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Source-Separated Urine Nitrification and Ion Accumulation in Sub-Irrigated Planters Using Domestic Wastewater

David A. Forrest

David Forrest
Candidate

Civil, Construction, and Environmental Engineering
Department

This thesis is approved, and it is acceptable in quality and form for publication:

Approved by the Thesis Committee:

Andrew Schuler, Chairperson

José M. Cerrato

Ricardo González-Pinzón

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David Forrest

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Thesis

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ABSTRACT

Wastewater reuse is a field at the crux of the food-energy-water nexus. This nexus is a modern attempt at qualifying relationships between resource availability and stewardship, and the choices and needs of market-driven culture. This study aims to develop simple low-tech systems to use domestic wastewater or source-separated urine, treated onsite, for domestic container garden water and nutrient supply. Four experiments were conducted to assess specific aspects of this technology to help develop relevant stages in this reuse chain.

The objective of the first experiment was a proof of concept to assess compatibility of sub-irrigated planters (SIP) with treated domestic effluent. Domestic blackwater was treated with aeration; random sampling values ranged between 0.34-0.57 mg/L DO and had a hydraulic residence time (HRT) of about seven days. This effluent was used in two SIPs as the sole source of water and nutrients. After one successful growing season, soil samples showed transport of elements from planter reservoir through the capillary fringe, in addition to plant uptake in the rhizosphere.

The objective of the second experiment was to assess parameters in using the planter reservoir for treatment of dilute urine as feed for SIP. Fresh dilute urine was used directly in an aerated and oversized SIP reservoir. After one growing season, soil samples showed some transport of elements from planter reservoir through the capillary fringe in addition to plant uptake in the rhizosphere. Planter evapotranspiration was also recorded and seen to be the primary factor determining reservoir HRT, treatment time, and thus suitability as feed solution.

The objective of the third experiment was to assess the effects of media choice on nitrification of human urine. Time for biological nitrification of four media were tested (loose potting soil, confined soil, perlite, plastic MBBR) in separate batch reactors filled with fresh dilute urine (0.8 μS). Confined soil completed partial nitrification (oxidation to nitrite) first in 31 days followed by loose soil at 39 days. The MBBR media completed partial nitrification last at 61 days. After day 61, all reactors were daily pH adjusted to 8. Full nitrification (oxidation to nitrate) was first seen at day 79 with MBBR media, on day 80 both soil reactors show full nitrate speciation, and day 85 for the perlite media.

The objective of the fourth experiment was to determine the aforementioned nitrified urine's stability against denitrification at various DO concentrations. The contents of reactors 1, 2, and 3 from Experiment 3 were combined and the resultant nitrate solution used in four different SIP reservoir configurations. DO values for the four reservoirs (R1-R4) ranged between 0.50 mg/L and 8.1 mg/L during the 20 days of observation. Day nine showed R2 with a 6% nitrate concentration drop when compared to initial concentrations, and was associated with 5 days of

DO between 0.50 – 1.5 mg/L. On the other hand, R4, which had DO between 1.75 - .50 mg/L

from day 8 to day 20, finished with a nitrate concentration almost identical to that on day 1.

These experiments may be useful in establishing parameters for some configurations of backyard

bioreactors and SIP. In particular, nitrification of source-separated urine for onsite food

production seems to hold vast potential for decentralized food security.

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1.1   Background

The food-energy-water nexus is a modern attempt at qualifying relationships between resource availability and stewardship, and the choices and needs of market-driven culture. Historically, stable populations co-evolved within ecosystems whose mindful modification could preference the satisfaction of human needs. Conversely, destruction of the supporting ecosystem or watershed destroyed or impoverished a communities' material and genetic success (Hillel, 1991). This causal relationship often generated a land ethic of biotic reciprocity that guided decision making in communities regarding ecosystem modifications (Kimmerer, 2013).

Industrial advance now enables the fixing of atmospheric nitrogen for fertalizer, usually using fossil fuel, the Haber-Bosch process has allowed food production and thus population growth to expand beyond previously available natural limits. Estimates indicate this process consumes at least 2% of global energy annually (Lui, H. 2014). The global nature of our commodity market allows such movement of resources that the consequences of our consumer choices are often not visible in our own bio-region.

Human populations generate quantities of potential soil organic matter and agricultural nutrients. Appropriate application of these materials in decay could be used to support bio-regionally appropriate systems of agroforestry. An increase in local recycling of water and nutrients for de-centralized food production will make communities more resilient and sustainable.

Wastewater reuse in agriculture includes use of both liquid and solid streams. Potential shortcomings of these strategies include: salt accumulation, metal accumulation in soil and in crops and humans, and pathogen transfer to humans and livestock (Metcalf & Eddy, 2014). Wastewater is typically treated in liquid bioreactors at a central collection facility.

As the majority of earth's people now live in urban environments, at least 5 billion people are interested in household food security. Urban gardening is an increasing phenomenon where in the UK 87% of people have access to private gardens. In Australia, more than half of urban households grow some of their own food, accounting for 34% of residential water consumption (Semananda, 2016).

Sub irrigated planters (SIP) and wicking beds (WB) are an emerging method of small scale urban cultivation. These beds can provide increased yields, decreased labor, and water savings (Semananda, 2016). Using capillary action, moisture is delivered to the plant's rhizosphere from below by a fibrous wick or soil plug in a saturated zone below. This results in a steady supply of water and constant moisture gradient in the unsaturated zone above.

Soil capillarity is a complex interaction between water's physical and chemical traits, soil surfaces, and gas exchange. Often referred to as three-phase transport, this dynamic is further complicated by the roots of plants and soil microorganisms inhabiting this zone. Overall, the constituents in the water are transported by convective flow into the unsaturated zone. This flow is a result of the physical and electro-chemical properties of the water molecules interacting with solid surfaces. Once in the rhizosphere, adsorption, precipitation, and uptake by plant roots add to the transport mechanisms (Hillel, 1998).

1.2   Surface Biosolid Application

The first and most ancient synthesis of human waste in agriculture is the application of bio-solids to the surface of soil used in crop production. This can take many forms, from the untreated 'night-soil' of east Asia to the sediment of bioreactors from Sweden to New Mexico. Yet depending on the location and upstream industry, unwanted elements may be deposited along with more useful bio-solids. Cadmium is one element of concern, because it is easily taken up by plants and highly toxic to humans (Lindberg, 2007). After advocacy in the 1980s effectively banned sludge use on Swedish farmland, policies were initiated that forced industrial polluters of the waste stream to treat their effluent on site. This resulted in a sludge certification program in 1996 which continues today, with Swedish farmers recommending only the use of sludge from certified WWTP's. And other countries are following suit: In 2008, 77% of the UK's sewage sludge was used as fertilizer (Water UK, 2010).

1.3   Constructed Wetlands

A second and more recent trend is the broad category of constructed wetlands for wastewater treatment. Such wetlands can vary in configuration, size, hydraulic residence time (HRT), macrophyte guild, type of influent, etc. The primary mechanisms of this form of water treatment are: gravity settling, oxidation, adsorbtion, absorption, precipitation, natural attenuation, media surface area for biofilm formation, and proximal macrophyte's rhizosphere to uptake elements and purify water (Ford, 2011). Constructed wetlands are often considered the best approach to mitigate toxic streams and/or in remote sites. In the case of these wetlands, agriculture production is minimized in exchange for effluent quality and passive treatment simplicity. As

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habitat for many native species of plant and animal, these system's other ecological benefits are difficult to quantify (Hammer, 1989).

In one instance, a free water surface (FSW) constructed wetland has been used to treat petroleum hydrocarbon-contaminated wastewaters from Amoco's Mandan, North Dakota facility since 1975. The wastewater flows into a constructed wetland from a conventional oil separator and a 6-ha lagoon. The wetlands consist of 11 ponds with a total area of 16.6 ha. An important consideration for the design of wetlands is the removal of biological oxygen demand (BOD), which is driven by microbial substrate availability. Chemical oxygen demand (COD) defines the oxygen requirement to chemically oxidize wastes (Metcalf & Eddy, 2006). The lagoon-constructed wetland treatment system achieved the removal of BOD (98%), COD (93%), ammonia (84%), sulfides (100%), phenols (99%), oils and grease (99%) at the hydraulic loading rate of 1.2 cm/day (Vymazal, 2014). Although the installation, size, and biodiversity of this system is large, it is a passive treatment system once the ecology has equilibrated.

In addition, various configurations of constructed wetland have been used to passively treat effluents from tanneries and pharmaceutical plants, breweries, and dairies (Vymazal, 2014). Depending on the site chemistry and available resources, passive treatment is generally preferable in remote locations. Nyquist et al. (2009) found a 50% decrease in copper concentrations of mine runoff diverted through a wetland. Site-specific chemistry informs the configuration of a wetland's various components. Further, the reactions involved in normalizing pH values and metal sequestration are more difficult for biological systems to perform (Ford, 2003). Often these processes rely on adsorbtion, oxidation, and precipitation by physical rather

than biological agents. Thus, wetland performance for effluent heavy with organic compounds is usually better than for a stream laden with inorganics, metals, and hydrogen ions.

One wetland configuration relevant here is called a constructed wetland with horizontal sub-surface flow. In this system the influent is introduced into a porous medium under the surface of a bed that is planted with appropriate macrophytes. This configuration has many similarities with SIP, except with regards to the common aim of creating an anoxic and anaerobic treatment environment (Vymazal, 2008). In this application, we see the goal of denitrification of effluent rather than maximizing fertilizer and/or macrophyte value.

Individual process of wetland treatment can be parsed out, such as nutrient removal by various hydroponic and biofilm techniques. Vaillant et al. (2003) used nutrient film technique in a soilless hydroponic trough to treat raw domestic wastewater to effluent standards with a recirculating batch system. Planted with *Datura innoxia*, this group saw effluent water quality standards met after just 24 hours. Yang et al. (2015) pretreated urine with urease enzyme, struvite precipitation, and air stripping before dilution and use as hydroponic feed solution. Struvite (NH_4MgPO_4) precipitation is emerging as a common link between wastewater and agriculture, and in this study the magnesium was introduced using seawater. One explanation for using this pretreatment is its ability to treat high volumes of high concentration flow quickly, rather than by sole reliance on nitrifying bacteria and macrophytes. It should be noted, though, that the addition of the sodium and chloride in seawater decreases the solution's suitability for fertilizer.

Yang et al. (2015) also used a flow-through trough to polish treated urine to effluent standards in a 21-day batch system. Their best-performing dilution ratios range between 20 and 50 times. At these ratios, it would seem that the cost in potable dilution water negates the value of the treated effluent. In another case, Sooknah et al. (2004) found that floating beds of water hyacinth purified a batch pond of dairy sludge, removing 92 percent of total Kjeldahl nitrogen (TKN) in 31 days. Here, the value of the macrophyte is negligible, so nutrient value is wasted in exchange for simplicity and effluent quality. Also, neither of these approaches is interested in creating or maintaining an ideal plant nutrient solution from a wastewater source. This emphasis on effluent quality is a trait shared by industrial-scale waste streams and centralized treatment facilities. Yet household scale domestic flows are such that both water and nutrient can be fully consumed in many regions landscape and garden installations throughout much of the year. Of course, simple household septic systems are already widely used, yet these provide neither fertilizer nor macrophyte value.

1.4 Modern Wastewater Reuse

This most high technology reuse route involves treating wastewater to almost potable standards, then using a separate set of infrastructure to deliver this water to regional farmers, golf courses, and the like. Water quality reports from the San Diego north and south-side plants show typical electrical conductivity (EC) values between 1200-1600 μS and a chloride permit maximum of 300 mg/l with actual values around 250 mg/l, while total nitrogen is around .01mg/l and phosphorus reduced to .002 mg/l (San Diego City, 2016). Electrical conductivity reflects the ability of water to conduct current and is directly related to the concentration of salts dissolved in the water. This approach involves a treatment train more complex than one designed for typical

wastewater, and removes almost all desired nutrients while leaving undesirable salts, recycling the water only and requiring additional treatment and delivery infrastructure expense. These permit numbers seem to be the current economically feasible compromise between treatment expense and effluent value.

1.5 Sub-Irrigated Planters

Raised bed agricultural platforms (*chinampas*) have been maintained in American wetlands since before European contact (Seimens, 1983). Among the advantages of this configuration is the capillary fringe moisture delivery system to the rhizosphere. Within the last couple of decades, several consumer SIP products have appeared utilizing this water delivery system and touting many advantages, such as increased yield, decreased labor, and decreased water use. Not until 2016 were these producer claims assessed with scientific rigor by Dr. Niranjani Semananda at the University of South Australia.

In an article published in *Horticulturae*, Dr. Semananda compares the performance of various SIP configurations against precision drip surface irrigation of pots (Semananda, 2016). In addition to determining an optimum bed depth for planters with tomato plants of 11.8 inches, it was shown that SIP performed as well or better than precision drip irrigation for fruit yield, fruit quality, labor input, and water use efficiency. In addition, SIP are simpler and more robust than precision irrigation. Though container material and importation of potting soil detract from the complete sustainability of this system, the increase in urban gardening may make container style applications more attractive.

