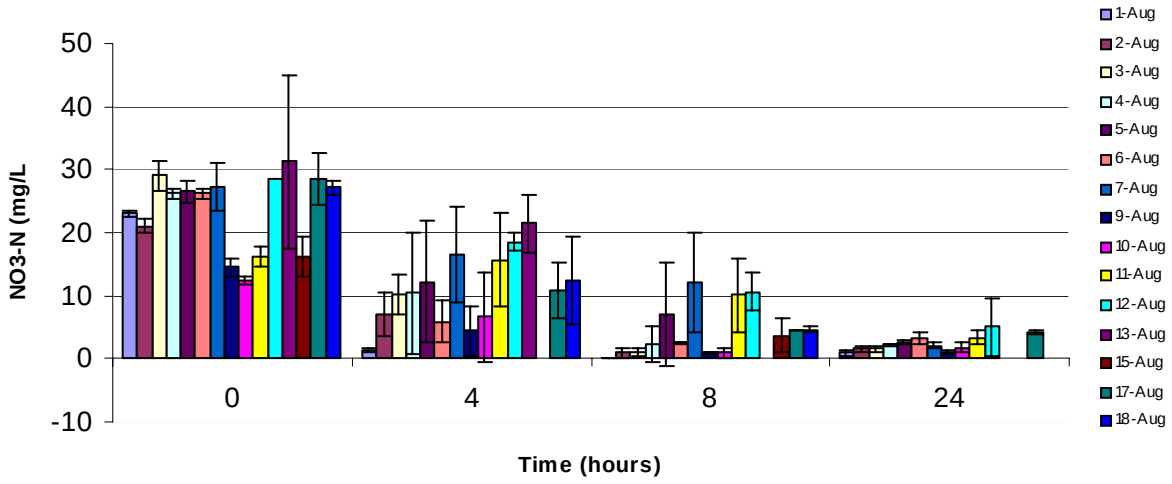


Figure 3-5. NO_3-N (mg/L) removed within 4 h, 8 h, and 24 h after dosing with 25 mg/L NO_3-N during the summer 2004 studies with DOC saturated.

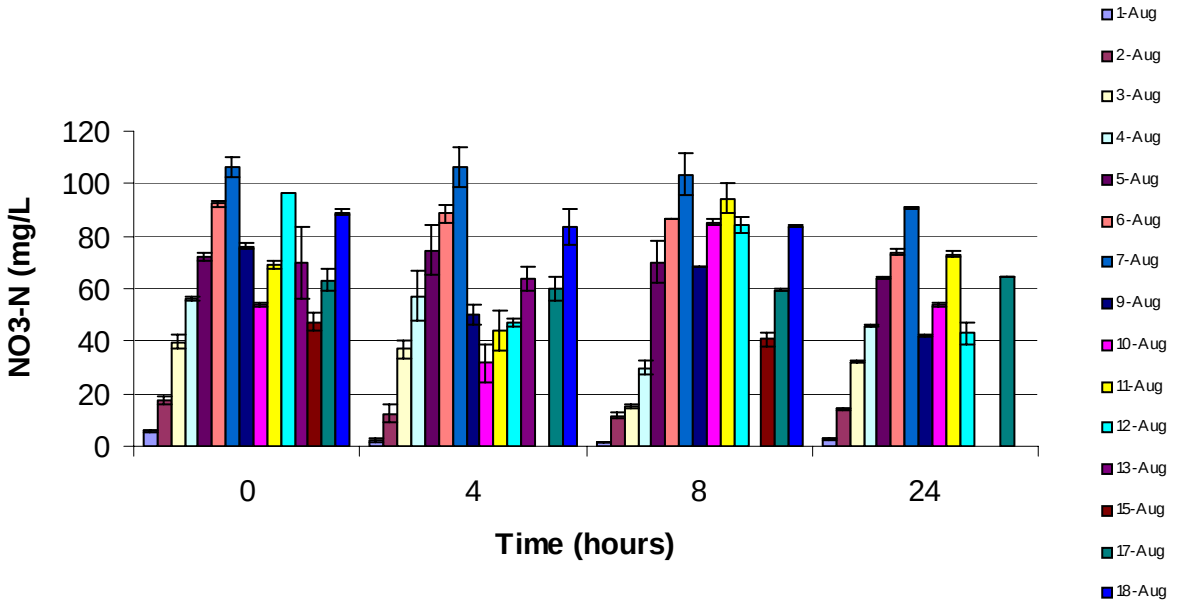


Figure 3-6. NO_3-N (mg/L) removed within 4 h, 8 h, and 24 h after dosing with 25 mg/L NO_3-N during the summer 2004 studies with DOC depleted.

