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Continuous Flow Applications for Managing Source-Separated Urine Nutrient Recovery

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CONTINUOUS FLOW APPLICATIONS FOR MANAGING SOURCE-SEPARATED URINE NUTRIENT RECOVERY

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The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

ABSTRACT

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Continuous Flow Applications for Managing Source-Separated Urine Nutrient Recovery
Thesis directed by Professor Karl Linden

Urine-diverting toilets are a common method to collect human waste and recover valuable nutrients. While researchers have investigated methods to recover nutrients from urine, most of these methods utilize stored urine that is held in large storage tanks and has undergone the conversion of nitrogen to ammonia (ureolysis), causing spontaneous precipitation of some phosphate and release of odorous NH_3. Continuous-flow applications with fresh and stored urine can decrease the need for urine storage and supplement nutrient recovery technologies.

Two experimental designs were used to analyze continuous-flow applications for urine management: (1) an engineered ureolysis biological filter and (2) solid media phosphate recovery filter. Two biological filters were fed fresh urine, and bacteria producing urease encouraged ureolysis. Maximum ureolysis rates were 66.9 kg $N/m^3/day$ for the column filled with biofilm carriers sampled from a wastewater treatment plant ("activated") and 7.52 kg $N/m^3/day$ for the column filled with fresh carriers ("urine-only"). Urea decay demonstrated a first-order rate constant of 0.036 min^{-1} in the activated column. While increasing urine concentration had little effect on overall ureolysis rate in the columns, it increased solids build-up in the columns. Based on these data, biological filters can be designed for flow rate, cross-sectional area, and total volume to control the amount of ammonia entering nutrient recovery schemes for various process requirements.

Additionally, DNA sequencing of microbial communities over time displayed a decrease in community diversity in the activated column, with Proteobacteria dominating in the mature column, specifically Rhizobiales, Burkholderiales, and Pseudomadales.

Solid phosphate recovery experiments packed columns with various magnesium-containing media and pumped ureolyzed urine through them. In a test with 1 liter of urine, magnesium sulfate and compost mixture captured 0.17 moles phosphorus per mole of magnesium initially present in the media; magnesium-rich soil captured 0.12 moles P per mole Mg; and dolomite stone captured 0.027 moles P per mole Mg. Due to leaching of magnesium from the solid phase into the liquid effluent, a sharp decrease in phosphorus recovery occurred after ~3 hours for magnesium sulfate and 5 minutes for soil.

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Chapter I: Introduction

The Problem

Sanitation Coverage in the World

The growing population brings an increase in population density and a greater demand for food. With 2.5 billion people lacking access to improved sanitation, the push for sanitation has infiltrated much discourse around development and public health (CDC, 2013). As the Millennium Development Goals come to a close in 2015, the Sustainable Development Goals (SDGs) have become the new lens to look through, with goals to be met by 2030. Though the goals are not yet finalized, goal 6 proposes to "ensure availability and sustainable management of water and sanitation for all" (United Nations, 2014). Under this umbrella is not only access to proper sanitation, but also decreased pollution of waterways and safe recycling and reuse opportunities (United Nations, 2014). However, toilet projects are often met with cultural aversion and lack of desire for such improvements. As a result, a variety of development actors have sought economic incentives for improved sanitation, such as converting human waste into a resource with monetary value. Though urine contains the majority of productive agricultural nutrients in wastewater, beneficial reuse of urine has been sparse. Important difficulties include the cost of storage and transport of large volumes of liquid (Gebauer, 2013), land application difficulties, such as clogged drip irrigation lines (Zandee, 2012), and foul smell during storage (Shaw, 2010).

Fertilizing the World

Goal 2 of the proposed SDGs is to "end hunger, achieve food security and improved nutrition, and promote sustainable agriculture" (United Nations, 2014). With resource

depletion and growing population, this goal will only become more difficult between now and 2030. Most prominent crop fertilizers include nitrogen, phosphorus, and potassium. Heffer and Prud'homme (2014) estimated that by 2018, world fertilizer demand would reach 200 metric tons (Mt), made up of 120 Mt nitrogen, 46.2 Mt phosphorus, and 34.2 Mt potassium. Heffer and Prud'homme (2014) also describe increased capacity for ammonia production coming from East Asia, Africa, West Asia, and Latin America as well as a large increase in urea-producing plants in China. Phosphate rock supply is predicted to grow between 2013 and 2018 with the majority of supply coming from Morocco, China, and Saudi Arabia. In addition to new rock supply discoveries, processing plants are anticipated to open up in these same countries (Heffer and Prud'homme, 2014). Despite these hopeful outlooks for supply of nutrients for agriculture, resource extraction has been the cause of numerous international conflicts and extensive environmental damage. Dependence on fertilizer imports causes great susceptibility of fertilizer and food prices to geopolitical issues. Even with an available supply of fertilizer, much of this nutrient value is too expensive and out of reach for poor communities, jeopardizing food security. In addition, continued production of non-renewable fertilizer does not fall under the goal of promoting sustainable agriculture.

Phosphorus fertilizer is derived from phosphate rock, a finite resource, making it a nutrient of high concern when considering sustainable agriculture. Morocco contains approximately 75 percent of known reserves, making it the main exporter of phosphate ore (Schoumans et al., 2015). A recent estimate of global phosphate reserves indicates 67 billion tons, and approximately 224 million tons of P were mined in 2013 (Jewell and Kimball, 2014). If phosphorus is mined at the rate in 2013, the global phosphate reserves

will last for approximately 300 years, but with increasing population and food demand, consumption is likely to increase. A 2014 study by Ulrich and Frossard discussed the literature and research about global phosphorus supply between 1800 and 2002. After sifting through large data sets, the authors concluded that estimates of phosphorus supply are varied and imprecise, and furthermore, that the more serious problem is not resource exhaustion but economics. The per capita cost and socio technical factors reduce the practicality of chemically-produced phosphorus fertilizers for poor communities. The paper advises that the problem must be represented more in terms of socio-economic and environmental issues than in terms of geological phosphorus availability.

As a result of the large demand for phosphorus in the world and the limited resources, a recent proposal was made for effective nutrient management, called the 5R strategy (Withers et al., 2015). The five Rs are: Realign P inputs, Reduce P losses to waters, Recycle P in bio-resources, Recover P from waste, and Redefine our food system. As a result, a number of management strategies have been developed to implement the 5R strategy (Schoumans et al., 2015). Recovering phosphorus from urine and feces provides one opportunity for addressing issues described by Ulrich and Frossard (2014) and implementing the fourth R in the 5R strategy, "recover P from waste". Mihelcic et al. (2011) studied the global phosphorus potential from human urine and feces based on 2009 and 2050 values and projections of population and diet. Based on 2009 data, phosphorus available from urine was approximately 1.68 million metric tons, or 11 percent of global phosphorus demand. This potential appears great, but logistical issues must be addressed, such as nutrient losses during urine application, the shift of populations from rural to urban areas, and cultural taboos associated with human waste.