1.6   Nitrogen Forms and Transformations

Nitrogen naturally exists in several different chemical forms and oxidation states. Urea (CH_4N_2O) is the most abundant constituent in urine and is the primary source of nitrogen in domestic wastewater. Urease enzymes already present in urine, begin to hydrolyze urea to ammonia under non-sterile conditions (Eq. 1-1) (Maurer, 2006). Ammonia (NH_3) and ammonium ions (NH_4^+) comprise an acid/base pair, and represent nitrogen in its reduced oxidation state (-3). They are produced from hydrolysis of urea and degradation of organic nitrogen compounds, Equation 1-1. The chemistry equilibrium equation between ammonia and ammonium ions can be written as Equation 1-2.

$$CH_4N_2O + H_2O \rightarrow CO_2 + 2NH_3 \tag{1-1}$$

$$NH_3 + H_20 \leftarrow \rightarrow NH_4^+ + OH^- \tag{1-2}$$

The ammonia/ammonium equilibrium equation is pH and temperature dependent. In a temperature range of 10° to 20° C and a pH range of 7 to 8.5, around 95% of the reduced form of nitrogen is NH_4^+ (Gerardi, 2002).

Nitrification is defined as the oxidation of reduced nitrogen (e.g. ammonia) to oxidized forms of nitrogen (e.g. nitrite and nitrate). Nitrification commonly occurs as a two-step process, with the first step (nitritation) performed by ammonia oxidizing bacteria (AOB), such as *Nitrosomonas sp.* (Eq. 1-3), and the second step performed by nitrite oxidizing bacteria (NOB), such as *Nitrobacter* (Eq. 1-4).

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+ \tag{1-3}$$

$$NO_2^- + 0.5\,O_2 \rightarrow NO_3^- \qquad\qquad (1\text{-}4)$$

The net nitrification reaction is:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2\,H^+ \qquad\qquad (1\text{-}5)$$

Suzuki et al. (1974) reported that ammonia (NH_3) rather than ammonium ion (NH_4^+) serves as the substrate of ammonium oxidizers (AOB). From equations 1-1 and 1-2, it is seen that hydrolysis of urea increases pH, which in turn increases the NH_3 speciation. Ammonia (NH_3) can inhibit nitrification (Anthonisen et al., 1976), as discussed below.

During the oxidation of ammonium ions to nitrite, hydrogen ions are released and reduce the pH (Eq. 1-3). Nitrite exists in equilibrium with un-ionized nitrous acid (free nitrous acid, FNA) as an acid/base pair according to Eq. 1-6 with pKa = 3.3. Decreased pH in turn increases FNA speciation, which can create inhibition of nitrification at low pH and concentrations in the range of 0.22 to 2.8 mg N/L, discussed below (Anthonisen et al., 1976).

$$H^+ + NO_2^- \rightarrow HNO_2 \qquad\qquad (1\text{-}6)$$
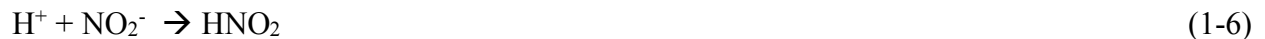
The alkalinity consumed during nitrification can be estimated by including bicarbonate (HCO_3^-) consumption in the net nitrification reaction (Metcalf & Eddy, 2014).

$$NH_4 + 2HCO_3^- + 2O_2 \rightarrow NO_3^- + 2CO_2 + 3H_2O \qquad\qquad (1\text{-}7)$$

Assuming the empirical formula of bacterial cells to be $C_5H_7O_2N$, a synthesis yield of 0.12 g VSS/ g NH_4^+ oxidized (volatile suspended solids/gram N), and the addition of alkalinity as $NaHCO_3$, the following equation can represent the net stoichiometry of nitritation (Metcalf & Eddy, 2014):

$NH_4^+HCO_3^- + .985NaHCO_3 + .0744CO_2 + 1.4O_2 \rightarrow 0.01485C_5H_7O_2N + 0.985NaNO_2^- +$

$2.94H_2O + 1.985CO_2$ (1-8)
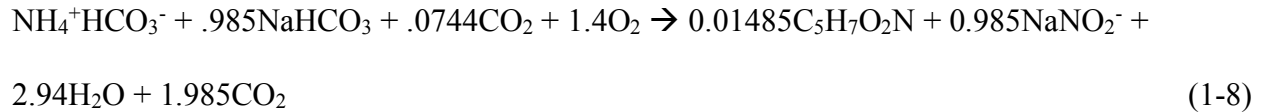
Assuming a synthesis yield coefficient of 0.04 g VSS/ g NO_2, the overall nitrification can be represented as follows:

$NH_4(HCO_3) + .985Na(HCO_3) + .099CO_2 + 1.867O_2 \rightarrow 0.01982C_5H_7O_2N + 0.985NaNO_2 +$

$2.92H_2O + 1.985CO_2$ (1-9)
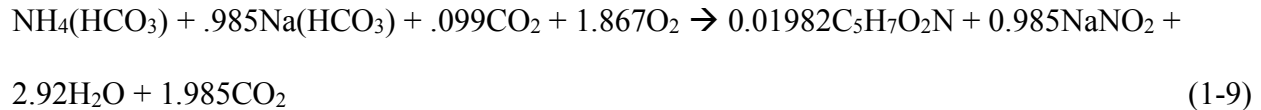
As seen in the previous discussion, various factors affect the behavior of this equation including species concentration, temperature, and pH. The journey from urea to nitrate can take weeks in single batch configuration. Since the ideal conditions for completion of each stage are different, sequenced batch reactors can accelerate the process by maintaining robust and separate populations of AOB and NOB.

1.7 Nitrifying Bacteria

As previously discussed, nitrification is executed by nitrifying bacteria. These ammonium or nitrite oxidizers (AOB and NOB, respectively) are autotrophic microorganisms. They obtain carbon from dissolved CO_2 and energy for growth and other functions from the oxidization of ammonium and nitrite. One mole of carbon dioxide to be assimilated into nitrifying bacteria requires approximately 30 moles of ammonium ions or 100 moles of nitrite ions to be oxidized (Gerardi, 2002). Due to a relatively large energy requirement for growth, nitrifying bacteria have a low generation rate.

AOB, which are responsible for the ammonia oxidation, have up to 25 species isolated from activated sludge (Koops and Pommerening-Roser, 2001), among which *Nitrosomonas* is the most extensively studied. Different AOB species have different salt requirements and substrate affinities.

NOB which are responsible for the nitrite oxidation have at least 8 species isolated from active sludge (Koops and Pommerening-Roser, 2001). They differ in eco-physiological requirements. *Nitrobacter* is the representative NOB genus. Table 1-1 compares some basic features of AOB (*Nitrosomonas*) and NOB (*Nitrobacter).*

Table 1-1   Basic features of *Nitrosomonas* and *Nitrobacter* (Madigan, 2015).

Feature	*Nitrosomonas*	*Nitrobacter*
Cell shape	Coccus (spherical)	Bacillus (rod)
Cell size (μm)	0.5 - 1.5	0.5 - 1.0
Habitat	Soil and water	Soil and water
Reproductive mode	Binary fission	Budding
Carbon source	CO_2	CO_2
Oxygen requirement	Strict aerobe	Strict aerobe
Temperature growth range	41°F - 86°F	41°F - 102°F
pH growth ranges	5.8 - 8.5	6.5 - 8.5
Generation time	8 - 36 hours	12 - 60 hours

Table 1-2   Nitrifiers preferred environmental parameters (Metcalf & Eddy, 2014).

Parameters	Optimum range
DO	2-3 mg O_2/L
pH	pH = 7.2 - 8.0
Temperature	70 °F to 89 °F
NH_3	<10 mg/L for *Nitrosomonas* inhibition, <.1 mg/L for *Nitrobacter* inhibition
HNO_2	<1 mg/L for both *Nitrobacter* and *Nitrosomonas* inhibition

1.8   Ammonia and Nitrous Acid Inhibition:

Anthonisen et al. (1976) and others have investigated the inhibitory parameters that affect

nitrification at relevant ion concentrations and pH. Mieklejohn (1954) broadly states the

scenario:

> "The electrolytes to which the nitrifiers are sensitive include their own substrates,
> and in each case, the substrate of the other species was found to be much more
> toxic than the organisms' own substrate. Nitrite depressed both respiration and
> growth of *Nitrosomonas* [. . .] *Nitrobacter* was sensitive to the ammonium ion,
> but even more so to free ammonia [. . .] Nitrate was only slightly toxic to both
> species."

This set of relationships involves multiple types of microbe preference as well as the pH

dependence of nitrogen species' fractions and transformations.

To plot an environmental sweet spot to support full nitrification in terms of ammonia and nitrite concentrations, Anthonisen et al. (1976) offers two equations. The free ammonia fraction can be determined from the total ammonia concentration, pH, and temperature as follows:

$$\text{NH}_3 \text{ (mg/L)} = \frac{17}{14} * \left(\frac{total\ ammonia\ as\ N \left(\frac{mg}{L} \right) * 10^{pH}}{\frac{K_b}{K_w} + 10^{pH}} \right) \tag{1-10}$$

K_b = equilibrium constant of ammonia, K_w = equilibrium constant of water.

The ratio of K_b to K_w can be related to temperature:

$$\frac{K_b}{K_w} = e^{\left(\frac{6344}{273 + ℃} \right)} \tag{1-11}$$

Similarly, the concentration of free nitrous acid can be determined:

$$\text{HNO}_2 \text{ (mg/L)} = \frac{46}{14} * \left(\frac{NO_2^- \ as\ N \left(\frac{mg}{L} \right)}{K_a * 10^{pH}} \right) \tag{1-12}$$

The equilibrium constant of nitrous acid varies with temperature:

$$K_a = e^{\left(\frac{-2300}{273 + ℃} \right)} \tag{1-13}$$

These equations provide inhibition boundaries as a function of total concentration and pH. FA inhibition on *Nitrosomonas* range from 10-150 mg/L while those for *Nitrobacters* are from 0.1 mg/L to 1 mg/L. FNA inhibition affects both, in a range from 0.22 mg/L to 2.8 mg/L (Anthonisen et al., 1976). These boundaries create a triangular shape beneath which uninhibited nitrification can occur.
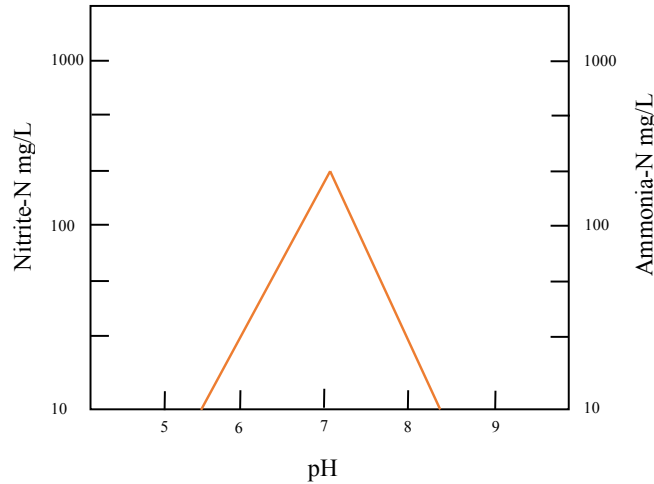
Figure 1-1   Nitrification inhibition boundaries (developed from Anthonisen et al., 1976).

1.9   Nitrification of Source Separated Urine

Source separated urine is an emerging strategy in the wastewater industry. With a slight increase in fixture complexity, various local needs could be met. The majority of nitrogen and phosphorus in the wastewater stream are from urine, so multiple strategies have emerged to help recycle these nutrients. One advantage of urine separation is that the high N and P concentrations facilitate nutrient recovery, as through chemical precipitation such as struvite.

After hydrolysis, Verhave (2009) refers to partial nitrification as a self-regulating reaction. Because there is only enough alkalinity produced during hydrolysis to facilitate nitrification of about half the initial nitrogen concentration (Equations 1-1 and 1-7). After this, the pH settles near 5.7, which inhibits further nitrification. This solution state is sometimes referred to as partial nitrification equilibrium.

Udert et al. (2003b) studied three lab-scale systems for treating diluted urine: 1. a moving-bed biofilm rector (MBBR), 2. a continuously stirred tank reactor (CSRT) and 3. a sequencing batch reactor (SBR). External alkalinity was not added in this urine treatment, so nitrification will only oxidize about half the ammonium in the urine before the pH dropped to 6. The effluent from the MBBR contained ammonium and nitrite in equal proportion. The final ammonia loading of the MBBR was 750 ± 50 g $N/m^3/d$ with an influent nitrogen concentration of 7100 ± 100 g N/m^3. The effluent from the CSTR was similar to that from MBBR and the nitritation rate was found to be 790 g $N/m^3/d$ at a steady state when the HRT was 4.8 days. In the SBR, the overall nitritation rate was low, at only 280 g $N/m^3/d$ with an influent nitrogen concentration of 2240 g N/m^3.