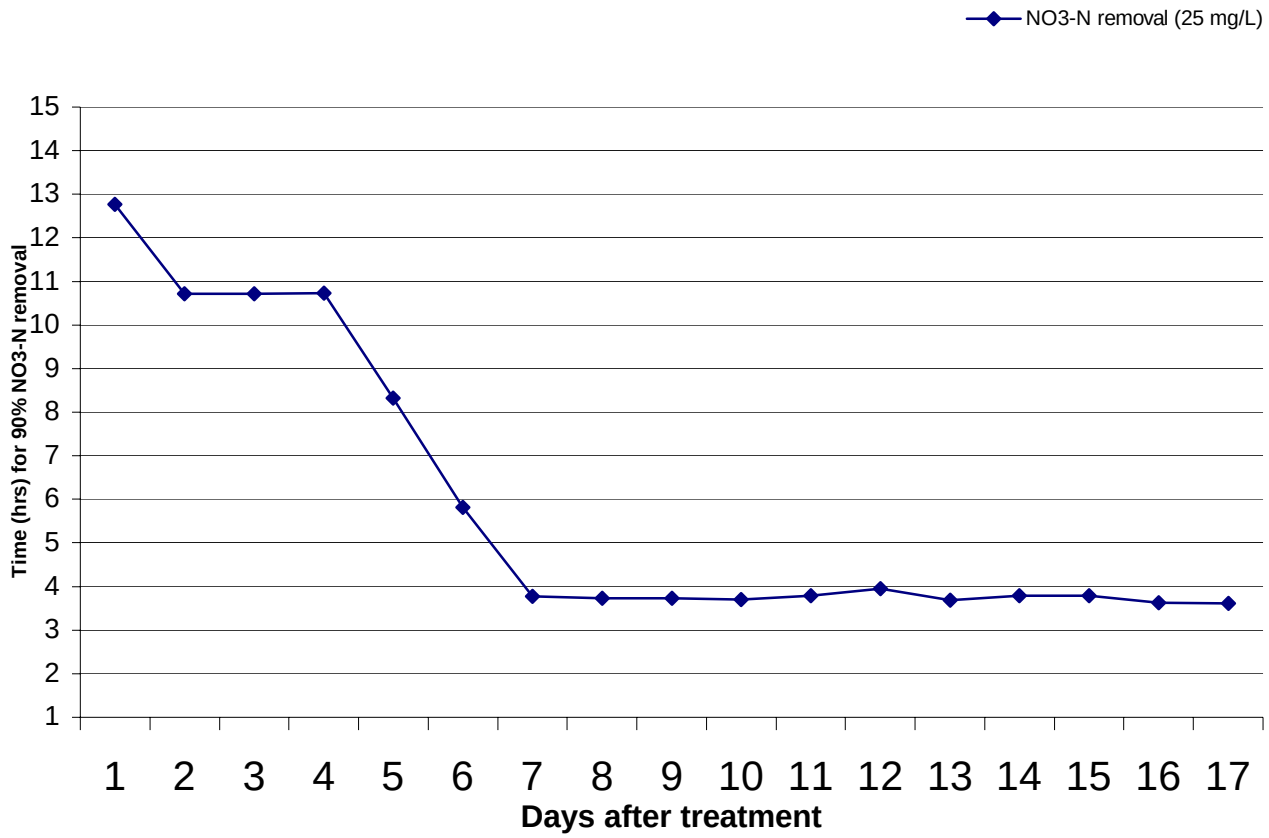


Figure 3-7. Time required for removing 90% of 25 mg/L NO_3-N during the spring 2005 confirmation study under optimal NO_3 and DOC scenarios.