Urine-Diversion

Over the past two decades, urine diversion at the toilet level has become a much more prevalent topic of discussion in the sanitation arena. In the case of dehydrating toilets, diversion of urine assists in reducing odors, decreasing moisture in the vault of waste to be dehydrated, cutting down treatment time, and producing a relatively pathogen-free fertilizer effluent (Reick et al., 2012). Between 1995 and 2000, the Stockholm Water Company conducted a focused research project on urine diversion, at a time when urine diversion was only common among extreme environmentalists or "tree-huggers" and rural ecovillages (Kvarnstrom and Emillson, 2006). This research provided much of the initial detail on health, agricultural reuse, and social aspects of urine reuse. This research was conducted during the early years of the Millennium Development Goals (MDGs), which greatly emphasized the importance of proper water and sanitation services for development. Kvarnstrom et al. (2006) also describe how meeting the sanitation goal through urine diversion and fertilizer production could impact many additional MDGs, including reducing poverty through income from produce or fertilizer; addressing hunger; improving nutrition to reduce child and maternal mortality; reducing major disease risks; and environmental protection by reducing pollution and recycling nutrients. From a strictly technical point of view, diverting urine also reduces odors and dehydrating time from waterless sanitation facilities, which are quite common in water-scarce areas.

Motivation for urine diversion varies based on different contexts, as described by Kvarnstrom et al. (2006). For on-site water and sanitation in rural to urban edge areas, the main motivators are improved sanitation and nutrient recycling. Compared to areas with existing sanitation infrastructure, it is simpler to introduce this new technology to areas

where little sanitation infrastructure exists. In densely populated areas, such as Jakarta, with a density of 1,200 people per hectare, the main driver for urine diversion is improved sanitation, as diverting urine improves the functioning of dry sanitation systems. Fertilizer production in this context may be a secondary motivator, but with the abundance of people, the potential for microenterprises is much higher. The third context is prevalent on the continent of Africa and includes piped water and sewage distribution systems without adequate wastewater treatment. In this context, the main benefit is diverting large nutrient loads from recipient water bodies. Finally, in contexts with effective distribution and treatment systems in place, urine diversion provides the benefit of reduced nutrient loads on treatment plants, leading to lower energy consumption. In the case of nitrogen, wastewater treatment plants (WWTPs) are required to remove ammonia and nitrate, commonly through nitrification and denitrification. Total energy consumption for these processes is approximately 109 MJ/kg N (Maurer et al., 2003). With urine containing 50-90 percent of nitrogen in human waste, the savings in WWT can be substantial.

Urine-diversion provides a number of potential benefits in all contexts. In the context of feces treatment via dehydration, diversion of urine reduces the liquid load to the vault, decreasing the time required for dehydration. In a centralized wastewater treatment context, diverting urine diverts approximately 90 percent of the nitrogen in wastewater, thus greatly reducing the need for energy-intensive nutrient removal processes for conversion of ammonia to nitrate (nitrification) and then to nitrogen gas (denitrification). Finally, diverted urine can be used as a valuable fertilizer, increasing the economic value of toilets and creating more incentive to have a toilet, which is particularly important in a context lacking in improved sanitation options.

Urine Makeup

Urine is a complex mixture, which has been mostly characterized by Kirchmann and Pettersson (1995), Putnam (1971), and in the urine-diverting systems at Eawag (Udert et al., 2003). Fresh urine contains large concentrations of urea, which are converted to ammonia during storage, increasing the pH and alkalinity of urine solution. Stored urine has a pH of approximately 8.9 and the following main ionic species: Na, K, NH_4, and Ca cations and Cl, SO_4, PO_4, and HCO_3 anions. Table 1 shows various urine concentrations determined by the previously-mentioned authors in stored and fresh urine. Based on approximately 1 to 2 liters per person per day, estimated plant nutrients excreted in urine per person per year are 2.5-4.3 kg nitrogen, 0.67-1.0 kg phosphorus, and 0.9-1.0 kg potassium (Kirchmann and Pettersson, 1995). Separating urine and feces ensures recovery of 60 to 90 percent of N, P, and K in solution (Kirchmann and Pettersson, 1995).

Table 1. Characterization of fresh and stored urine (from literature values)

Citation	Putnam (1971); Laboratory assessment	Udert et al. (2003); Avg. of samples collected from 12 participants	Udert et al. (2003); Urine-diversion system, collection tank	Kirchmann and Pettersson (1995); Averages from 2 reported urine-diverting toilets
Source	Fresh	Fresh	Stored	Stored
Ammonia ($g\ N/m^3$)	200-730	320	1720	1420
Urea ($g\ N/m^3$)	9300-23300	7280	73	-
Phosphorus (g/m^3)	470-1070	463	76	205
Calcium (g/m^3)	30-390	149	28	15
Magnesium (g/m^3)	20-205	99	1	1.6
Sodium (g/m^3)	1170-4390	3200	837	960
Potassium (g/m^3)	750-2610	2210	770	1010
Sulfate ($g\ SO_4/m^3$)	180-2025	1050	292	225
Chloride (g/m^3)	1870-8400	4530	1400	2370
Total COD ($g\ O_2/m^3$)	5570-10600	8925	1650	
pH	7.0	6.6	9.0	8.9

Ureolysis

Ureolysis describes the conversion of organic nitrogen in the form of urea (CH_4N_2O) to inorganic total ammonia (NH_3/NH_4^+). The reaction is catalyzed by a specific hydrolase enzyme called urease (urea amidohydrolase) (Udert et al., 2003). As shown below, the reaction adds water to a urea molecule, producing NH_3/NH_4^+ and carbamate. Carbamate spontaneously decomposes to carbonic acid and produces another molecule of ammonia (Mobley and Hausinger, 1989). Thus, for each molecule of urea, two molecules of NH_3/NH_4^+ are produced. As ureolysis adds alkalinity, pH of urine increases from near neutral to approximately 9 during this process. The pKa of ammonia at room temperature is 9.34, which is the point at which total ammonia is split between ammonium ion (NH_4^+) and free ammonia (NH_3). It is important to note that as the ureolysis process occurs, the increasing pH leads to a greater proportion of NH_3, which is the volatile state.

$$CH_4N_2O + H_2O \rightarrow NH_3 + CH_3NO_2$$

$$CH_3NO_2 + H_2O \rightarrow NH_3 + H_2CO_3$$

In the EAWAG urine-diverting system, research has been done to determine the rate of hydrolysis within piping networks leading to their storage tanks (Udert et al., 2003). This was accomplished by sampling the biomass accumulating within the pipes and observing ammonia production over time in a controlled, completely mixed setting. The average rates were approximately 4610 g N/m^3/day and 9860 g N/m^3/day and approximately 5400 g N/m^2/day when normalized to surface area of biofilm (Udert et al., 2003). These rates were determined by collecting samples from the transport pipe and storage tank and observing ureolysis in mixed batch reactors. In the pipes, the samples

were biofilm from the pipe surface over a specific surface area, allowing for the ureolysis per surface area of biofilm estimation. In the storage tank, stored, ureolyzed urine was mixed with fresh urine to observe the presence of urease enzyme in storage (Udert et al., 2003).