Udert and Wachter (2012) showed that an increase in pH set point of their MABR resulted in nitrite accumulation and consequent nitrous acid inhibition of nitrification (Figure 1-2). To remedy this, aeration was stopped and acetate added for six days in order to stabilize the reactor.
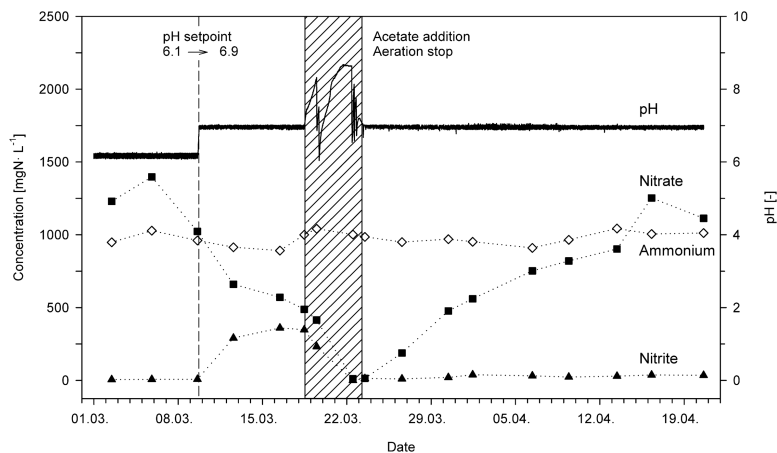
Figure 1-2   Nitrite build-up after sudden increase of pH
set point from 6.1 to 6.9. Udert and Wachter (2012).

All research papers here reviewed used stored urine, in which cases the urea has undergone

complete hydrolysis. At this stage the pH is near 8, and the nitrogen is primarily ammonia. In

this form, nitrogen loss can be high from ammonia volatilization, depending on pH. At pH of

6.7, Udert (2003b) reported 24% nitrogen loss in a membrane-aerated biofilm reactor; he

speculates that heterotrophic denitrification occurred in addition to volatilization.

Feng et al. (2008) operated a packed-bed SBR with aeration and no pH control initially, to

achieve partial nitrification (Figure 1-3). Once all of the alkalinity produced during hydrolysis

(Eq. 1-1) was used, the pH measured 5.8 and about half of the nitrogen was nitrite (day 10).

Beginning on day 12, the pH was daily adjusted to 8; this facilitated the transformation of the

remaining nitrogen to nitrate.
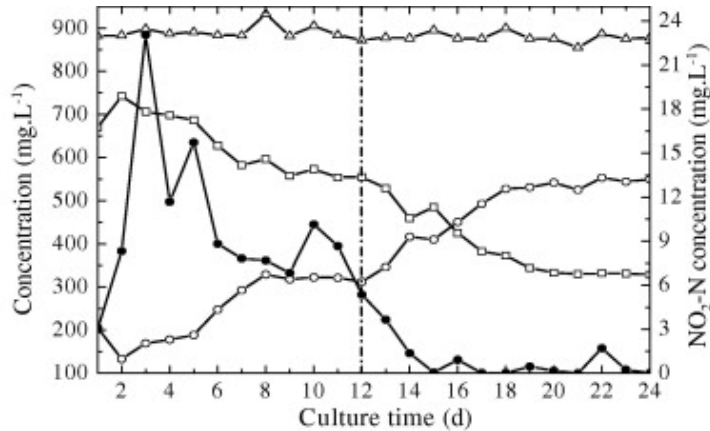
Figure 1-3   Synthetic urine nitrification.
Δ = Total N, O = Nitrate, □ = Ammonia
● = Nitrite. Feng et al. (2008).

Anthonisen et al. (1976) operated two aerobic bioreactors side by side, one with and the other without pH adjustment after day 16. Both units contained about 50% ammonia and 50% nitrite after 15 days. After 35 days, the reactor with no pH adjustment contained only nitrite. The other unit began pH adjustment on day 16 with a similar 50:50 ammonia:nitrite ratio. Figure 1-4 (right side) shows a continued increase in nitrite until day 30, after which nitrate begins to form. By day 37, all of the nitrogen is in the form of nitrate. Since nitrate is the desired end result for agriculture, this data informs both the timescales and alkalinity stoichiometry of our efforts.
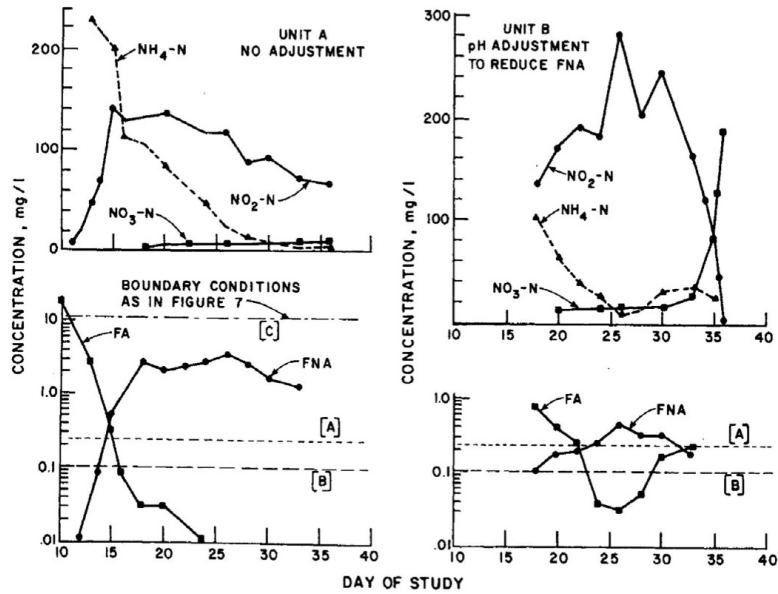
Figure 1-4   Nitrification with and without pH adjustment. (Anthonisen et al., 1976).

1.10   Research Needs and Objectives

Currently no published work is available combining simple domestic wastewater treatment trains with SIPs. The solutions envisioned involve a minimum of wastewater treatment, while maximizing macrophyte value, using wicking capillary beds with various domestic wastewater streams. One goal of this project is to see if this configuration can be harnessed to provide low cost sequestration of undesirable wastewater constituents while utilizing the agricultural nutrients in a waste stream with a minimum of treatment.

The following are research needs that have been identified:

- It is unknown whether treated blackwater is suitable as a nutrient and water supply for SIP in household agriculture.

- It is unknown whether fresh dilute urine is suitable as a nutrient and water supply for aerated SIP in household agriculture.

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- It is not known how ion accumulation occurs as a function of depth in the planter bed of SIP.

Objectives:

- Assess effectiveness of aerobically treated domestic blackwater and/or diluted fresh urine as water and nutrient feed for reuse in SIP systems.

- Determine salt (ion) accumulation as a function of depth in the SIP planter bed.

- Determine the effect of four different media on nitrification of fresh dilute urine, as treatment for feed of SIP systems.

In the first experiment, domestic blackwater was treated with aeration; random sampling values ranged between 0.34-0.57 mg/L DO and had a hydraulic residence time (HRT) of about seven days. This effluent was used in two SIPs. After one growing season, soil plugs were extracted at regular depths to assess element migration and accumulation within the planter.

In the second experiment, fresh dilute source-separated urine (0.8 μS) was used directly in an aerated and oversized SIP reservoir, random sampling values ranged between 4.32 – 5.67 mg/L DO and HRT varied with seasonal plant metabolism. After one growing season, soil plugs were extracted at regular depths to assess element migration and accumulation within the planter.

In the third experiment, four media were tested (loose soil, confined soil, perlite, plastic MBBR) in four batch reactors filled with fresh dilute urine (0.8 μS), and the rates compared for the 3 stages of complete biologic nitrification.

The fourth experiment monitored the nitrate solution from Experiment 3, for 20 days in four different SIP reservoir configurations. Daily solution DO measurements were taken and

compared to solution nitrate concentrations to assess stability against possible denitrification through time.

This study examines systems which may help shorten human food and waste transportation distance, close and shorten relevant nutrient loops, and reduce dependence on high-energy, centralized systems for human sanitation and subsistence. The solutions envisioned involve a minimum of wastewater treatment while maximizing macrophyte value, using SIP with various domestic waste streams.

Chapter 2　Experiments

2.1　Experiment 1: Treatment of domestic blackwater in aerated septic tank as feed to unaerated SIP.

Research needs:

- It is unknown whether treated blackwater is suitable as a nutrient and water supply for SIP in household agriculture.

- It is not known whether salt accumulation occurs in the reservoir or planter bed of such systems.

Objective:

- To demonstrate that aerobically treated domestic blackwater can be used as water and nutrients with SIP.

- To determine where salt (ions) accumulate vertically in the planter profile.

2.1.1　Materials and Methods

Domestic blackwater from a single family home of three adults was aerobically treated onsite

with a simple treatment train consisting of: settling/skimming, aerated bioreactor, settling/pump

tank. This is a typical two compartment 1500 gallon septic tank with faint aeration in the

downstream side and a 300 gallon collection/pump tank after. Recycled activated sludge (RAS)

was separated by a size exclusion filter and relative flow controlled manually with ball valves.

Dissolved oxygen measurement in the aerated tank portion ranged between 0.20 – 0.45 mg/L

from random sampling. This treated effluent was slightly cloudy in color and contained

conductive ions measuring 1.36 μS on the day of planter installation. Figure 2-1 shows the basic

schematic.

21

Ion	Well Water (mg/L)	Waste Water (mg/L)
Ca	22.9	31.8
K	1.5	24.5
Mg	3.5	29.15
Na	26.5	75.97
Si	10.99	6.1
P	0.12	5.7
NH_3	0	39.6
NO_2^-	0	5.8
NO_3^-	0	0.6
EC (μS)	0.1	1.36

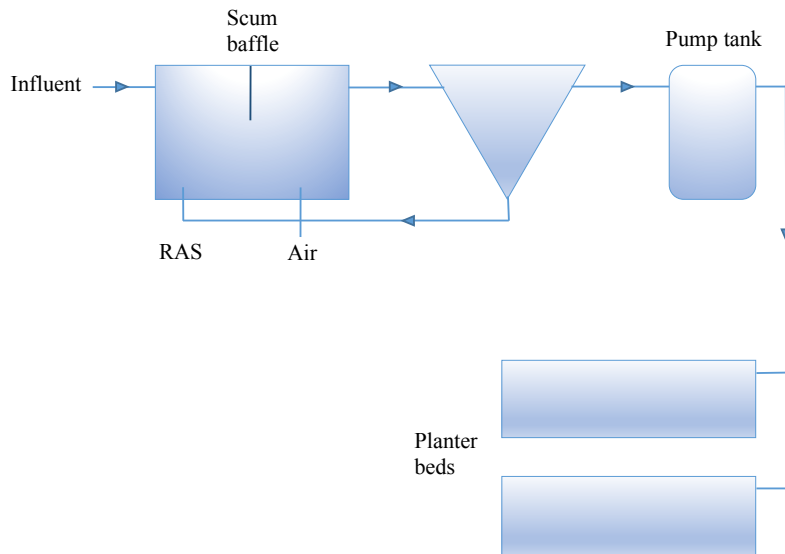Table 2-1   Well and wastewater concentrations.

Figure 2-1   Experiment 1 wastewater treatment schematic.

Figure 2-2   Planter Installation.

To this effluent stream were connected two sub-irrigated planters of 3'L x 12"W x 18"H, Figure 2-2. The planer reservoir is a wood frame measuring 9"H x 12"W x 3'6"L draped with pond liner. The planter bed is a wood frame of 9"H x 12"W x 3'L dimensions with an apron of landscape fabric stapled along the bottom edge. Horizontally between these frames is a layer of ¾" expanded metal lath with 3" holes cut to receive the wicking columns. Onto this lath is laid a 0.1" rubber grid also with 3" cutouts for the wicks. The wicks are made of 3" PVC perforated with ½" holes, then packed with the same soil used in the planter bed. The green lidded ports on the lower edge (Figure 2-2) allow access to the reservoir. The reservoir fluid level is maintained using a float valve, this is tied to the treated effluent dispersal system which feeds this zone 2 or 3 times per week, depending on household water use. The total soil column, from bottom of reservoir to top of planter, was 18 inches, and from the free water surface to the top of planter between 10 and 12 inches (Figure 2-3). This configuration allowed assessment of multiple variables simultaneously, many of them subjective.

The planter beds and wicks were filled with a standard potting soil mix (Black Gold, Natural & Organic). The advertised soil composition is 30% sphagnum, 30% perlite, 30% compost, 10% aged bark compost. Tomato (*Solanum lycopersicum*) and butternut squash (*Cucurbita moschata*) were grown in them from June through October, one outdoor season of four months. The ambient temperatures ranged between 60°- 95° F. No well water was added to the reservoirs during this time, and no effluent water left the reservoir. Less than 4" of rain precipitated on the planters over the course of the four-month growing period. Any wastewater ions not taken up by plant roots accumulated in the soil, with the likely exception of some ammonia which volatilized to the atmosphere.