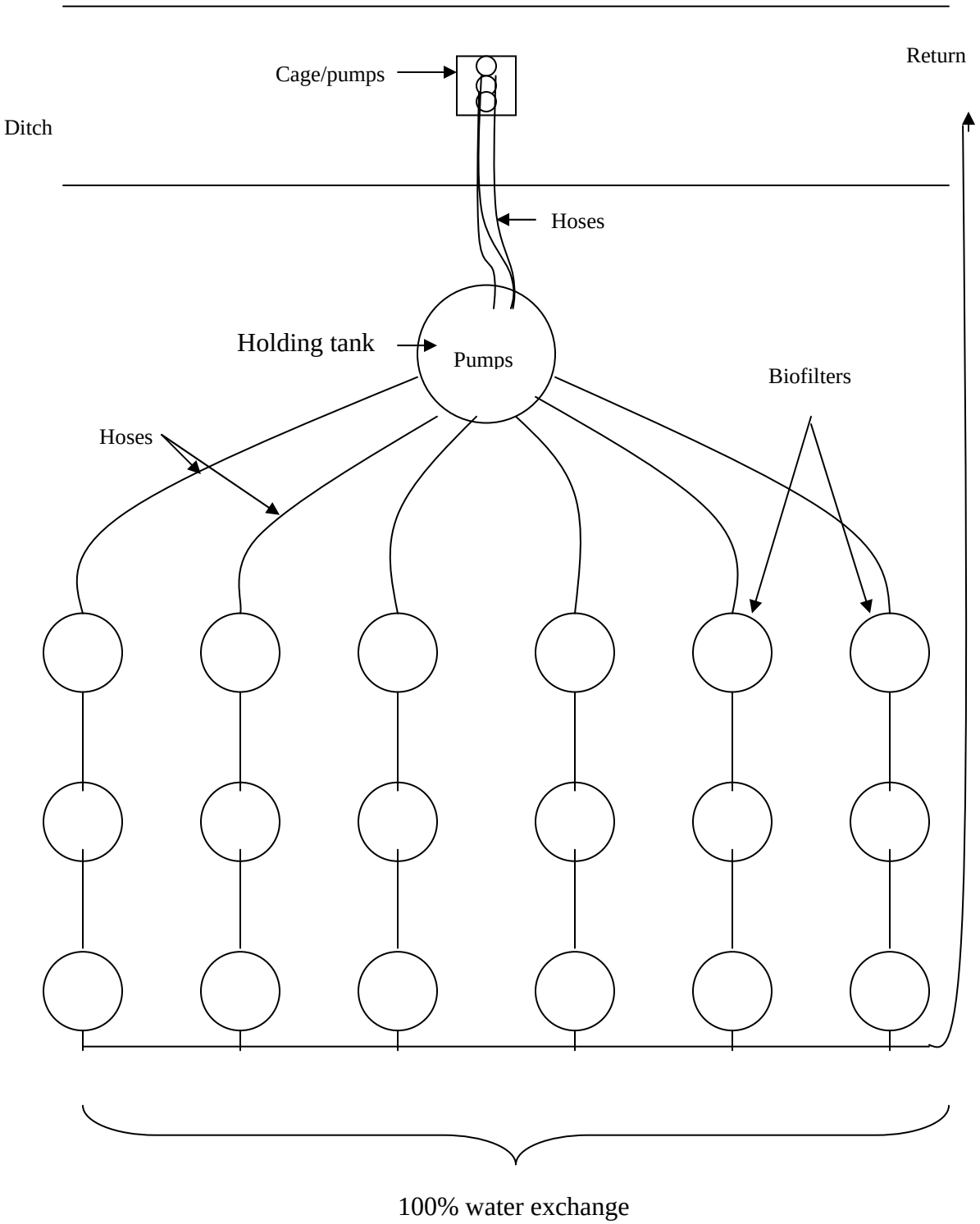


Figure 3-8. Native microflora harvesting apparatus.

APPENDIX A
FERTIGATION EVENT WATER VOLUME INPUT AT THE FOLIAGE NURSERY FLOW STUDIES. 1) SUMMER. 2) FALL

Table A-1

Zone	Emitter volume/zone (Liters)	Emitters/zone	Total volume/zone (Liters)
1	5.56	576	3,203
2	4.35	2,208	9,605
3	5.31	960	5,098
4	4.33	264	1,142
5	5.60	560	3,136
6	4.15	960	3,984
7	3.90	2,340	9,126
8	3.20	2,688	8,602
9	4.38	320	1,400
10	4.35	960	4,176
11	3.75	1,400	5,250
12	4.43	708	3,133
Total			57,853

Table A-2

Zone	Emitter volume/zone (Liters)	Emitters/zone	Total volume/zone (Liters)
1	2.54	576	1,463
2	2.05	1,152	2,356
3	1.88	960	1,800
4	2.17	264	573
5	2.53	660	1,667
6	2.17	1,060	2,300
7	1.75	2,340	4,095
8	2.29	2,688	6,142
9	2.59	420	1,086
10	2.05	1,060	2,168
11	1.92	1,400	2,688
12	1.98	708	1,398
Total			27,735