Urease Enzyme and Production

The urease enzyme is often described as ubiquitous throughout the environment, due to the more than 200 microorganisms, including bacteria, yeasts, filamentous fungi, and algae reported that produce it (Mobley and Hausinger, 1989). The variety of organisms that produce the enzyme creates great variation in the activity and properties of urease. Some of these differences include repression of urease synthesis either in the presence or absence of ammonia. Given this fact, without knowing which organisms produce the urease, it is difficult to determine whether the high nitrogen-content in urine will enhance or inhibit urease synthesis. Mobley et al. (1995) described organisms that constitutively express urease (*M. morganii, Bacillus pasteurii, Sporosarcina ureae, Anabaena variabilis*). These organisms produce urease regardless of environmental conditions, whereas other organisms are regulated by environmental conditions, namely the presence or absence of nitrogen and, specifically urea. *K. aerogenes* urease is synthesized under conditions of nitrogen starvation, and *Proteus* and *Providencia* urease production is induced by the presence of urease substrate (Mobley et al., 1995). These details demonstrate the various methods of urease production in the environment and demonstrate the opportunity for urease production within urine-diverting systems.

Ureolysis in Urine-Diverting Systems

Outside of a laboratory setting, sterile conditions are impossible, as microorganisms are ubiquitous within the environment. This fact opens the door for spontaneous ureolysis in urine-diverting systems. In addition, urine often travels through pipes, which provide surface area for biofilm growth, further increasing the chances for colonization of urease-producing organisms. The optimum pH of urease activity and the pH of fresh urine both lie near neutral, with Jack Bean urease acting at an optimum pH of 7.4 (Cesareo and Langton, 1992). With an average concentration of 8750 mg urea-N per liter, urease synthesis in urine will occur through organisms that are nitrogen-regulated, such as *Proteus*, described above (Udert et al., 2003). The majority of urease is located within the cytoplasm of cells, but urease activity in soil is mostly due to extracellular enzymes. Thus, within urine-diverting systems, urease may come directly from bacteria or may be present as a free enzyme.

Nutrient Recovery Technologies

General methods of nutrient recovery or recycling of phosphorus and nitrogen in urine and other wastewater streams include mineral precipitation, ion exchange, ammonia stripping, isobutylaldehyde-diurea precipitation, anammox process (Maurer et al., 2006), algae production (Zhang et al., 2014; Tuantet et al., 2014), and adsorption (Chintala et al., 2014; Fang et al., 2014). For the purposes of this thesis, the technologies focused on here are struvite precipitation, nitrification, algae production, and phosphorus adsorption.

Struvite Precipitation

Struvite precipitation has been used in wastewater treatment plants as a method of controlling undesirable precipitates from forming and clogging pipes. However, the same technique has been utilized as a method of nutrient recovery in traditional wastewater treatment plants and in source-separated urine. Zsofia Ganrot (2005) investigated the combination of precipitation and adsorption for efficient nutrient recovery. Magnesium oxide was added to urine to encourage struvite (NH_4MgPO_4) precipitation, recovering 98-100 percent of phosphorus, 22-64 percent of potassium, 2-5.6 percent of calcium, and 25 percent of nitrogen. The recovery of elements other than those found in struvite indicates the formation of additional fertilizer minerals. Additionally, Ganrot investigated the addition of zeolite and activated carbon as agents of adsorption. Mineral adsorption in combination with struvite precipitation recovered between 64 and 80 percent of nitrogen in laboratory tests, with optimum N and P recovery with 0.1 grams of MgO and 15 to 30 grams of zeolite added per liter of stored and diluted urine. Finally, the study considered the availability of recovered nutrients to wheat plant (*Triticum aestivum L.*). The study found struvite/zeolite mixtures to act the same as slow-release fertilizers, Diammonium Phosphate and Calcium Phosphate.

In a similar concept, Grau et al. (2012) developed a fully automated struvite reactor in the field at eThewkini, demonstrating the potential to recovery up to 93 percent of total phosphorus in the form of struvite. This reactor used turbidity and conductivity as proxies for phosphate concentration, as demonstrated by Wylie (2009), Maurer and Gujer (1995), and Etter et al. (2011). Magnesium was added at a ratio of 1.1:1 (mol Mg: mol P), and the solution was mixed in a stirred batch reactor.

One limitation to struvite precipitation as a method of recovery is the lack of sustainable magnesium sources. As a result, Lee et al. (2003) demonstrated struvite precipitation using bittern, a concentrated by-product from salt production. The study analyzed three sources of magnesium, including magnesium chloride ($MgCl_2$-H_2O), seawater (1,200 mg/L Mg^{2+} and 400 mg/L Ca^{2+}), and bittern (32,000 mg/L Mg^{2+} and 8,000 mg/L Ca^{2+}). Through a jar test, bittern was found to recover 76 percent of phosphate, as compared to 75 percent for $MgCl_2$ and 81 percent for seawater.

Nitrification and Distillation

In 2011, Udert and Wachter demonstrated complete nitrogen recovery through nitrification and distillation of stored, source-separated urine. The process utilized a membrane-aerated biofilm reactor and achieved a maximum nitrification rate of 1.8 g N/m^2/day. Following nitrification, the solution was distilled in a laboratory scale, producing a solid residue of ammonium-nitrate, potassium, sulfate, and phosphate. Biological nitrification as a process requires a balanced interplay of ammonia oxidizing bacteria and nitrite oxidizing bacteria, which proved a challenge in the study. Excessive oxygen was supplied to avoid denitrification, and pH was controlled by controlling influent, to avoid high ammonia losses at high pHs. Despite control and operations challenges, this study demonstrated promise of nitrification and distillation as a method for complete recovery of nitrogen from human urine.

Use of Algae for Nutrient Recovery and Biofuel Production

While all of the above studies used stored urine, a number of studies have grown microorganisms on fresh urine as a method of recycling urine's nutrients. Two studies

released in 2014 cultivated microalgae on fresh human urine to recover phosphorus and nitrogen. Zhang et al. (2014) observed instantaneous urea hydrolysis and precipitation as obstacles for sustained algae growth. Additionally, bacterial contamination occurred due to high carbon content of urine, but microalgal cells eventually regained dominance. Tuantet et al. (2014) cultivated *Cholorella sorokiniana* on urine in a short light-path photobioreactor over 8 months. Microalgae were responsible for 90 percent of nitrogen and phosphorus removal, and were increased with increasing N:P ratio, shortening reactor light path, and enriching with magnesium. This study also monitored organic reduction in urine, demonstrating a 71 percent removal of COD and the presence of a heterotrophic population.