Soil samples were collected from the planter beds after four months of growing using only wastewater feed. Two-inch diameter by two inch length soil plugs were obtained after the first killing frost using a garden trowel—see white circles on Figure 2-3. A measurement datum was established at the bottom of the planter bed, samples from the wicking column are given a negative depth and those from the planter bed a positive one. The – 6" point was always fully saturated below the reservoir surface. These soil samples were compared against unused potting soil saved from the beginning of the season as a control.

Soil samples were oven-dried at 100° C for 48 hours to remove all moisture. Then, the samples were pulverized for five minutes using a shatterbox. Each sample was weighed to near 1.50 g. Using 8 ml nitric acid and 4 ml hydrochloric acid, the samples were digested for 24 hours at 100° C. Samples were diluted to 25 ml with 2% nitric acid. Sample duplicates were made and diluted 100 times with the same 2% nitric acid to assess aluminum and potassium concentrations, which were beyond the equipment's scale in the first sample set. These were then run on ICP-OES (PerkinElmer Optima 5300DV) to generate solid concentration results as mg/Kg solid.

Electrical conductivity and pH were measured using Blue Lab electrical conductivity pen and pH pen. Analysis of NH_3^- was performed using Hach test and optical analyzer (Hach DR2700). Solutions were further characterized for NO_2^-, NO_3, and Cl^- by ion chromatography (Thermo Fisher Dionex ICS-1100) and ICP-OES (Perkin Elmer Optima 5300DV) yielding results in mg/L of solution.
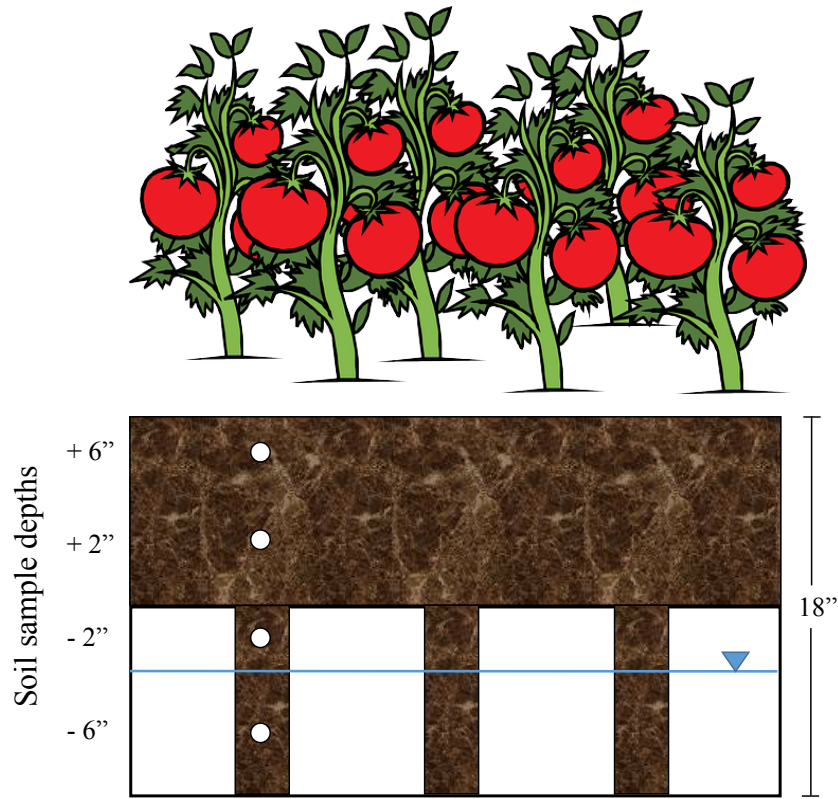
Figure 2-3   Sub-irrigated planter model

2.1.2   Results and Discussion

To consider the data, it is helpful to organize the elements into two primary categories: those that are beneficial and useful to plant metabolism, and those that are found in abundance in wastewater. In some cases these categories overlap, as with phosphorous, while in other cases they do not, as with sodium. What is obviously missing in this analysis is nitrogen. Clear assessment of its forms in soil is difficult. Nitrogen transformations in water will be addressed later.

For this experiment, four points were chosen at regular intervals from the soil profile: one in the saturated zone, and three in the unsaturated zone (Figure 2-3). The reservoir depth varied up to three inches during operation so the capillary fringe and soil saturation levels oscillated with the reservoir's level. The highest point (+6") generally represents the phase change zone where residual salts accumulate from evaporation. The zero datum represents the bottom of the bed where it meets the soil wicks, 2-5 inches above the free water surface. The graphical grouping of ions is solely a function of relative concentration similarities. The choice of ions is a function of both abundance and distribution variance.

It is apparent that almost all ions are taken up indiscriminately by the plants in the +2" root zone, with the possible exception of sodium, Figures 2-4, 2-5 and 2-6. It is also clear that almost all ions accumulate at the +6" capillary fringe in higher concentrations than is found in and adjacent to the reservoir. Chromium and zinc showed very little difference in concentration across the soil column; this makes sense as there was none in the waste stream (see Appendix A).
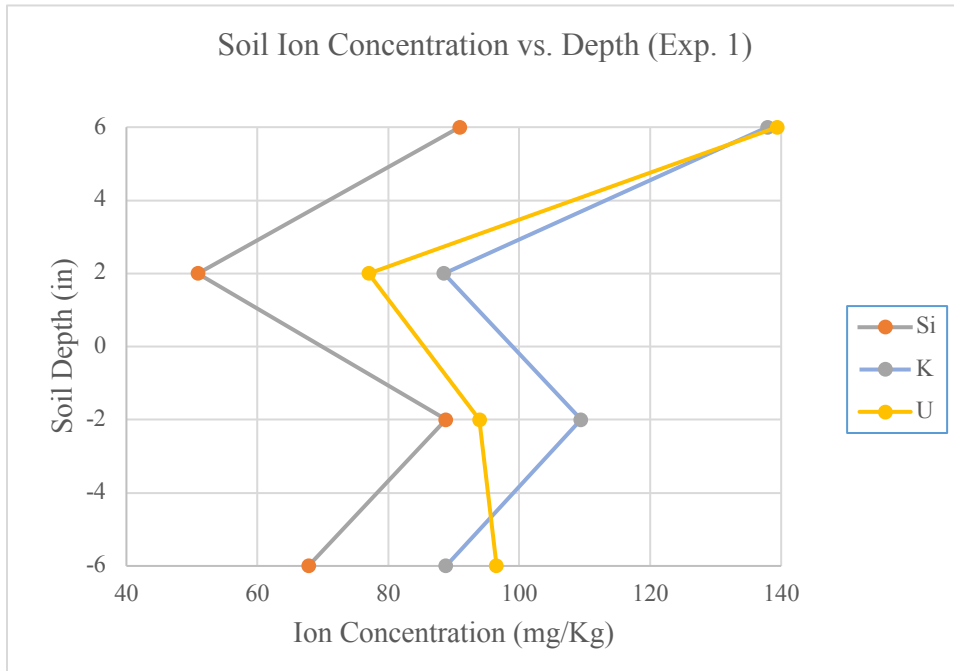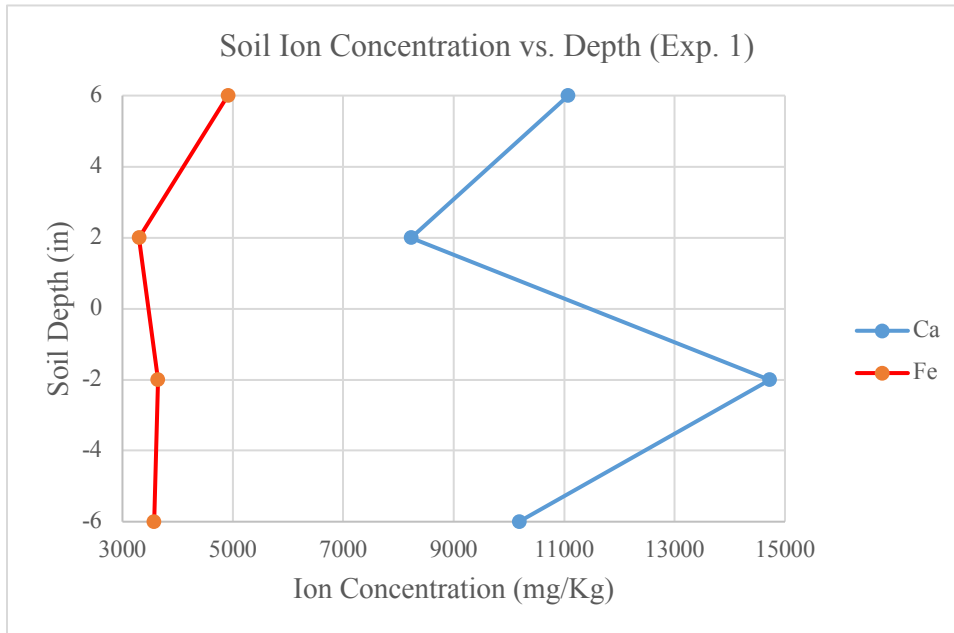
Figure 2-4　Soil ion concentration.

Figure 2-5　Soil ion concentrations.
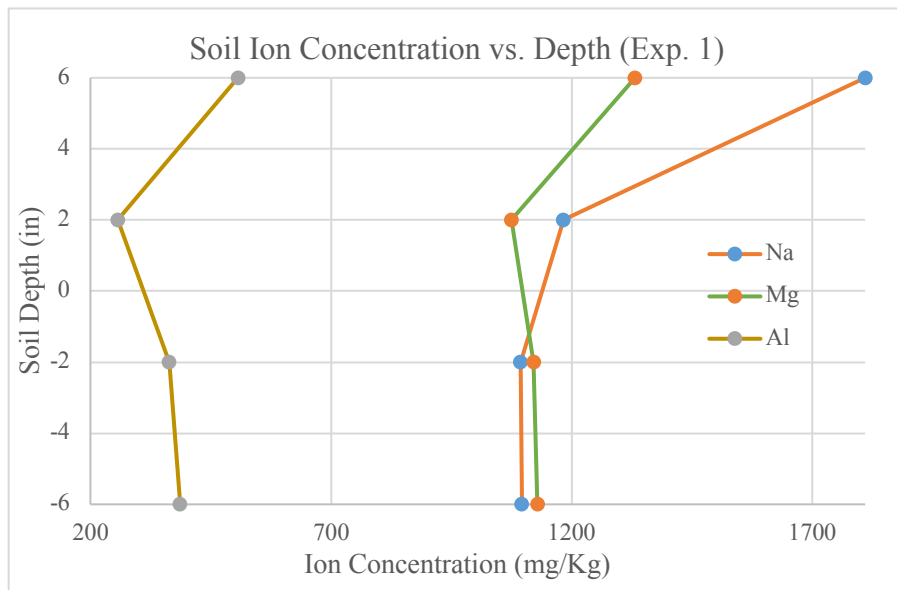
Figure 2-6   Soil ion accumulations.

Figure 2-7   End of season fruit and foliage (day 115).

2.1.3 Conclusions

The performance of this system overall showed it to be a viable configuration for treated

domestic wastewater combined with SIP. A relative lack of surface precipitate crust indicates

that the planter soil may be reused for another season. The overall soil ion concentration trends

spread in an expected gradient for capillary fringe. The generally lower soil concentrations in the

+2 root zone indicate little plant preference for uptake of both desirable and undesirable elements

in the soil.

2.2 Experiment 2: Diluted fresh urine as feed for aerated SIP

Research needs:

- It is unknown whether fresh dilute urine is suitable as a nutrient and water supply for aerated
SIP in household agriculture.

- It is not known where ion accumulation occurs in the planter bed of such systems.

Objectives:

- Apply diluted fresh urine feed as water and nutrient feed for an aerated SIP system.

- Compare overall growth in this system relative to growth in the unaerated, treated blackwater
system of Experiment 1.

- Assess rates of urea hydrolysis and nitrification in an aerated SIP system.

- Determine rates for evapotranspiration in an aerated SIP system.

- Compare final planter ion accumulation in aerated SIP system fed with dilute fresh urine to the
system used in Experiment 1.

2.2.1   Materials and Methods

Figure 2-8   Partial nitrification planter installation.

In this iteration, an SIP was constructed with an oversized and aerated reservoir. The dimensions were 2'W x 3'L x 11"H of reservoir and planter each (Figure 2-8). The same pond liner, metal lath, rubber grid, soil and soil wicks were used as in Experiment 1. The reservoir was filled with 20 gallons of fresh urine diluted to 0.8 μS with well water (about 10x dilution), and topped with the same based on the planter's daily evapotranspiration (ET) rate. Four air stones in the reservoir maintained dissolved oxygen levels above 4.0 mg/L and ensured mixing. The soil cube and wicks used Black Gold Natural and Organic standard potting soil. This system was intended as a self-regulating, partial nitrifying reactor, and so no pH adjustment was used. The wick bed was planted with yellow pear tomato (*Solanum lycopersicum*) and grown outdoors for four months under ambient conditions.