APPENDIX B

NO_3-N LOADINGS APPLIED TO CROPS DURING THE FLOW STUDIES AT THE FOLIAGE NURSERY. 1) SUMMER. 2) FALL

Table B-1

Zone	Total water vol (Liters)	NO_3-N conc. (mg/L)	Total NO_3-N load (mg)
1	3,203	41.0	131,305.0
2	9,605	56.0	537,868.8
3	5,098	46.0	234,489.6
4	1,142	23.0	26,261.4
5	3,136	41.3	129,516.8
6	3,984	29.5	117,528.0
7	9,126	110.5	1,008,423.0
8	8,602	182.4	1,568,931.8
9	1,400	42.0	58,800.0
10	4,176	28.7	119,851.2
11	5,250	111.3	584,325.0
12	3,133	110.3	345,558.9
Total	57,853	Average: 69 +/- 49	4,862,860 =4.86 Kg

Table B-2

Zone	Total water vol (Liters)	NO_3-N conc. (mg/L)	Total NO_3-N load (mg)
1	1,463	30.0	43,891
2	2,356	23.0	54,184
3	1,800	27.1	48,780
4	573	31.1	17,817
5	1,667	82.1	136,820
6	2,300	104.4	240,141
7	4,095	138.0	565,110
8	6,142	160.5	985,804
9	1,086	92.8	100,753
10	2,168	86.5	187,506
11	2,688	144.2	387,610
12	1,398	148.5	207,648
Total	27,736	Average: 89 +/- 52	2,976,064 =2.98 Kg

APPENDIX C

NO_3-N RUNOFF CONCENTRATIONS AT THE FOLIAGE NURSERY. 1) SUMMER. 2) FALL

Table C-1

Time (min)	Pipe 1 (m/L)	Pipe 2 (mg/L)	Pipe 3 (mg/L)
0	46.6	52.8	27.6
10	47.7	49.3	30.8
20	50.2	47.7	37.5
30	51.3	48.0	38.8
40	49.3	45.3	39.2
50	48.1	48.9	32.3
60	52.8	53.6	39.7
70	51.0	56.3	38.9
80	56.6	58.1	38.9
90	54.0	56.8	40.6
100	134.7	52.8	39.6
110	159.6	149.9	50.1
120	196.5	154.7	50.1
130	243.2	161.0	50.0
140	236.7	151.9	65.8
150	211.5	85.3	52.6
160	189.6	76.9	54.9
170	192.2	76.2	55.4
180	141.3	76.6	55.5
190	121.0	80.7	47.3
200	129.1	83.1	50.0
210	133.7	96.8	44.5
220	139.4	99.7	50.1
230	201.5	115.6	21.0
240	153.7	153.6	45.1
250	167.6	153.4	40.0
260	230.2	144.1	42.7
270	240.3	159.6	43.1
280	274.0	156.3	42.6
290	250.7	160.2	42.1
300	256.0	162.0	-
310	251.3	158.0	-
320	253.7	159.6	-
Average	152.0	102.6	43.6
Standard deviation	79.3	46.4	9.2
Minimum	46.6	45.3	21.0
Maximum	274.0	162.0	65.8

Table C-2

Time (min)	Pipe 1 (mg/L)	Pipe 2 (mg/L)	Pipe 3 (mg/L)
0	113.8	81.6	100.4
10	188.5	141.5	82.9
20	210.7	163.8	70.5
30	252.7	155.7	81.8
40	244.8	168.2	89.1
50	241.2	160.5	97.3
60	252.5	167.0	100.3
70	141.4	159.0	100.6
80	115.7	90.3	102.7
90	111.6	74.2	104.6
100	123.8	89.0	102.6
110	136.9	97.6	109.1
120	146.6	99.2	109.2
130	144.5	99.1	116.5
140	136.4	95.3	111.9
150	139.7	89.7	118.4
160	143.1	99.9	117.8
170	150.9	101.9	121.4
180	150.2	106.3	121.4
190	152.2	105.2	-
200	155.2	101.7	-
210	155.5	110.9	-
220	161.8	105.4	-
230	163.9	110.0	-
240	171.8	108.6	-
250	160.7	108.6	-
260	165.6	-	-
270	-	-	-
280	-	-	-
290	-	-	-
300	-	-	-
310	-	-	-
320	-	-	-
Average	164.1	115.0	103.1
Standard deviation	41.4	29.1	14.2
Minimum	111.6	74.2	70.5
Maximum	252.7	168.2	121.4

APPENDIX D

NO_3-N LOADINGS APPLIED TO CROPS DURING THE FLOW STUDIES AT THE BEDDING NURSERY. 1) SPRING. 2) SUMMER

Table D-1

Zone	Total water volume (Liters)	NO_3-N conc.(mg/L)	Total NO_3-N load (mg)
1	3,962	0.51	2,022.6
2	3,879	0.52	2,026.5
3	3,856	0.55	2,118.1
4	3,914	0.47	1,915.1
5	3,961	1.24	4,897.2
6	3,863	1.05	4,073.7
Total	23,435	Average = 0.73	17,053.2