Phosphorus Adsorption

Phosphorus adsorption has been studied on a number of materials, such as calcium carbonate (Perassi and Borgnino, 2014), lanthanum hydroxide materials (Xie et al., 2014), and biochar (Fang et al., 2014; Hale et al., 2013). Biochar, a high surface area adsorbent made from waste organic materials has the greatest potential for adsorption in a developing community context, due to its use of waste products in manufacturing. Biochar is produced by heating organic materials in an oxygen-free environment at high temperatures. In practice, biochar functions similarly to activated carbon, and it has been an effective adsorbent of organic, hydrophobic compounds in particular (Mohan et al., 2014). Chintala et al. (2014) investigated phosphorus adsorption to biochars from 3 different feed stocks. The maximum phosphorus uptake with corn stover biochar was approximately 3.4 mg P/kg char, and the Ponderosa pine wood residue biochar exhibited an order of magnitude lower uptake. Pre-treatment of biochar with divalent cations could

increase capacity, as demonstrated by Fang et al. (2014). Fang et al. (2014) produced Ca and Mg-loaded corncob biochar for phosphorus recovery from biogas fermentation liquid. Ground corncob was soaked in $MgCl_2$ and $CaCl_2$ solutions prior to pyrolysis at high temperatures, leading to a maximum uptake of 326 mg P/g char with the 600˚C biochar (Fang et al., 2014). This study also assessed the desorption properties of phosphorus to assess the agricultural availability, demonstrating that desorption of P was slow but provided a continuous, steady release of P, particularly in an acidic environment (Fang et al., 2014).

Recovered Urine Considerations

Pathogen Risks

Due to filtration in the kidney, excreted urine is typically considered sterile. However, urinary-tract infections and fecal cross-contamination can introduce pathogens in urine. *E. coli*, *Klebsiella spp*, *Enterococcus faecalis* were detected in urine of people with urinary tract infections (Das et al. 2006). Hoglund et al. (1998) detected an average of <10 CFU/mL *E. coli* and Clostridia and 10^3 CFU/mL *fecal streptococci* in urine with fecal contamination. Due to the risk of pathogenic contamination, storage of source-separated urine for 6 months at 20˚C is recommended (Schonning, 2001). During the 6-month storage period, complete ureolysis takes place, creating a concentrated ammonia environment toxic to microorganisms of concern.

While storage is suitable in some instances, development of productive fertilizer technologies requires disinfection options with shorter time requirements. Three important parameters for pathogen inactivation include temperature, pH, and ammonia

(Bischel et al., 2014), and various studies have examined the impact of these parameters on various microorganisms, including viruses. At temperatures below 20˚C, inactivation of ascaris and viruses was very slow (Vinneras et al., 2008). Vinneras et al. (2008) also demonstrated that free ammonia (NH_3) at a critical concentration of 600 mg/L shortened bacterial and viral inactivation by months. Biological treatment was also found to impact pathogen removal in a urine nitrification reactor (Bischel et al., 2014). In a moving bed biofilm reactor (MBBR), Bischel et al. (2014) demonstrated 3-log reduction of *Enteroccus spp* and 5-log inactivation of *S. typhimurium* over 6 days, resulting from biological process, such as competition for nutrients, sorption to biofilms, and predation.

Agricultural Effectiveness

Depending on the nutrient-recovery technique, nutrient availability varies. For stored urine that is directly applied to land, nutrients are in their ionic forms and readily available to plants. However, due to high nitrogen content, the N/P and N/K ratios exceed those of typically applied commercial fertilizers (Richert et al., 2010). Johnston and Richards (2003) examined the effectiveness of different precipitated phosphates and phosphorus sources for plants. The study evaluated 11 precipitated phosphates and compared them to monocalcium phosphate ($Ca(H_2PO_4)_2$), a fully-available, water soluble source of phosphorus. Pot experiments were performed using each phosphorus source, and phosphorus offtake in harvested grass calculated. Both $MgNH_4PO_4$ and $MgKPO_4$ were effective slow-release struvites, yielding statistically similar dry matter yields and phosphorus offtakes to that of monocalcium phosphate. Overall, the study demonstrated that water solubility does not necessarily mean crop availability, and the slow-dissolution of struvite does not make it unavailable for plants (Johnston and Richards, 2003).

Additionally, Bonvin et al. (2015) assessed the availability of nutrients recovered in the form of struvite and synthetic nitrified urine fertilizer, obtained from the nitrification reactor. P and N from urine-derived fertilizers were as available to ryegrass as mineral fertilizers, with ryegrass recovering 26 percent of P applied and 72 and 75 percent of N applied as struvite and synthetic nitrified urine fertilizer, respectively (Bonvin et al., 2015).

Social Acceptance of Urine Reuse and Recovery

As with any new technology, people may have aversions to adopting a new kind of fertilizer; however, in the case of human waste recycling, these acceptance barriers are often much larger. Along with cultural and religious taboos related to human waste, personal preferences such as anal cleansing practice must be considered when designing for urine-diversion and waste reuse. Lienert et al. (2003) conducted a mail survey of 467 Swiss farmers to assess the social acceptance of urine-based fertilizers. 57 percent of recipients stated that they thought it was a very good idea, and 42 percent were willing to purchase the product. In addition, 30 percent of farmers interviewed expressed concern with micro-pollutants added by synthetic fertilizers, therefore indicating an interest in urine as a hazard-free fertilizer (Lienert et al., 2003). Though this survey demonstrated that urine nutrient recovery has potential for fertilizer usage in the Swiss context, these attitudes will differ from one context to the next. Additionally, economic assessment of fertilizer value can help justify urine reuse and recovery, as performed by Etter et al. (2011) in Nepal. This study assessed the monetary value of struvite recovered from source-separated urine compared to the cost of fertilizer available nearby, ultimately demonstrating the need for reducing cost of a magnesium source used in struvite recovery.

Experiences with Urine Diversion and Nutrient Recovery

The following list, while not exhaustive, describes numerous large-scale urine diversion projects, demonstrating the urine-diversion trend beginning in the 1990s in a variety of environments.