Soil samples were oven-dried at 100 degrees C for 48 hours to remove all moisture. Then, the samples were pulverized for five minutes using a shatterbox. Each sample was weighed to near 1.50 g. Using 8 ml nitric acid and 4 ml hydrochloric acid, the soil samples were digested for 24 hours at 100 degrees C. Then the samples were diluted to 25 ml with 2 % nitric acid. Sample duplicates were made and diluted 100 times with the same 2% nitric acid to assess aluminum and potassium concentrations. These were then run on ICP-OES (PerkinElmer Optima 5300DV) to generate solid concentrations in mg/Kg.

Electrical conductivity and pH were measured using Blue Lab electrical conductivity pen and pH pen. Analysis of NH_3^- was performed using Hach test and optical analyzer (Hach DR2700). Solutions were characterized for NO_2^-, NO_3, and Cl^- by ion chromatography (Thermo Fisher Dionex ICS-1100), and ICP-OES (Perkin Elmer Optima 5300DV) was used to assess other solution ion content, both giving results in mg/L of solution.

Ion	Fresh Dilute Urine (mg/L)	Udert (stored urine, mg/L)
Ca	36.8	16
K	83	1410
Mg	14.8	< 5
Na	139.7	1740
P	75.3	208
Cl	165	3210
NH3	25	2390
SO42-	0	778

Table 2-2   Characteristics of urine (Udert, 2012).

Ion	Well Water (mg/L)	Fresh Dilute Urine (mg/L)
Ca	24.9	36.8
Cl	0	165
K	1.5	83
Mg	3.5	14.8
Na	26.5	139.7
Si	6.7	4.54
P	0	75.3
NH_3	0	25
NO_2^-	0	0
NO_3^-	0	0

Table 2-3   Well water and fresh urine ion concentrations.

2.2.2   Results and Discussion

During the early vegetative part of the season, the tomato plants grew well, as seen in Figure 2-9. We know that ammonia and nitrite can be toxic at moderate concentrations in hydroponic systems. Analysis, from Experiment 3 to follow, shows that full urea hydrolysis in these conditions takes five to seven days. Thus, the initial fill with no initial reservoir topping acted as a batch reactor with the solution. In the time it took for the plants to establish themselves (14-21 days), ammonification and partial nitrification had taken place in the reservoir. During the first half of season, the ET rate was such that ammonia and nitrite concentrations did not seem to reach toxic levels when fresh urine was introduced.

After two months of reservoir topping, Dr. Andrew Schuler deemed it relevant to conduct a pulse test in the reservoir. The liquid level was allowed to drop to 75% capacity, and five gallons of fresh dilute urine were introduced. The nitrogen curves of that event is in Figure 2-11. During this event, noticeable plant stress occurred as the plant's roots perceived a toxic ion

concentration spike. As a result, the planter's ET rate decreased drastically (Figure 2-10). In a situation like this, it is possible that some metabolic equilibrium is reached/forced between plant water use and reservoir ion concentrations.

It took more than two weeks after this test for the plant's ET rate to match that of prior to the test. This test begins to illustrate the times involved for ammonification of urea, and for AOBs to reduce the ammonia to less toxic levels: at least two weeks in these conditions. After this, the plant ET rate recovered and continued to increase. Although the plants now exhibited a constantly stressed appearance, they produced fruit of ½ - ¾ of normal size, Figure 2-12. The fruit, however, had a salty, astringent taste, not sweet like the fruit from the soil garden. The end-of-season concentrations of the reservoir were: nitrite = 159 mg/l, nitrate = 153 mg/l, chloride = 204 mg/l, ammonia = 42 mg/l, phosphate = 64.3 mg/l. These numbers indicate no disproportionate salt accumulation in the reservoir.

Figure 2-9   Lush early season growth (day 45).
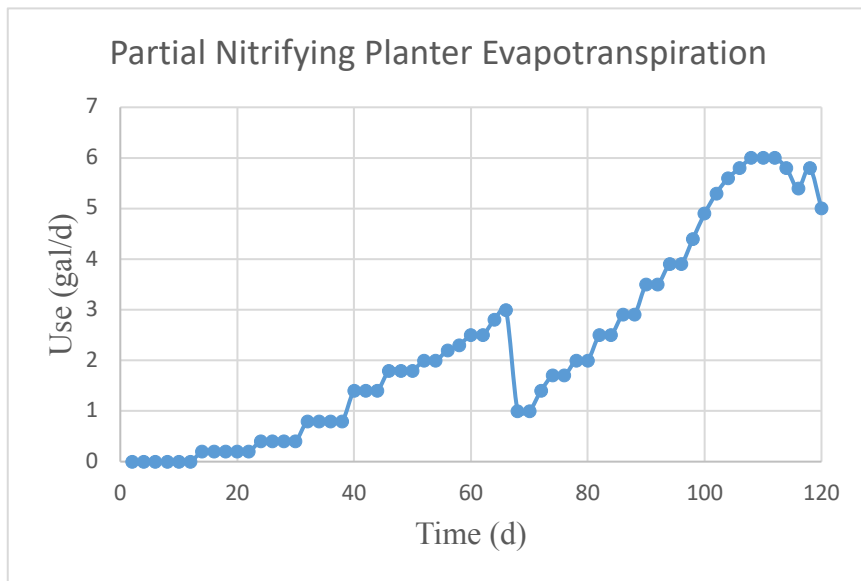

Figure 2-10   Evapotranspiration of partial nitrifying planter reservoir.
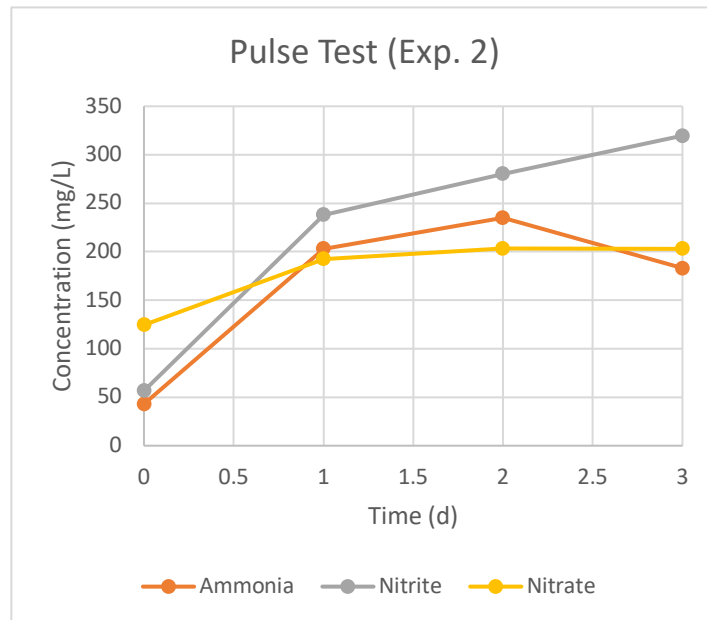
Figure 2-11   Pulse of 25 % volume of fresh urine into reservoir.

At the end of season, full root penetration of the capillary bed and wick columns was apparent. In addition, a set of air roots was established above the water where the aeration bubbles surfaced. Figure 2-13 shows extensive air roots below the soil bed. This could be a plant strategy for dealing with the non-ideal reservoir solution. From this image it seems possible that the plant's primary root zone may not have been concentrated in the +2" region. The pH of the liquid bottomed near 5.7 and remained thus for the remainder of the growing season. Influent urine likely provided enough alkalinity to maintain some nitrification. Also, the acidic solution may have affected the plants rhizosphere uptake strategy. This experiment confirms the need for some amount of treatment of fresh urine before introduction into the planter reservoir consuming greater than 10% reservoir volume daily.

Figure 2-12   End of season fruit and foliage, endemic stress response (day 125).

Figure 2-13   End of season root growth above reservoir aeration zone.

Figure 2-14   Soil ion concentrations.

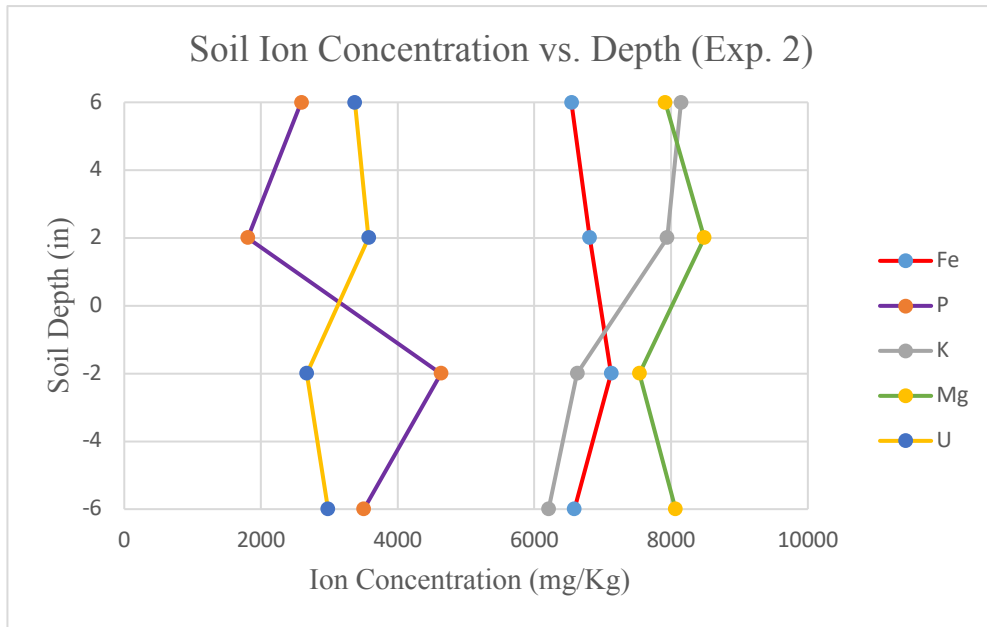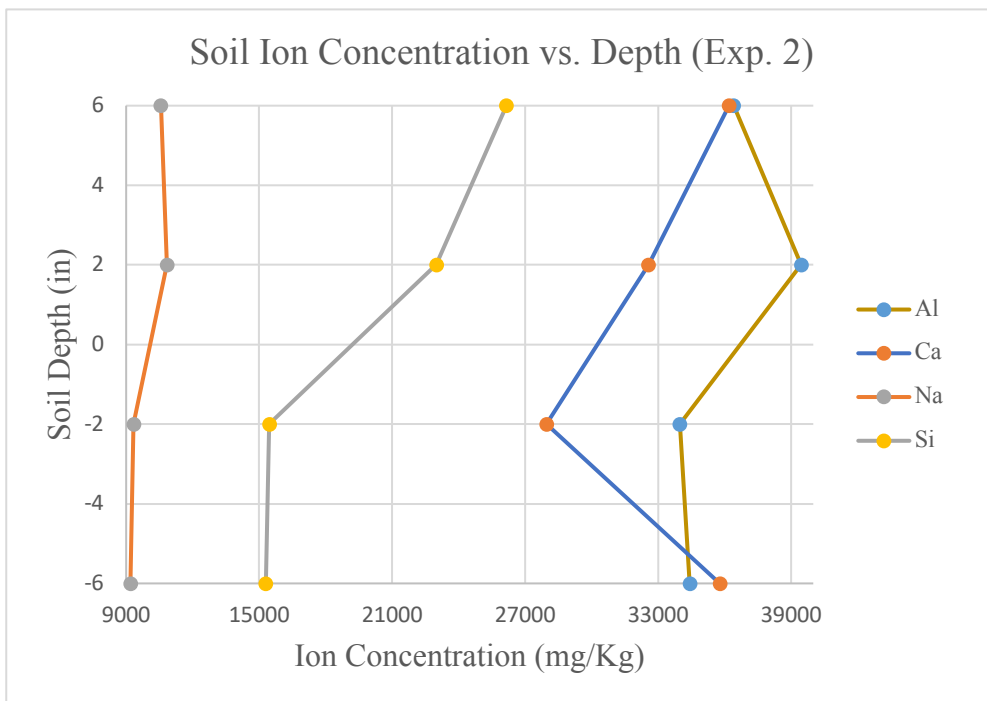Figure 2-15   Soil ion concentrations.

Since this wastewater is solely dilute urine, we expect to see lower levels of some ions. For some reason the soil composition was altered by the manufacturer, so the control concentrations were noticeably higher for some elements like Si and Al (Appendix A).

2.2.3   Conclusions

Unlike Experiment 1, the highest ion concentrations were seen in the +2" zone rather than as expected in the +6". One possible explanation is the oversizing of the planter. Semananda recorded a maximum capillary rise in a simulated soil column of near 12"; at this height the soil moisture was only 15%. As this planter was 11" tall and the free water surface two to three inches below this, it is possible that capillarity was insufficient to move elements to the upper layer. It is also possible that the establishment of the air roots enabled the plants to uptake directly from this zone, thus obviating the normal strategy of root uptake from the +2 root zone.