Table D-2

Zone	Total water volume (Liters)	NO_3-N conc. (mg/L)	Total NO_3-N load (mg)
1	3,524	2.13	7,505
2	2,874	1.98	5,691
3	3,493	1.65	5,763
4	3,193	2.91	9,291
6	2,810	3.16	8,881
Total	15,894	Average = 2.37	37,131

APPENDIX E

NO_3-N RUNOFF CONCENTRATIONS AT THE BEDDING NURSERY. 1) SPRING. 2) SUMMER

Table E-1

Time (min)	Pipe 1 (mg/L)	Pipe 2 (mg/L)
0	20.6	3.2
10	20.3	1.7
20	19.1	1.6
30	16.0	1.7
40	18.7	1.6
50	21.3	2.0
60	20.4	2.1
70	23.8	4.2
80	26.3	7.6
90	21.4	10.1
100	24.1	11.6
110	12.3	15.1
120	15.6	15.7
130	15.0	16.0
140	14.3	15.3
150	15.6	17.9
160	16.3	19.7
170	13.9	18.7
180	14.3	16.7
190	12.6	15.3
200	14.8	16.3
210	17.3	16.7
220	17.9	11.3
230	19.1	13.1
240	14.7	13.8
250	19.6	14.8
260	16.4	-
270	19.2	-
280	19.5	-
290	19.5	-
300	19.8	-
310	19.6	-
320	13.2	-
330	-	-
340	-	-
350	-	-
360	-	-
370	-	-
380	-	-
390	-	-
400	-	-
Average	18.0	10.9
Standard deviation	3.4	6.4
Minimum	12.3	1.6
Maximum	26.3	19.7

Table E-2

Time (min)	Pipe 1 (mg/L)	Pipe 2 (mg/L)
0	0.9	1.2
10	0.8	5.1
20	0.8	7.7
30	0.7	7.3
40	5.3	6.9
50	8.5	5.9
60	7.6	5.7
70	7.6	6.2
80	7.8	7.5
90	7.9	7.9
100	7.7	8.5
110	7.5	8.3
120	7.1	9.0
130	6.9	9.9
140	6.6	10.0
150	6.4	9.9
160	6.1	9.5
170	5.9	8.3
180	5.6	7.5
190	5.3	7.4
200	5.1	7.5
210	4.8	8.0
220	4.6	8.4
230	4.4	8.6
240	4.2	8.8
250	4.1	8.9
260	4.0	-
270	3.8	-
280	3.7	-
290	3.5	-
300	3.3	-
310	3.2	-
320	3.0	-
330	2.9	-
340	2.8	-
350	2.7	-
360	2.6	-
370	2.6	-
380	2.6	-
390	2.5	-
400	2.4	-
Average	5.3	7.7
Standard deviation	2.3	1.8
Minimum	0.7	1.2
Maximum	8.5	10.0