1. Eawag, Switzerland: As a partner in the Valorization of Urine Nutrients in Africa (VUNA) project, the facility provides much of the research into nutrient recovery from both technical and social points of view. All toilets in Eawag's main building divert urine to a separate storage tank, where the urine is used in pilot studies of nutrient recovery technologies. This research was completed between 2011 and 2014 and included research into struvite recovery, nitrification and distillation, electrolysis, social acceptance, collection, and agricultural use.
2. eThekwini Municipality: In 2002, the eThekwini Municipality installed urine-diverting dry toilets (UDDTs) as part of an Integrated Planning Development process to provide basic water and sanitation to peri-urban and rural areas (Etter et al., 2015). As a result, 82,000 households received UDDTs by 2014 (Etter et al., 2015). In 2010, the water utility combined with Eawag in the VUNA project to further optimize the utility of these urine diverting toilets. The household UDDTs serve as a field application site for research done in Eawag. Urine collected in the municipality is processed at the Newlands-Mashu DEWATS Demonstration Plant, which holds trials for the various nutrient recovery and fertilizer application projects.
3. Rich Earth Institute: Located in Vermont, USA, this represents the first large-scale urine collection and recycling operation in the US. The site conducted one year of

urine collection and field trials on crops not for human consumption, as per the Vermont regulations. Urine is collected from homes in large trucks, like those used to empty septic tanks. Collected urine is disinfected by storage at 21˚C for 1 month or pasteurization at 70˚C for 30 minutes. The Rich Earth Institute serves as a research ground for urine treatment, field trials, and market studies of urine fertilizer.

4. El Salvador government provision of toilets: Between 1990 and 2006, ministry of health provided 120,000 urine-diverting pit latrines, double vault urine-diverting toilets, and solar urine-diverting toilets. However, urine use has not been promoted here, and the collected urine is typically led to a soak away pit.
5. Eschborn, near Frankfurt, Germany. GTZ (German Technical Co-operation): The main building was equipped with waterless urinals and water-flushed urine diversion toilets (Kvarnstrom et al., 2006)
6. Stockholm, Sweden, 1997: Large scale implementation of urine-diversion in which condo organizations were responsible for collection tanks in residential areas and Stockholm Water Company for storage and spreading on farmland. Tenant farmers were involved in decisions. This project impacted 130 households and 1 conference center, allowing collection of 150-170 m^3 each year. Collected urine was stored in 3 150 m^3 PVC balloon tanks (Kvarnstrom et al., 2006).

The push for research in the urine-diversion world has increased the prevalence of urine-diversion experiences over the past few decades. While urine-diversion has a rich history, dating back to 19th century England with the development of Rev. Thomas Moule's "Earth

Closet", further technical, social, and economic research and development is required to increase mainstreaming of this technology (Steinfeld, 2007).

Research Goals

This thesis attempts to address gaps in the urine recovery research by utilizing fresh urine in continuous flow systems that can be used at a household level for treatment and recovery. Employing continuous flow, on-site nutrient recovery systems reduces the need for large storage tanks and transportation of large volumes of liquid, which is particularly advantageous in densely populated areas.

The hypotheses which this research will address include:

1. Urine can be a substrate for biological growth, and specific microorganisms can thrive on its supply of nutrients, high salt concentration, and pH.
2. Ureolysis can be engineered by cultivating microorganisms such as those described in hypothesis 1 and utilizing the kinetics of ureolysis in the system.
3. Solid magnesium-supplemented filters can act as an alternative to well-mixed batch reactors for initial and potential longer term effective struvite recovery from source-separated urine.
4. Different magnesium sources will exhibit varying struvite recovery potential based on solubility and availability of magnesium to the solution.

Arrangement of Thesis

This thesis is divided into sections based on the different experiments conducted:

- “Chapter II: Biological Filter for Engineered Hydrolysis of Fresh Urine” describes experiments which cultivated microorganisms on human urine and attempted to characterize the ureolysis occurrence.
- “Chapter III: Phosphate Recovery Filter” presents the initial findings of continuous flow struvite recovery filters with various magnesium sources.
- “Chapter IV: Synthesis: Improved source-separated urine management and nutrient recovery during continuous flow applications” ties chapters 2 and 3 together by describing potential applications for these filters and providing insight into future research that should be carried out.
- “Chapter V: Conclusions” presents the resulting conclusions based on the original hypotheses made.

Chapter II: Biological Filter for Engineered Hydrolysis of Fresh Urine

Introduction

The proposed Sustainable Development Goal (SDG) number 6 seeks to "ensure availability and sustainable management of water and sanitation for all" by 2030. This includes not only access to proper sanitation, but also decreased pollution of waterways and safe recycling and reuse opportunities (United Nations, 2014). With 2.5 billion people still lacking improved sanitation, development of proper sanitation systems is urgent (CDC, 2013). Given the additional stresses of water scarcity and poor infrastructure in developing communities, centralized, water-intensive sanitation options lack practicality. As a result, decentralized methods of human waste collection and treatment have been pursued. Converting this waste stream to a useful product further increases the appeal of toilets for those living below the poverty line. However, urine fertilizer is often undesirable for cultural, aesthetic, and logistical reasons such as taboos, odors, and the weight of large volumes of liquid, restricting transportation.

Urine is a unique substrate for biological growth, with high concentrations of carbon, nutrients, and salts. While various research exists around urine as a substrate for algae production (Zhang et al., 2014; Tuantet et al., 2014), no research has been carried out with fresh urine as a bacterial substrate. Species that grow with urine as their main substrate must be able to withstand the high salt concentrations and the pH variations within urine collection systems.

In this research, we investigated fresh urine as a biological substrate and the potential of a biological filter to control the spontaneous hydrolysis of urine and precipitation of

phosphate minerals in storage systems. This could supplement phosphate recovery technologies and mitigate the need for storage of urine and transport of large volumes of liquid. The specific objectives of this research were to:

1) Assess the process of engineered hydrolysis of urine (ureolysis) in a biological trickling filter
2) Describe filter characteristics and operating conditions that impact the rate of ureolysis in the column
3) Analyze bacterial community utilizing urine as a growth substrate and causing ureolysis in the filter
4) Assess the ureolysis trickling filter as a supplemental tool for use in urine disinfection and recovery of phosphorus and other nutrients

Urine Hydrolysis

Urea hydrolysis (ureolysis) is the conversion of organic nitrogen in the form of urea (CH_4N_2O) to inorganic ammonia (NH_3). The reaction is catalyzed by a specific hydrolase enzyme called urease (urea amidohydrolase) (Udert et al., 2003). Over 200 microorganisms synthesize urease, and though its production is known to be dependent on certain environmental characteristics as pH and urea concentration, urease-producing organisms are considered ubiquitous (Mobley et al., 1989). As shown below, the reaction adds water to a urea molecule, producing ammonia and carbamate (CH_3NO_2). Carbamate spontaneously decomposes to carbonic acid and produces another molecule of ammonia (Mobley and Hausinger, 1989). Thus, for each molecule of urea, two molecules of ammonia are produced, increasing the pH from approximately 6.5 to 9 and increasing alkalinity.

$$CH_4N_2O + H_2O \rightarrow NH3 + CH_3NO_2$$

$$CH_3NO_2 + H_2O \rightarrow NH_3 + H_2CO_3$$

The majority of urine-diverting systems pipe urine to a storage tank, where it is stored for an extended period of time to ensure pathogen die-off. Due to microbial growth in piping and storage containers, ureolysis occurs in all urine storage systems. The amount of ureolysis in piping and storage was quantified by Udert et al. (2003) by sampling solids from pipes, adding fresh urine to stored urine, and determining ammonia production. From these experiments, hydrolysis rates of 1820/m^3/day and an average of 5350/m^3/day in untreated stored urine and piping, respectively were achieved (Udert et al., 2003).