The data also indicate an ET range under which this type of system operates effectively. So long as the initial mixing provides enough time before the plant ET requirements set in (15 days), in order to allow full hydrolysis and partial nitrification to begin. Additionally, if the daily ET requirements of the system stay below 10% or the reservoir volume, at the concentrations used here this system will run at a tolerable performance. Yet, when the daily ET requirements approach 25% reservoir volume the plant vigor is diminished and toxic stress is visible. The plant stress triggered by the pulse test may have been due to ammonia and/or nitrite toxicity.

2.3 Experiment 3: Compare nitrification of fresh dilute urine in four batch reactors using: loose potting soil, confined potting soil, perlite and plastic MBBR media.

- Objective: Determine the effect of four different media on nitrification rates of fresh dilute urine, as treatment for SIP systems.

2.3.1 Materials and Methods

In an effort to produce suitable treatment of fresh dilute urine, from the failings of Experiment 2, four batch reactors (BR) were constructed and filled with different media. These bench scale reactors utilized four five-gallon buckets with air stones on the bottom and were filled with five gallons of dilute fresh urine measuring 0.8 μS, in addition to the media.

Reactor 1 received ½ gallon of loose potting soil (Black Gold Natural & Organic) as surface area for microbe colonization. Into Reactor 2 went one of the soil wicks from Experiment 2's partial nitrification reactor (potting soil packed into 10" length of 3" perforated pipe). Reactor 3 was topped with ½ gallon of nursery perlite. Reactor 4 received ½ gallon of MBBR media (½" ø smooth round plastic with honeycomb infill).

The same fresh urine source and well water were used as in Experiment 2. The well water is pH = 7.4, so this is the starting value of the data from an approximate 10 times dilution of fresh urine. All reactors received 4 oz of fluid from the partial nitrification reservoir used in experiment 2 on day 0. Reactor aeration was by vigorous course bubble, which kept DO at or above 4 mg/l and provided steady mixing.

After all reactors reached partial nitrification equilibrium on day 63, with pH near 6, alkalinity was added daily to adjust pH to 8 to facilitate full nitrification. At day 51, reactors were moved indoors from the shed; this temperature increase resulted in noticeable evaporation, and hence concentration increase.

Electrical conductivity and pH were measured using Blue Lab electrical conductivity pen and pH pen. Analysis of NH_3^- was performed using Hach test and optical analyzer (Hach DR2700). Solutions were characterized for NO_2^-, NO_3, and Cl^- by ion chromatography (Thermo Fisher Dionex ICS-1100) generating results in mg/L of solution.

2.3.2   Results and Discussion

Hydrolysis begins immediately. The urea cracks to become two ammonias and one carbon dioxide, causing the perceived ion concentration to almost double, Figure 2-15. This is accompanied by an increase of pH toward 9, which is the upper limit for AOB's metabolism.

Although hydrolysis is the easiest step to complete in this process, Figure 2-16 shows that the media can affect the time for this reaction. The reactor using perlite completed hydrolysis in five days, whereas the one with MBBR media took nine days. The reactors using loose and packed soil both finished this phase in seven days.
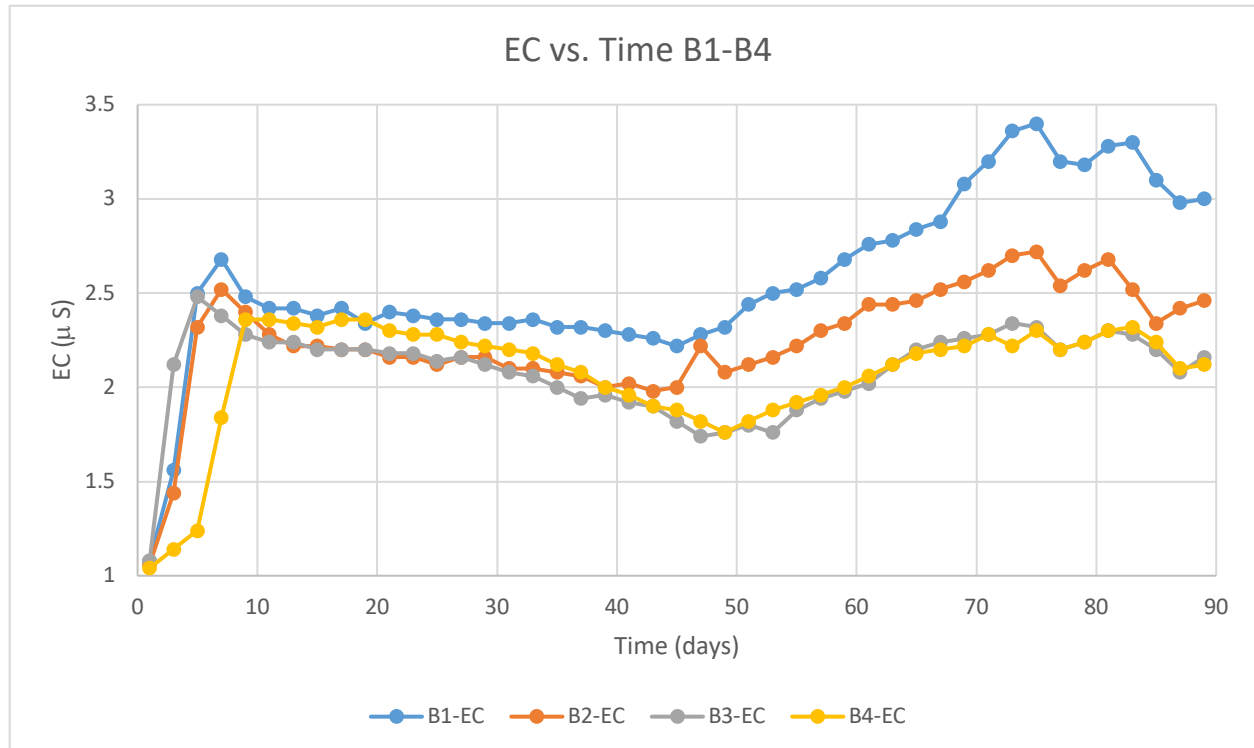
Figure 2-16   Electrical conductivity vs. time B1 - B4. Dilution water added at day 75 and 85.

After hydrolysis and before nitrification, the solution is most susceptible to nitrogen loss from ammonia volatilization. Figure 2-16 shows gradual decrease in electrical conductivity of the solutions between day 10 and day 45. This is likely attributable to air stripping of ammonia, before nitrification has transformed the majority of it to a more stable species. By day 50, evaporation becomes apparent and ammonia levels are near zero, so we see an increase in ion concentration in Figure 2-16.

Figure 2-17 shows pH through time; this parameter indirectly indicates the onset of nitrification by an increasing proton presence. Here we see that Reactor 2 achieved partial nitrification equilibrium by day 31, while Reactor 4 did not reach the lower bond of pH=6 until day 61. Although all reactors were inoculated similarly, the packed soil column provided the best environmental parameters for this phase and configuration. It is possible the high rate of aeration was ameliorated by this arrangement of surface area. It is also possible that within the soil column, a concentration gradient exists, allowing AOBs to function within a solution that otherwise would be ammonia inhibited. Or, perhaps the columns' previous use in the partial nitrification reactor robustly colonized the media with nitrifiers, and these survived hydrolysis and high initial ammonia concentrations.
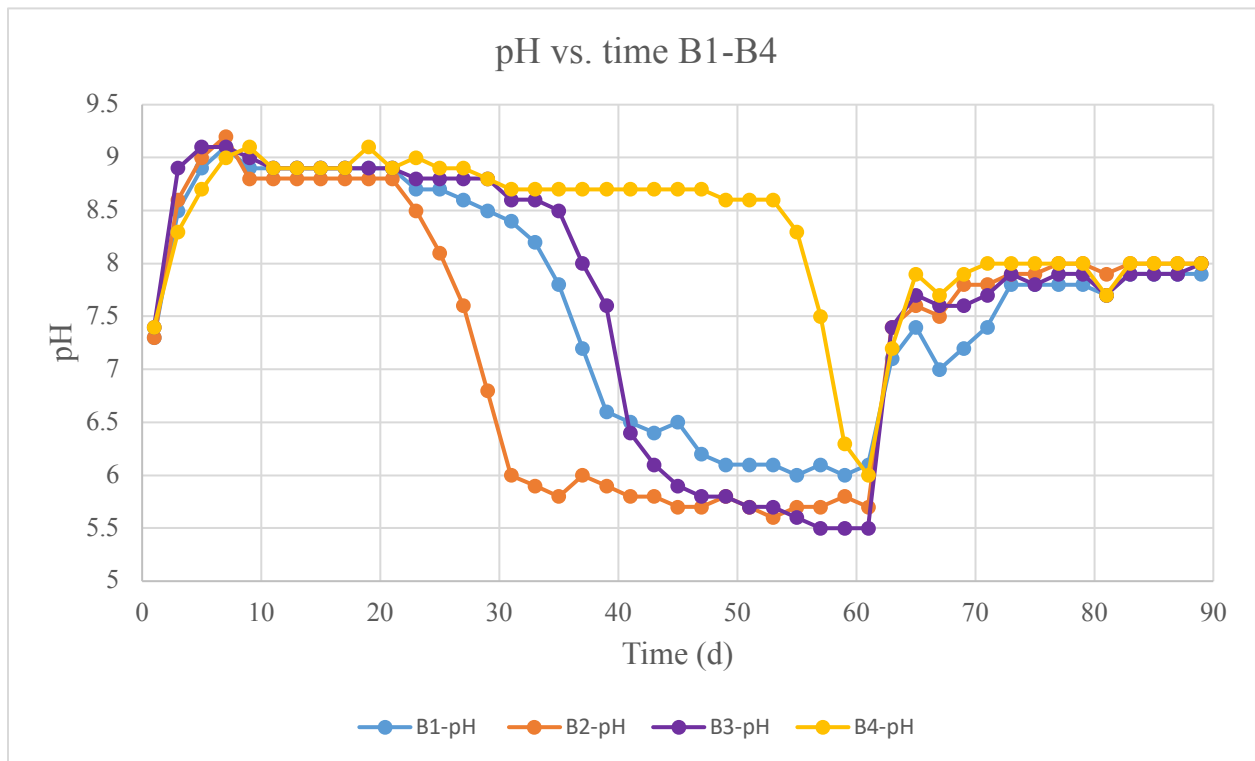

Figure 2-17   pH through time B1 - B4. pH drop signals onset of nitrification.

In the final phase of nitrification, starting at day 63, alkalinity was added daily to bring the pH up to 8. The first BR to reach full nitrate speciation was the MBBR media, seen in Figure 2-21 at day 78. This was followed closely by BR-1 with loose potting soil at day 80, seen in Figure 2-18. The final nitrate concentrations in these two, however, were drastically different. Nitrate concentrations in BR-1 finished near 700 mg/L, while those for BR-4 were just above 200 mg/L.

One factor in this is likely the slow onset of ammonia oxidization in BR-4. The prolonged period of nitrogen as ammonia was subjected to far more air stripping (30 days) before it was converted to more stable nitrite.

Figures 2-18 through 2-21 clearly show that the pH drop seen in Figure 2-17 does correspond with the onset of nitrification in each reactor. However, by waiting for all reactors to reach pH=6, BR-1, 2, and 3 spent considerable time at partial nitrification equilibrium. In each of these reactors we see nitrate dominance leading up to day 63 pH adjustment. Further, we see a decrease in nitrate concentration toward the end of this period. We know from the redox tower of microbial metabolism that oxygen as an electron acceptor is energetically more favorable than nitrate in a standard one molar concentration solution. Although our reactors were kept above 4.0 mg/L DO, Figures 2-18 through 2-21 show nitrate concentrations between 150-300 mg/L nitrate. With these concentrations nitrate metabolism may be more energetically favorable than oxygen, and that at low pH may also be nitrous acid inhibited.

For Reactors 1, 2 and 3 between days 40 and 60, ammonia levels are approaching zero. During this time the substrate for AOB becomes scant yet the pH requirement for full nitrification is

lacking. These conditions, then, may favor heterotrophic denitrification, which may be the cause of a decrease in nitrate concentrations in reactors 1-3 before day 63.

After day 63 pH adjustment, we see nitrogen speciation flip to a predominance of nitrite, just as Udert (2012) noticed nitrite accumulation after adjusting reactor pH set point up. Although the pH adjustment took the solution out of pH inhibition, it may also have shocked the NOB community, which then recovered in time.

It is unclear why both reactors with soil achieved nitrate concentrations well above expected total nitrogen levels from earlier sample times. This concentration cannot be accounted for from evaporation alone and was not evident in the two reactors without soil. Mineralization of soil organic matter is one possibility, as is microbial nitrogen fixing from the atmosphere. Further studies may help isolate this mechanism.