APPENDIX F

SUMMARY TABLE FOR WATER AND NO_3-N LOADS FOR ALL FLOW STUDIES

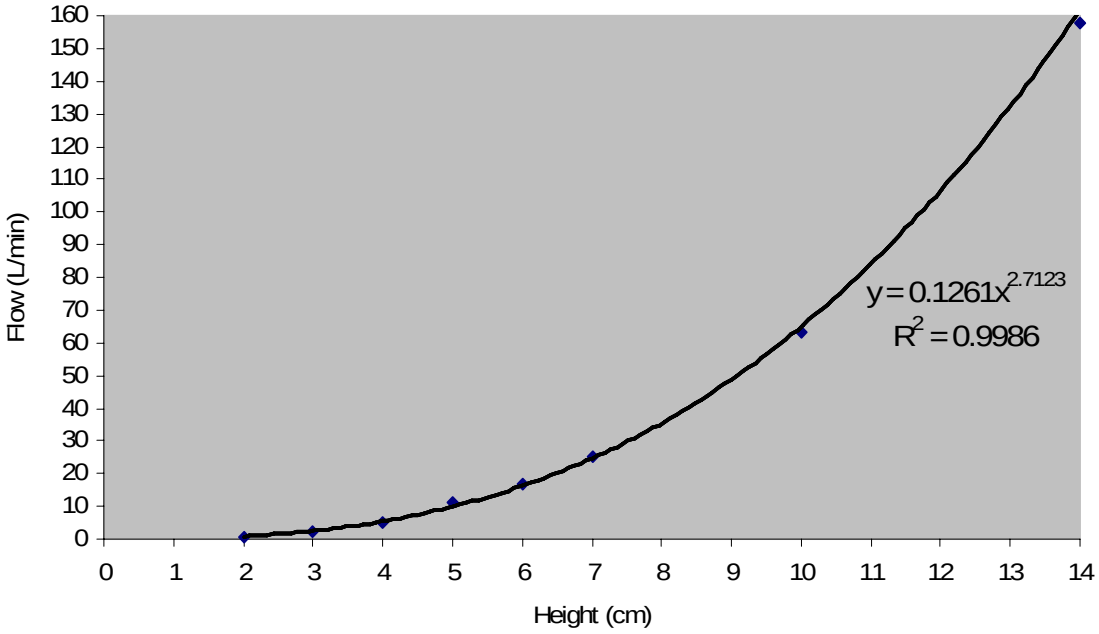
	FN- Summer	FN- Fall	BN - Spring (Irr)	BN-Summer (Irr)
NO_3-N input	4.80 Kg	2.98 Kg	17.05 g	37.1 g
NO_3-N runoff	3.00 Kg	1.99 Kg	111.42 g	51.2 g
NO_3-N runoff/acre	1.92 Kg	0.80 Kg	405.00 g	186.0 g
NO_3-N runoff %	62%	67%	665%	138%
Water input	57,861 L	27,736 L	23,435 L	15,894 L
Water runoff	20,935 L	13,668 L	7,523 L	8,190 L
Water runoff %	36%	49%	32%	52%

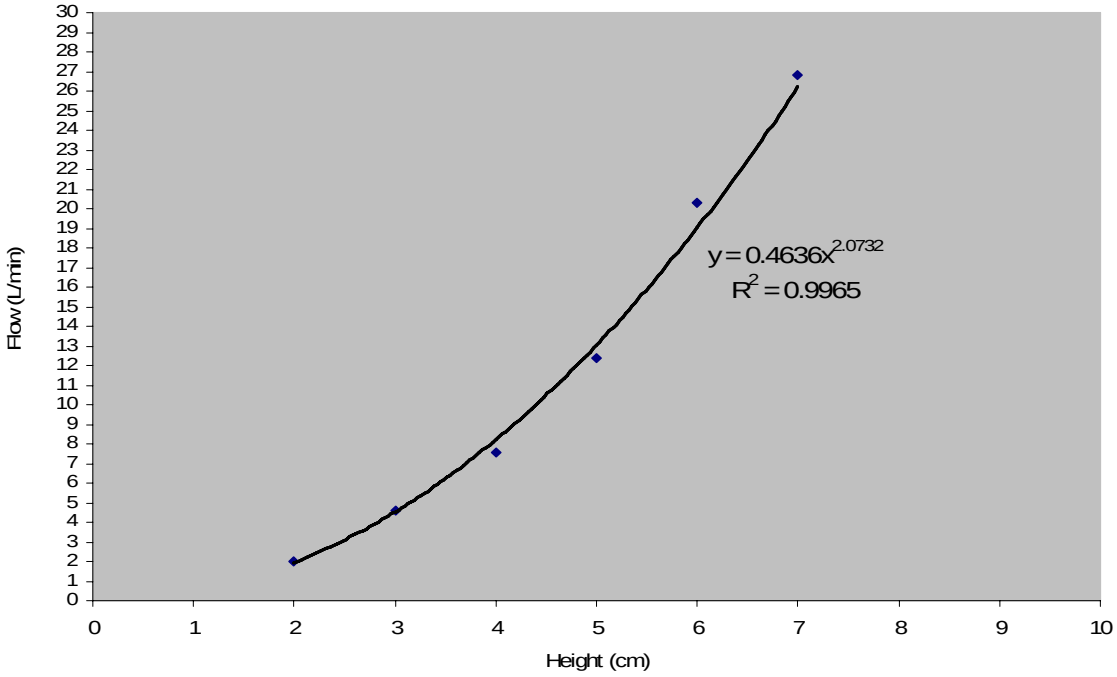
APPENDIX G

DISSOLVED ORGANIC CARBON (DOC) RESULTS DURING THE SPRING 2005 NO_3-N REMOVAL RATES STUDIES UNDER OPTIMAL DOSING SCENARIOS