Urine Treatment and Nutrient Recovery

With a growing population and increased demand for food, recovery of nutrients from human waste has become increasingly attractive, and urine contains anywhere from 50 to 90 percent of nitrogen, phosphorus, and potassium. As a result, large initiatives for nutrient recovery research have been underway over the past 5 to 10 years. Methods of nutrient recovery or recycling of phosphorus and nitrogen in urine and other wastewater streams include mineral precipitation, ion exchange, ammonia stripping, isobutylaldehyde-diurea precipitation, anammox process (Maurer et al., 2006), algae production (Zhang et al., 2014; Tuantet et al., 2014), and adsorption (Chintala et al., 2014; Fang et al., 2014). Struvite (NH_4MgPO_4) precipitation has been demonstrated as a proven method of recovering phosphorus, but in the case of urine, an additional magnesium source is required to meet the 1:1:1 (N:P:Mg) molar ratio (Ganrot, 2005). Struvite formation requires some level of ureolysis, and researchers have discovered that the onset of struvite

formation in urine-collecting systems occurs at a pH of approximately 7.2 (Udert et al., 2003), and efficiency of struvite formation increases as the pH increases up to approximately 9 (Ariyanto, 2013). Biological treatment of urine has been demonstrated through nitrification reactors, which forms ammonium-nitrate, followed by distillation, which produces a concentrated nutrient solution (Udert and Wachter, 2012). This process was successfully demonstrated at EAWAG with control of influent pH to ensure the ideal balance between ammonia- and nitrite-oxidizing bacteria.

Most nutrient recovery research revolves around stored and fully ureolyzed urine, because most applications treat urine via storage. However, during the process of storage, approximately 40 percent of recoverable phosphorus is spontaneously precipitated as struvite and calcium phosphate and lost to storage containers (Etter et al., 2011). In addition, the ureolysis process produces some nitrogen in the form of free ammonia (NH_3), which may be volatilized and lost to the atmosphere during transport and land application. Harnessing nutrients in fresh urine could increase the economic value of urine-diverting toilets. Algae production is the only nutrient recovery technology currently in the literature that utilizes fresh urine. Zhang et al (2014) and Tuantet et al. (2014) both studied urine as a source of P and N for algae growth and biofuel production. Both studies found heterotrophic microbial growth in the algae ponds, demonstrating the ability of bacteria to grow on fresh urine as a substrate. Beyond these studies, research on fresh urine as a microbial substrate is limited, and yet the potential is great.

Materials and Methods

Column Design

Figure 1 shows the experimental setup described here. Columns were constructed with 2-inch clear polyvinyl chloride (PVC) pipes and PVC fittings. The columns were connected to neoprene and vinyl tubing that was replaced regularly throughout testing. Masterflex Peristaltic Pumps were connected to flow regulators to ensure constant flow rate. The pump flow rates were checked regularly during testing by measuring volume captured in a graduated cylinder over a 5-minute period, as flow rates tended to shift over time.

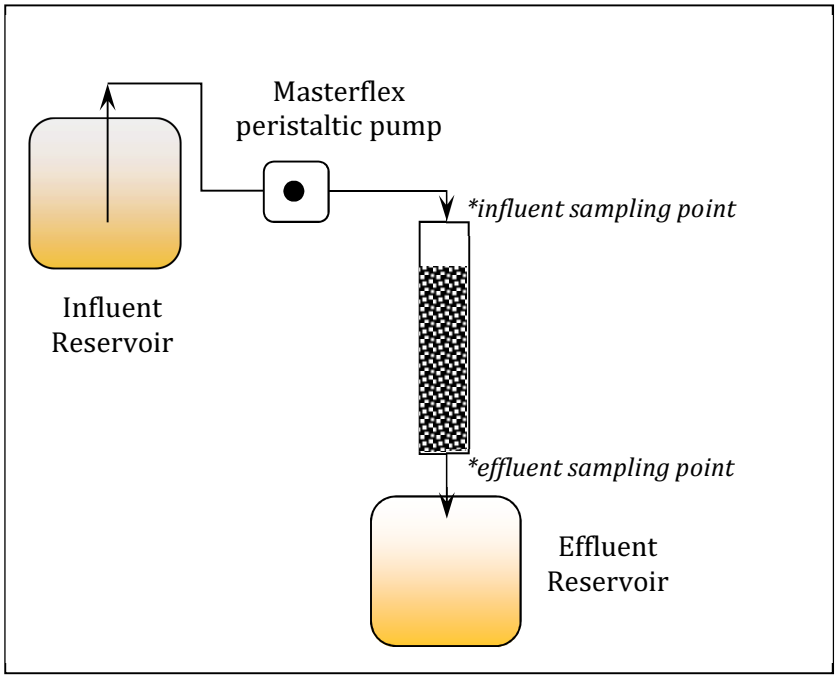

Figure 1. Experimental setup for ureolysis biofilter research

Media

Biofilm carriers utilized for bacterial growth included plastic, high surface-area Kaldnes rings (500 m^2/m^3). The rings have a diameter of approximately 9 mm, which made the media-diameter-to-column-diameter ratio approximately 5.6. Media depth was 8

inches, and clean dry carriers were placed in the first of two columns, which received only urine feed. This column will subsequently be referred to as the "urine-only" column. Approximately 3 weeks later, a second column was constructed using biofilm carriers from an aerobic MBBR at South Adams County Water and Sanitation District Wastewater Treatment Plant. This column will subsequently be referred to as the "activated" column.

Hydraulic Characterization

To assess the unique hydraulic regime in this design and obtain accurate residence times, residence time distributions were created for each column at the flow rates used throughout the experimentation period. Biofilms play an important role in liquid hold up in trickling filters and increase residence time compared to media without biofilm. In an in-depth hydraulic study of trickling filters, Sant'Anna et al. (1982) estimated that the mean liquid phase resident times for biofilm carriers with significant biomass build-up are 5 to 10 times greater than those for biofilm carriers without biomass. As a result, separate tracer studies were conducted on each column and each flow rate to estimate the most accurate average residence time possible.

Due to the high conductivity of urine, a wash-out tracer study was performed using deionized water. Following steady state operation of the columns with synthetic urine, deionized water was continuously pumped to the columns, and conductivity was measured with a programmed Thermoscientific meter at intervals of 8 to 10 seconds. Measurements were taken until the effluent conductivity leveled out and reached a minimum, signifying complete flushing of urine in the column. From the wash-out data, the exit-age distribution

could be determined, leading to a more accurate calculated average residence time, accounting for dispersion and liquid hold-up in biofilms.