There is a trend in each of the reactors at partial nitrification equilibrium to be predominantly nitrate and at pH=6. The data confirms the requirement for alkalinity to complete transformation of the remaining nitrogen, and that pH inhibition is limiting the reaction. It can also be seen that the first two stages of this reaction are basically self-regulating, and that the reaction stalls when pH nears 6. Further, if ammonia inhibition is hindering the AOB's initially, air stripping will eventually lower the concentration to tolerable levels for nitritation.
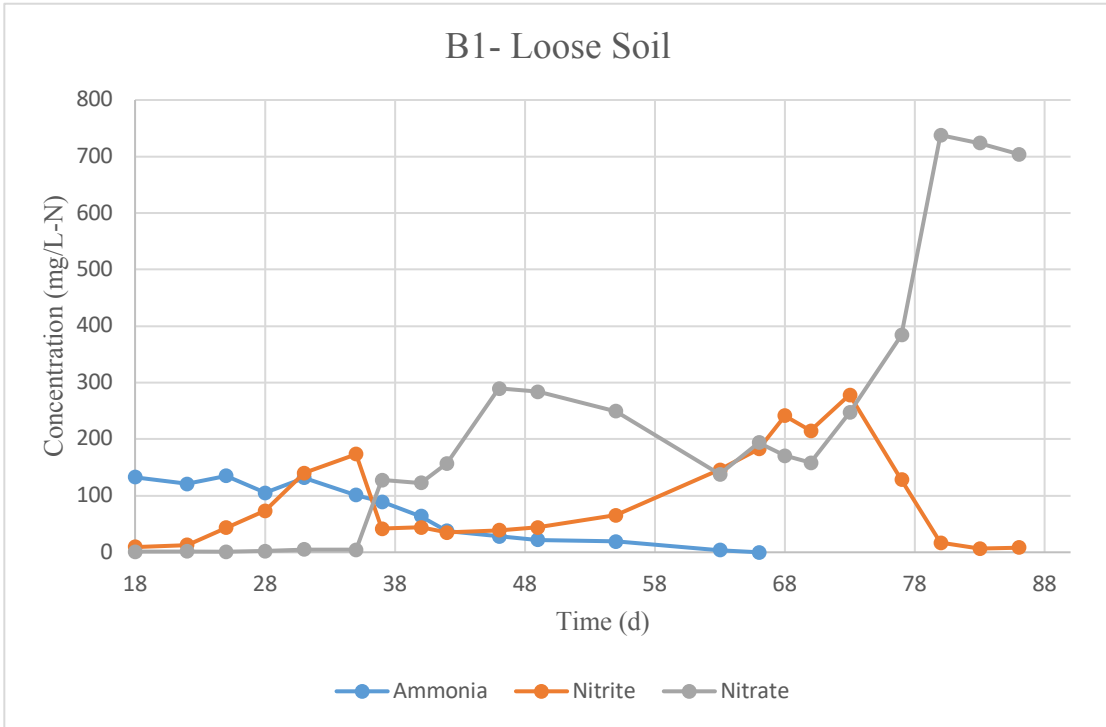
Figure 2-18   Urine nitrification using loose potting soil as media.
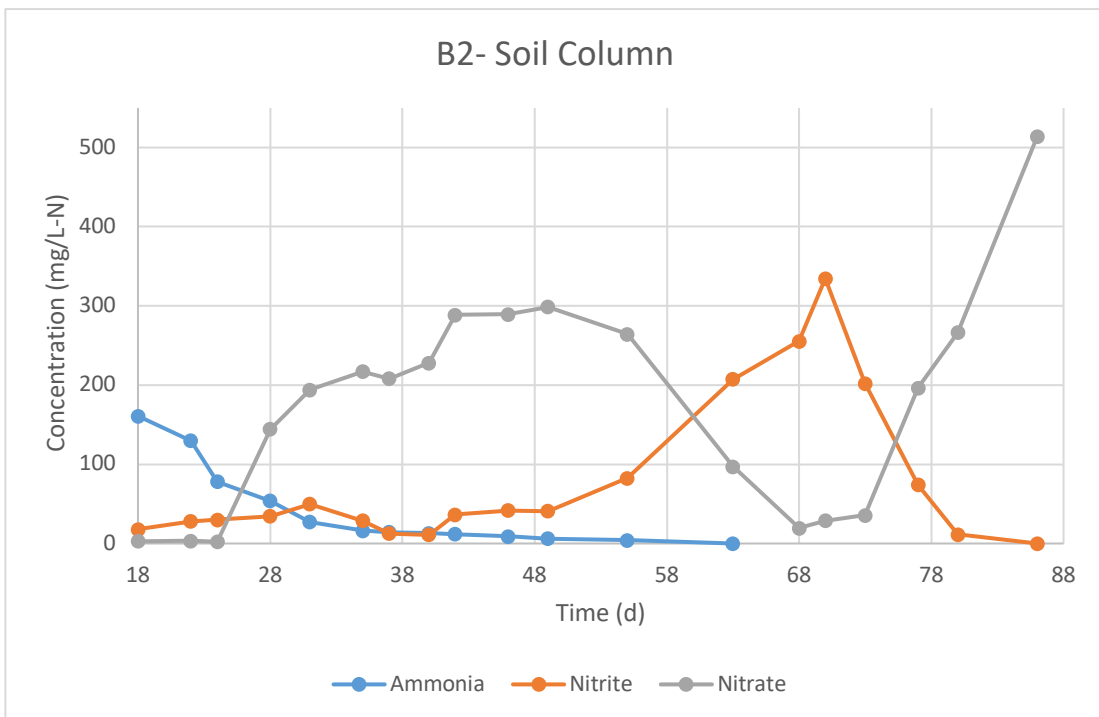

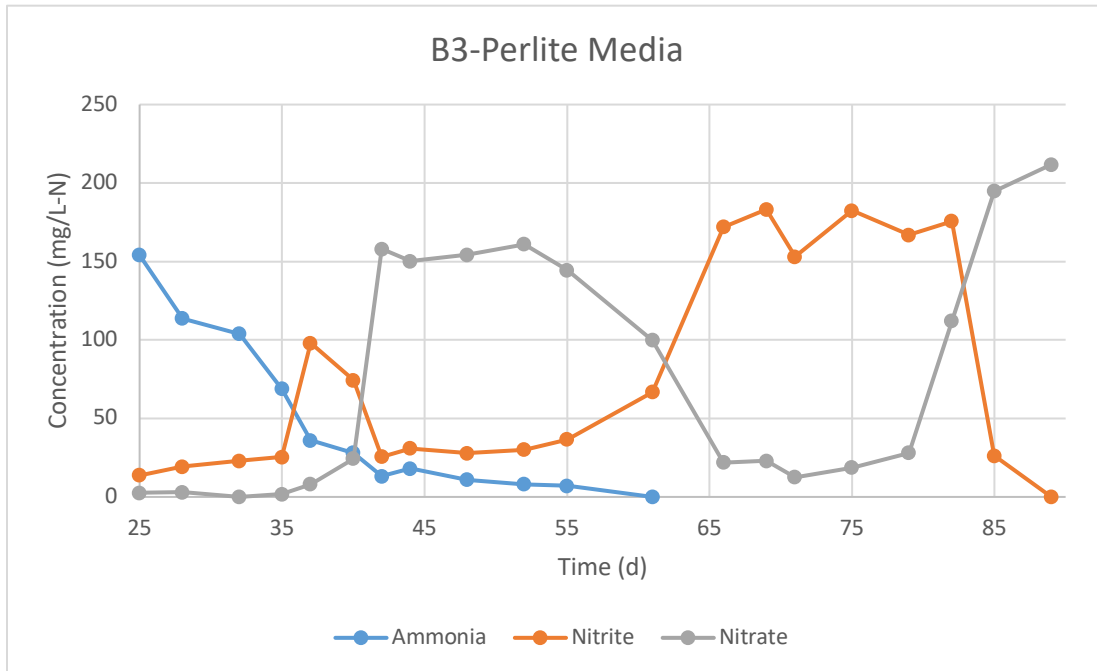Figure 2-19   Urine nitrification using packed soil column as media.

Figure 2-20   Urine nitrification using perlite as media.
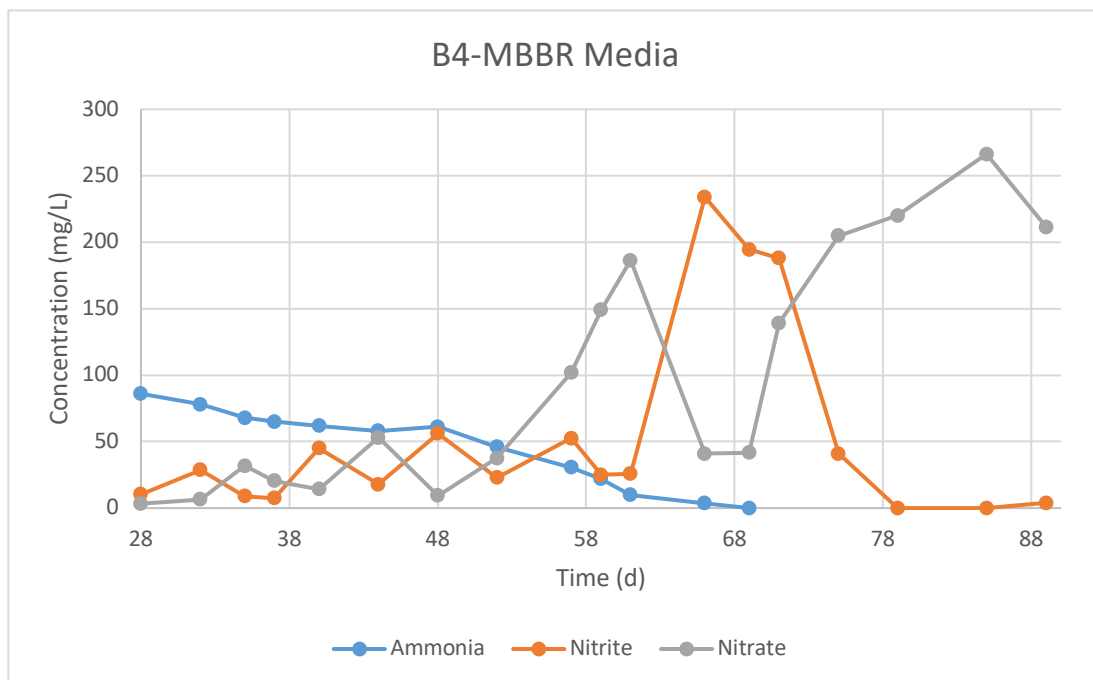
Figure 2-21   Urine nitrification using plastic MBBR media.

4.3.3 Conclusions

Hydrolysis and partial nitrification are basically self-regulating and occur easily under many conditions. The final stage of biologic nitrite oxidization requires alkalinity for completion. Streamlining these stages will result from experience of influent characteristics, flow requirements, and final fluid application, whether as hydroponic feed or liquid fertilizer.

Using the information from these batch reactors, the following treatment train seems one possibility for creating a continuous flow system, seen in Figure 2-22. From the ET curve of Experiment 2 we know that most applications will require a variable amount of solution depending on plant growth phase. Thus, the first and last reactors would be sized to accommodate flow variations.

Using the materials from this experiment, it seems best to use perlite in Reactor 3. Soil columns would be the cleanest media choice for the two nitrification reactors, depending on final solution use. These reactors could in fact be SIPs with large reservoirs and hardy, salt-tolerant macrophytes. For off-season storage of fully nitrified urine, various volume reducing strategies exist depending on climate. An ambient freeze thaw separation or solar evaporation are the least expensive options as far as energy is concerned in our latitude (Udert, 2012).

1. Partial hydrolysis/storage
2. Dilute 10x
3. Full hydrolysis
4. Partial nitrification
O₂
5. Adjust pH to 8
6. Full nitrification
O₂
7. Planter reservoir
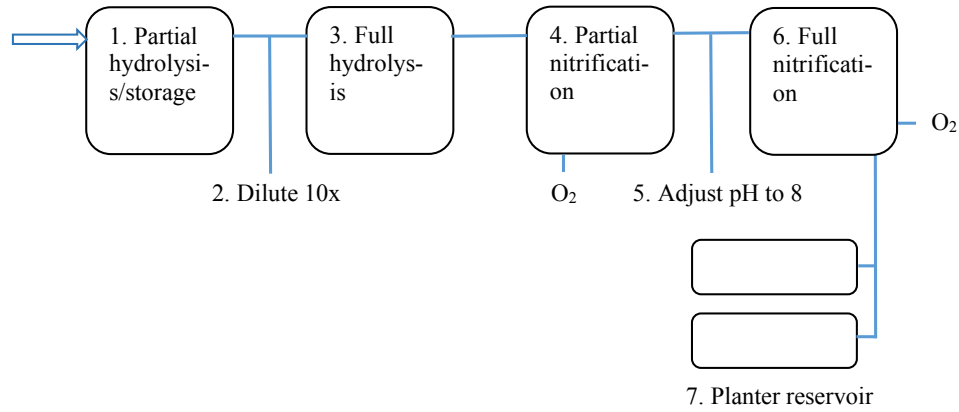
1. Full strength, fresh urine is collected; HRT ≈ storage capacity.