Days	1-DOC (mg/L)	1- STDEV	2-DOC (mg/L)	2-STDEV	3-DOC (mg/L)	3-STDEV
Day of treatment	302	29	481	80	679	79
Day 1 (3/16)	204	70	396	49	1671	312
Day 2 (3/17)	239	22	327	31	1501	154
Day 3 (3/18)	555	36	929	57	1371	104
Day 4 (3/19)	379	46	611	32	1224	61
Day 5 (3/20)	403	12	626	34	1331	102
Day 6 (3/21)	342	30	510	31	1193	88
Day 7 (3/22)	300	27	457	50	1208	73
Day 8 (3/23)	275	26	396	24	1128	100
Day 9 (3/24)	146	26	254	25	995	105
Day 10 (3/25)	467	49	194	18	816	59
Day 11 (3/26)	334	63	178	9	1090	38
Day 12 (3/27)	291	92	172	26	1071	86
Day 13 (3/28)	300	20	791	121	923	43
Day 14 (3/29)	84	28	323	24	990	37
Day 15 (3/30)	565	43	480	45	1145	58
Day 16 (3/31)	486	44	429	34	242	21
Day 17 (4/1)	290	87	223	49	75	37

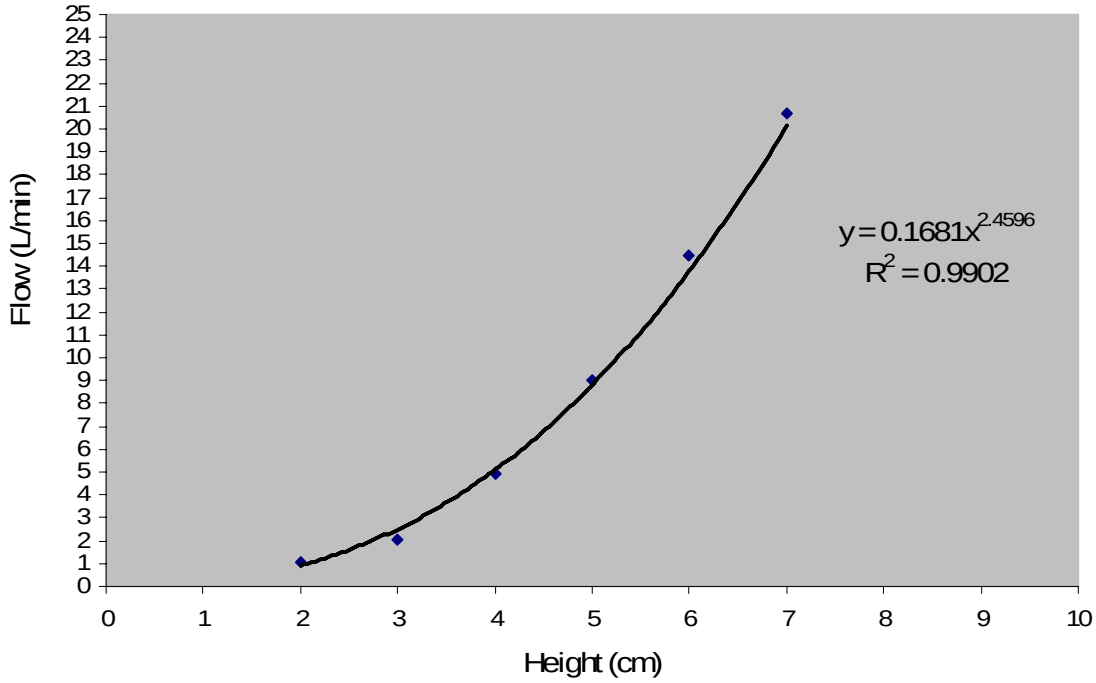
APPENDIX H
REGRESSIONS OBTAINED DURING THE CALIBRATIONS OF THE V-NOTCH WEIRS AT THE FOLIAGE NURSERY. A) PIPE 1. B) PIPE 2. C) PIPE 3