Tracer studies typically utilize concentration in calculations of average hydraulic residence time, requiring effective calibration of the conductivity meter to concentration. While most tracer studies utilize a well-characterized salt, such as sodium chloride, urine is a complex media with a number of ions contributing to conductivity. In response, calibration was conducted with fresh synthetic urine at various dilutions. Based on the fresh urine recipe used (Table 2), salt concentration could be calculated as the sum of the molar concentration of each salt ion present. Thus, the meter was calibrated to each molar concentration, producing a calibration curve.

Solution Preparation

The experiments utilized three different wastewater solutions for various phases of operation: real urine, acetate-supplemented secondary wastewater, and synthetic urine. Real urine and acetate wastewater were used during initial recirculation and inoculation of the filters during startup, while synthetic urine was used for the majority of filter life following startup. Real urine was obtained from a mixture of individual anonymous donors in containers disinfected with bleach prior to use. Acetate-supplemented secondary wastewater was utilized during the start-up of the activated column to ensure that existing biological communities were not shocked by the introduction of the high-salt, high nutrient content urine solution. Secondary wastewater was collected from South Adams County Water and Sanitation District Wastewater Treatment Plant, filtered with 0.45 micron filters, and mixed with sodium acetate to provide a carbon source for bacteria, 13.4 g/L of

sodium acetate was added to mirror the COD of human urine. After 6 days, ammonium chloride and sodium phosphate were added to the solution at a ratio of 30:4:1 (C:N:P), increasing the wastewater resemblance to urine and providing nutrients for further microbial growth. Following 1 week of acclimation, the column was fed synthetic urine for the remainder of the column life, beginning at a dilution rate of 1/10.

Synthetic urine was used for the majority of filter life and testing due to the consistency of nutrient concentrations and availability. Synthetic urine was prepared based on the recipe for fresh urine from Amstutz et al. (2012) in autoclaved containers. The ingredients used were lab-grade and are listed in Table 2. Ingredients were added to MilliQ water in the order listed, and each compound was allowed to completely dissolve prior to addition of the subsequent compounds. Synthetic urine was used in hydrolysis experiments to provide a consistent baseline and comparable concentrations across experiments. All solutions were stored at 4˚C until use and all dilutions were completed with 18 MilliQ water.

Table 2. Fresh Synthetic Urine Recipe (Amstutz et al., 2012)

Compound	Concentration (g/L)
Urea	16.0
NaAc anhydrous	10.25
Na_2SO_4 anhydrous	2.30
NH_4Cl	1.80
NaH_2PO_4 anhydrous	2.90
KCl	4.20
$MgCl_2$	0.370
$CaCl_2$	0.510
NaOH	0.120

Loading Rate Experiments

The biofilters were tested to determine the effect of urine loading rate on hydrolysis rates by varying flow rate and urine dilution rate. Initial flow experiments utilized a urine concentration of 1/10 (V_{urine}/V_{total}). Prior to assessment at a given flow rate, the columns were run for a few days at the new flow rate to allow for acclimation. The extent of hydrolysis was determined by collecting daily samples and analyzing for ammonia concentration and pH. Once this leveled out, experiments were conducted. In an experiment, diluted urine was fed to the column in 3500-mL batches. At least one influent and effluent sample was taken from each batch, and each experiment consisted of 5 batches, giving a total of at least 5 representative points to determine the steady state ureolysis rate. All samples were analyzed for ammonia and pH, most for urea, and a selection for chemical oxygen demand (COD). The urine-only column was tested at flow

rates between 3 mL per minute and 20 mL per minute, and the activated column also included a 30 mL per minute test.

When flow rate experiments were completed, 10 mL per minute was selected as the representative flow rate for urine dilution tests. These experiments were carried out as described above, with a few days of acclimation followed by systematic sampling. However, to test the effect of dilution rate, the flow rate was kept constant while the concentration of urine in the influent varied from 1/10 up to 5/10 (V_{urine}/V_{total}).

Influent and effluent samples were collected directly from the influent tubing immediately before the column input and directly from the effluent port, respectively. Sample volume was approximately 5 mL, and each sample was filtered with sterile 0.2 μm filters. To ensure accurate sampling, the first portion collected at each sampling point was filtered and wasted, and the second sample was filtered and collected in a sterile 40 mL centrifuge tube for analysis. Samples were stored at 4˚C until analysis. For dissolved oxygen (DO) measurement, influent and effluent samples were taken as described, but they were taken in a small vial and filled completely to the rim, with no headspace. The samples were covered during transport to the DO meter to reduce oxygen dissolution from the atmosphere and immediately read.

To confirm that additional hydrolysis did not occur after sampling and asses the presence of extracellular urease in effluent, some effluent samples were collected and incubated at 37˚C over the course of 24 hours. Half of the samples were mixed with 50 mg/L jack bean urease enzyme and the others were not. Samples were collected from the incubator at given times throughout the 24-hour period. With information from the first

test, additional samples were collected from the urine-only column effluent and mixed with 50 mg/L jack bean urease. Samples were incubated, and in this experiment, time points were within 5 minutes of each other for 1 hour, allowing for analysis of ureolysis rates by jack bean urease in urine.

Sample Analysis

Analyses of total NH_3-N, TKN, COD, and PO_4 were accomplished with HACH (Loveland, CO) TNTplus chemical kits. Samples were diluted with 18 µM milliQ water to ensure a readable value. The vials were read using the DR 3900 benchtop spectrophotometer and the DR 2800 benchtop spectrophotometer. Dissolved oxygen (DO) was analyzed with YSI Incorporated DO meter (model 52, SNL 96F50971).

Urea analysis was conducted using the diacetyl monoxime method. An acid solution made up of 8 N $H_2SO_4$and 4 N H_3PO_4, and 50 mg/L $FeCl_3.6H_2O$; and a color solution comprising of 1.4 g/L of diacetyl monoxime and 0.4 g/L of thiosemicarbazide was used. Measurement was done by adding 3.77 mL of acid solution, 1.13 mL of color solution, 2 mL of ultra-pure water, and 0.1 mL of sample for a total of 7 mL volume. The 10 mm glass tubes containing the test solution were manually mixed and put in a boiling water bath set at 98.9^{o}C for 30 minutes, followed by 30 minute of cooling down. After the samples cooled down, they were mixed and read at 527 nm in the DR 5800 benchtop spectrophotometer.