2. Dilute stored urine such that ammonia in 3 stays below 200 mg/L (10-15x).

3. Full hydrolysis takes place; HRT ≈ 5-10 d.

4. Partial nitrification takes place, AOB's reduce ammonia to 50 mg/L; HRT ≈ 15-30 d.

5. Adjust pH to 8.

6. Full nitrification, NOB's render nitrate solution; HRT ≈ 15-30 d.

Figure 2-22   Proposed continuous flow treatment train for fresh urine of large volume.

10x dilute fresh urine
1. Hydrolysis/storage
2. Partial nitrification
O₂
3. Adjust pH to 8
4. Planter reservoir
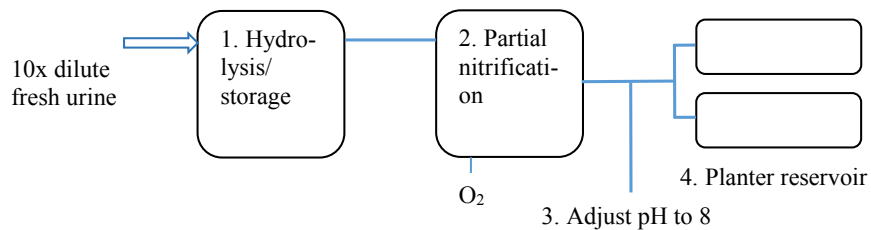
1. 10x dilute fresh urine is collected; HRT ≈ 5-10 d.

2. AOB's reduce ammonia to < 20 mg/L; HRT 15-25 d.

3. Adjust pH to 8.

4. Full nitrification takes place in planter reservoir.

Figure 2-23   Proposed low flow backyard bioreactor for fresh urine and SIP.

At a 10x urine dilution, the final chloride didn't exceed 250 mg/L. With nitrate at 500 mg/L and phosphate at 75 mg/L, this can provide appreciable recycled nutrient input for a variety of applications. Further investigation is needed to isolate the mechanism causing an increase in nitrate concentration in solutions with soil and nitrate.

Considering the dilution volume and total HRT, it may be more efficient to inject partially hydrolyzed urine directly into a typical irrigation system. At appropriate dosage and intervals, this would use the soil microbes and surface area for nitrification, and decrease treatment complexity, storage, and expense.

2.4 Experiment 4: Compare stability of nitrate solution in various SIP configurations and DO concentrations.

- Objective: Determine the effect of DO on nitrified nutrient solution over time in four SIP reservoirs of different configurations.

2.4.1 Material and Methods

The contents of BR-1, BR-2, and BR-3 were combined and mixed to produce the solution used in this experiment. The following design is intended to test the role of the capillary matrix on reservoir dissolved oxygen levels, and what effect these have on the nutrient solution's nitrogen, specifically the possibility of denitrification. Standard plastic SIPs were used, which have a four-gallon reservoir and 28" L x 14" W x 12" H soil cube (agardenpatch.com). Dissolved oxygen was measured daily using Hach HQ440d.

Reservoir 1	Live plants	Capillary matrix	Air stone
Reservoir 2	Live plants	Capillary matrix	
Reservoir 3		Capillary matrix	
Reservoir 4			

Table 2-4   Experiment 4 matrix.

The planters associated with Reservoirs 1 and 2 were established with spinach (*Spinacia oleracea*) prior to the experiment. Reservoir 3's planter was filled with moist potting soil and no plants. Reservoir 4 was simply a five-gallon bucket half filled with nutrient fluid, with the lid resting on top but the gasket not engaged. Reservoir 1 was equipped with an air stone providing constant course bubbles. After the initial reservoir fill, no fluid was added.

2.4.2   Results and Discussion

Figure 2-24 shows the range of dissolved oxygen in the four reservoirs over 20 days. Figure 2-25 tracks the nitrate concentrations of these reservoirs during that time. These results clearly show that the nitrate solution is very stable under all of these conditions. No denitrification seems to have occurred, even in R-4 with very low DO levels. Although R1 was potentially subject to more evaporation because of the air stone, nitrate concentration did not steadily increase during the 20 days of observation.

Reservoir 3 shows two interesting phenomena. One is the inexplicable increase in nitrate concentration, although there were no obvious inputs. This is akin to the increasing concentrations seen toward the end of Experiment 3 in BR-1 and BR-2. It is possible that some leaching of nitrate from the potting soil occurs in the presence of nitrate—or, that some microbe is producing it from organic matter decomposition, and/or N_2 fixation. The other interesting data from R3 is the variability in DO without any obvious input, Figure 2-23.
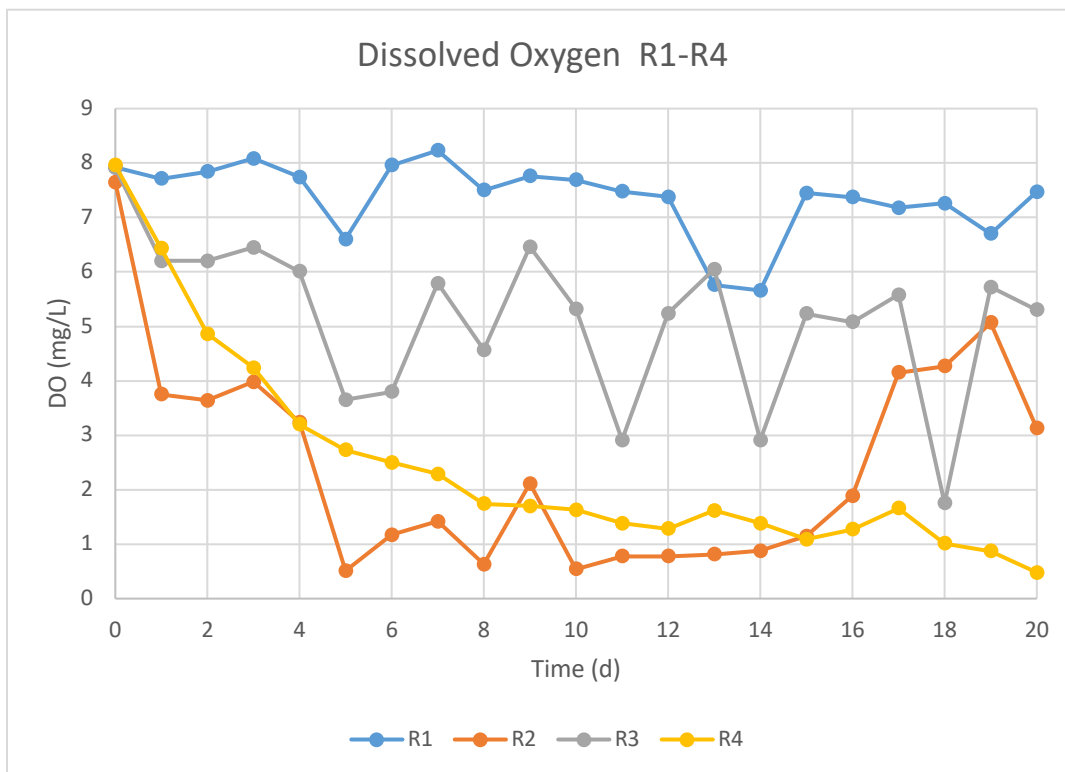
Figure 2-24   Dissolved oxygen in reservoirs 1-4.
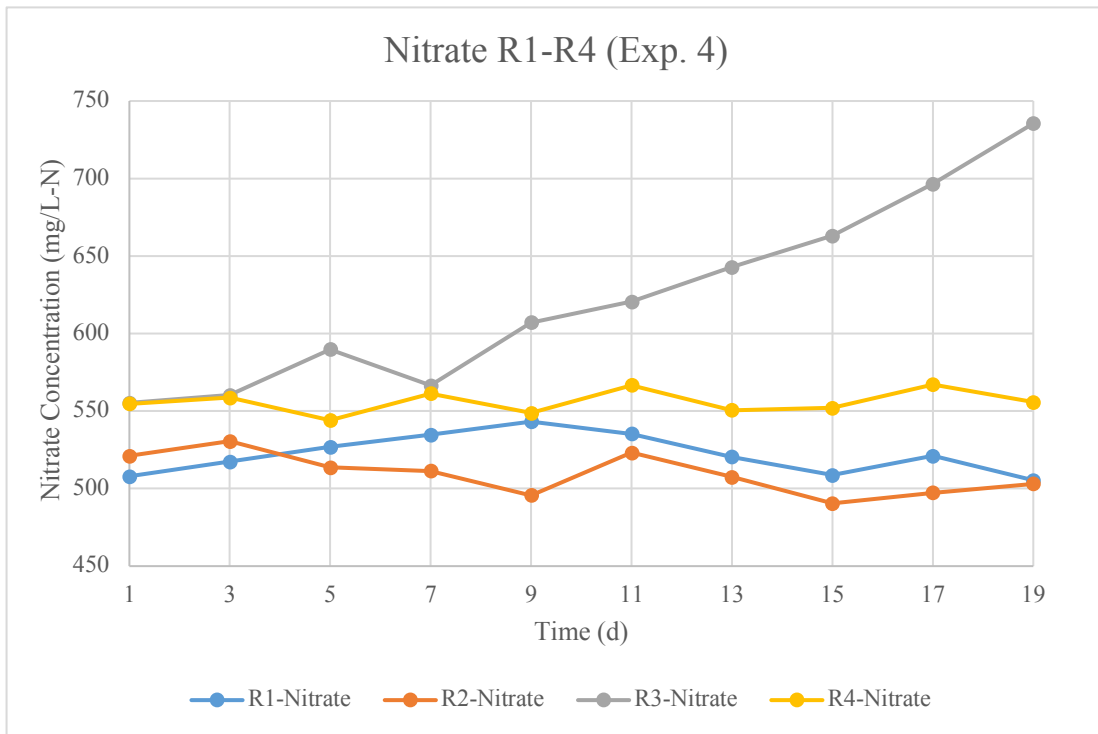
Figure 2-25   Nitrate solution in reservoirs 1-4.

2.4.3   Conclusions

Fully nitrified human urine has potential both as hydroponic feed and liquid fertilizer. These observations indicate stability of the nitrate under some potential application conditions. The chloride concentrations from this source are well below the permit maximum found in San Diego. The nitrogen and phosphorous would prove useful in many a yard or garden. The likely drawback to long-term use of this material could be accumulation of sodium-chloride. This would be highly dependent on soil characteristics and will need further long-term studies to define operating parameters.

2.5   Conclusion

The performance of the systems tested herein show multiple viable configurations for reuse of treated domestic wastewater with SIP. A relative lack of surface precipitate crust on the planter beds indicate that the planter soil may be reused for multiple seasons. Overall, soil ion concentrations were higher at the surface and decreased as a function of depth to the saturated zone.

Full biological nitrification of source separated urine was achieved in all batch reactors tested. The time required for each stage to complete may have been influenced by the reactor media characteristics. Reactor 2, with confined soil, achieved partial nitrification equilibrium by day 31. Reactor 4, with MBBR media, did not complete partial nitrification equilibrium until day 61. The shape, size, buoyancy, material surface, and microbial activity may contribute to the media's effectiveness. Future work will benefit from stage separation and precise media choice to achieve the desired outcomes.

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Appendix A: Soil Ion Concentrations (mg/Kg)

Depth-E2	Al	Ca	Fe	Na	Si	P	K
6	36402	36196.9	6540.8	10565.9	26158.3	2590.5	8144.3
2	39450.6	32564	6807.6	10837.9	22992.9	1806.3	7939
-2	33983.2	27961.5	7121.2	9338.6	15461.5	4632.3	6623.9
-6	34424.3	35784.3	6585.4	9192.9	15303.6	3504.3	6207.5

	Mg	U	Zn	Cr
6	7911.4	3372.3	58.4	177.1
2	8485.5	3572.5	58.6	17301
-2	7535.8	2670.3	44.3	157.8
-6	8063.1	2980.6	56.3	164.7

Depth-Std	Al	Ca	Fe	Na	Si	P	K
6	25967.1	39204.5	6103	11106.1	18943.4	1450.7	11509.9
1	37829.9	31732.6	6339.2	9643.2	23973.6	7798.1	10468
-3	42331.8	27321.8	6704.3	11014	25150.6	3005.8	8724.9
Control	31920.6	29961	6777.3	9086.2	15029.4	1621.7	8310.9

	Mg	U	Zn	Cr
6	7862.7	2223.1	269.8	57.6
1	8073	2668.6	60.7	137.7
-3	8716.1	3857.9	67.6	253.1
Control	7612.7	3035.3	43.8	183

Depth-E1	Al	Ca	Fe	Na	Si	P	K
6	506.4	11072.6	4908.5	1809.9	90.9		137.9
2	256.9	8226.5	3299	1182.9	50.9		88.5
-2	363.5	14723.3	3641.7	1094	88.8		109.4
-6	386.1	10186.4	3570.7	1096.7	67.8		88.8
Control	516.9	22888.4	3706.1	1216.7	65.4		174.7

	Mg	U	Zn	Cr
6	1331.7	139.4	20.5	4.3
2	1075	77	17.8	2.04
-2	1121.3	93.9	30.8	2.6
-6	1129.3	96.5	53.1	3
Control	1176.1	97.2	31.6	5.4