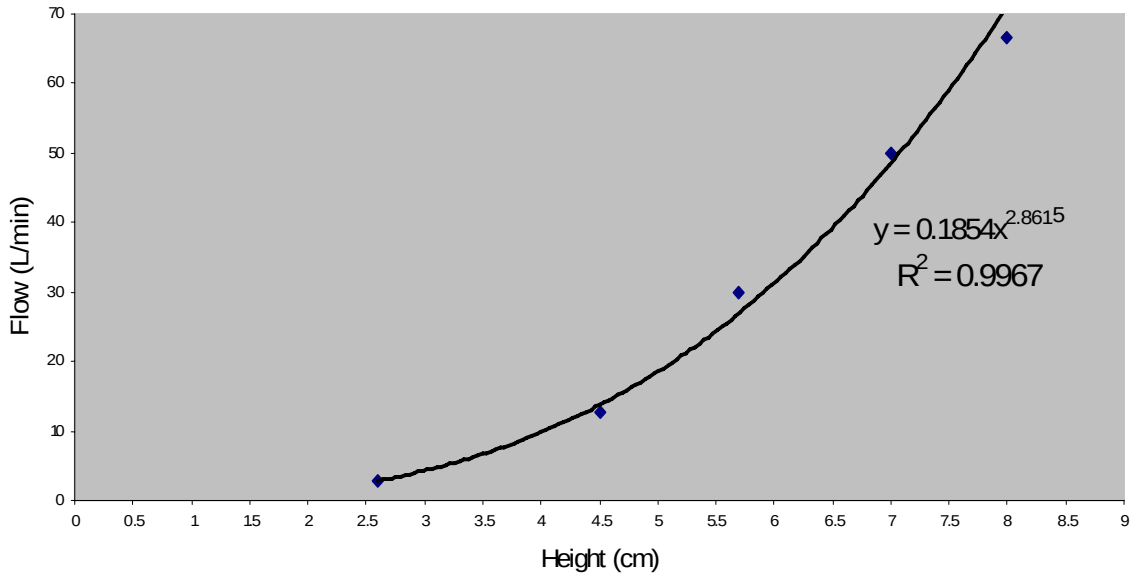


APPENDIX H. Continued

C



APPENDIX I
REGRESSIONS OBTAINED DURING THE CALIBRATIONS OF THE V-NOTCH WEIRS AT THE BEDDING NURSERY



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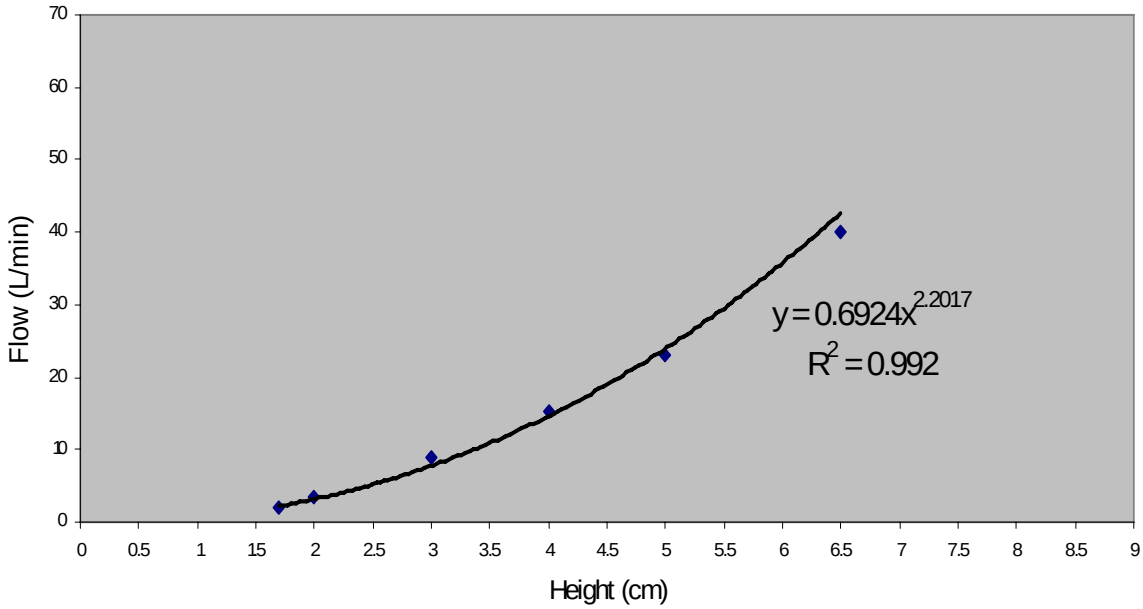

B

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BIOGRAPHICAL SKETCH

Miguel A. Mozdzen was born in a small village called el Pao, located within the Amazon basin, in Venezuela. Miguel lived and enjoyed his childhood within the wilderness while his father helped construct the third largest hydroelectric plant in the Americas. After his father returned to Chicago, USA (and later Florida), Miguel continued his fascination for the environment and natural sciences and after completing his active military service in the U.S. Army he attended the University of Florida and graduated in May 2003 with a Bachelors degree in Horticultural Sciences and in August 2007 earned a Masters degree in Environmental Sciences from the Soil and Water Science Department, while he continued to serve in the U.S. Army Reserves. Miguel currently serves as a biologist project manager for the U.S Army Corps of Engineers, Jacksonville District's Regulatory Division helping protect and conserve the nation's aquatic environment. Miguel's passion is his lovely wife and daughter.