Solids formed during column life were collected and refrigerated until analysis. X-ray diffraction (XRD), scanning electron microscope (SEM) imaging, and SEM energy dispersive spectroscopy (SEM-EDS) analyses were performed to investigate the identity of minerals formed. Samples collected were dried at 70˚C for 48 hours and then sputter-

coated with gold for SEM imaging and EDS. Previous research (Udert et al., 2003) demonstrated that struvite (NH_4MgPO_4), hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) , and calcite ($CaCO_3$) precipitate in urine-collecting systems and that ureolysis, in fact, can trigger this precipitation.

Characterization of Biofilms

Characterization of biofilms included confirming the presence of microbiological activity, identifying communities present over time, and visually inspecting biofilm structure. Bacterial activity was confirmed initially using LuminUltra ATP test kits and monitored during the first 3 months of operation. During ATP testing, 3 carriers were removed from the top portion of the filters, extracted, and analyzed with addition of luminase enzyme, according to the LuminUltra biofilm collector protocol. The LuminUltra tests yield values in units of microbial equivalents per carrier. This unit is based on the calculation of ATP concentration from the relative light units produced by the luminase enzyme added to each sample. From the ATP concentration, a cell count, or number of microbial equivalents, is determined as 1000 microbial equivalents per picogram of ATP.

Biofilm carriers were sampled at intermittent time points throughout column operation (initial, 1/19/2015, 2/18/2015, and 3/24/2015) and stored in a freezer at -20˚C until DNA extraction. Carriers were cut in pieces and then extracted using a protocol of phenol chloroform, buffer, and silica beads. DNA amplification reaction (PCR) was carried out according to the Earth Microbiome Project 16s rRNA Amplification Protocol (16s rRNA Amplification Protocol). Prior to amplification, the activated column DNA samples were diluted 1000 times, to ensure that highly-concentrated bulk nucleic acids did not interfere

with PCR. The amplification utilized the general rRNA region 515f/806r primers with unique indices to allow for multiplexing of multiple samples during sequencing. PCR products were quantified with gel electrophoresis to ensure that enough DNA was present for sequencing. Extracted DNA and PCR products were stored at -80 degrees C until future use. Following pooling and QA/QC of PCR products, 16s rRNA sequencing was performed on the Illumina MiSeq with 515f/806r primers. The raw data was filtered for quality purposes and analyzed in QIIME. Data was de-multiplexed, and chimeras were removed. The 2 X 150 run produced forward and reverse reads of approximately 150 base pair (bp) length, which were overlapped by 50 bps to produce reads of 250 bps. Since the samples contained different numbers of reads, 1000 from each sample were randomly selected for further analysis. The rarefication only led to one negative control being removed due to having less than 1000 reads. All singletons were then removed. Each read was assigned a phylogenetic description based on operational taxonomic units from the Greengenes database 13_8, and subsequent relative abundance analysis was performed.

To investigate differences in biofilm structure, carriers were also sampled and prepared for SEM imaging. Carriers with biofilm attached were fixed at 4^{o}C in 2.5 % glutaraldehyde for 24 hours and then dried using a graded ethanol sequence (25, 60, 80. 95% (2X) and 100% (2X)). The carriers were then cut and prepared for imaging with gold sputter-coating.

Bacteria Inactivation

Enterococcus faecalis was grown from a frozen culture (-80 ^{o}C) for 15 hours using 1 mL frozen culture in 50 ml of Brian Heart Infusion media (BHI) media at a concentration of

37 g/L. At the end of the 15 hours, 1 mL of the culture was added to 50 mL fresh BHI media (37 g/L) and grown for 4 hours. The culture was then centrifuged at 5000X for 5 minutes and the solids suspended in 50 mL of PBS media. The concentration of this solution was estimated at 10^9 CFU/mL and 2.4 mL of this solution was added to 2.4 liters of the 3/10 urine solution being fed to the urine and activated column at a flow rate of 10 mL/min. Sampling of column effluents was carried out prior to addition of the bacteria (background sample), and at 15, 30, 45, 60, 120, and 180 minutes following the bacteria addition to the urine input solution. The input urine solution containing *E. faecalis* was analyzed at time 0, and 180 minutes to determine the starting concentration as well as any inactivation over time within the input reservoir. In addition, the column effluent samples taken at 180 minutes were kept at room temperature for an additional 18 hours and plated to determine the *E. faecalis* count. *E. faecalis* count was done using DIFCO Enterococcus Agar. The agar was made by suspending 42 g of powder in 1 liter of UPW (ultra-pure water) and heating and stirring the solution to a boil for 1-2 minutes. 10 to 12 mL of the solution was transferred to 100 by 15 mm sterile plates and allowed to harden. *E. faecalis* samples were diluted as appropriate and plated by placing 25 μL of the sample on the agar plates and spreading aseptically over the surface with care not to reach the edge of the plates. Plates were incubated at 37^{o}C for 48 hours and counted. Each dilution was plated in duplicates and each sample had at least three different dilutions to ensure a wide range of detection. Ammonia and pH measurements were done for the 0, 60, and 180 minute column effluents following filtration through 0.22 μm nylon filters.

Results

Filter Startup

The initial start-up phase for both columns displayed increasing maturity over time as well as adaptation of bacterial community to produce the urease enzyme. The initial pH and ammonia concentration of the real urine in the urine-only column were 6.98 and 928 mg NH_3-N/L, respectively. This represented approximately 13 percent nitrogen in the form of ammonia at the beginning of recirculation. Over the course of 576 hours of recirculation, the effluent urine had a pH of 9.1, and the percentage nitrogen in the form of ammonia had increased to 90 percent. Though this provided evidence of some ureolysis, no COD reduction was observed in the effluent until the 576-hour mark.

The activated column was analyzed for biological activity via COD reduction rates over the first few days of recirculation. Over the course of 4 days, the COD was reduced from 17 g COD/L to 8.9 g COD/L. At this point, NH_4Cl (1700 mg/L) and NaH_2PO_4 (480 mg/L) were added to the acetate mixture to provide additional nutrient sources during acclimation at a ratio of 30:4:1 (C:N:P).

With this information and with data from initial ATP measurements, it was concluded that microbial communities were present within both columns. ATP values allowed for an estimate of an average of 8.5 microbial equivalents per device in the urine-only column and 8.6 microbial equivalents per device in the activated column. Finally, to assess the true presence of urease-producing microbes in the columns, a one-pass experiment was performed using 2 liters of fresh synthetic urine, diluted 1/10 and flowing at approximately 2.5 mL/min. After 390 minutes, the urine-only column saw a pH increase

from 6.02 to 8.88 and relative ammonia increase from 7.79 percent of total N to 11.50 percent. The activated column increased the pH from 5.83 to 7.41 and relative ammonia increase from 7.36 percent of total N to 9.35 percent. The data shown in the initial recirculation phase were not adequate to describe the columns, and it was assumed that the columns required additional acclimation to perform in a one-pass setting.

Following the initial one-pass experiment, a one-pass acclimation phase of approximately 2 weeks began. During this phase, the columns were acclimated to the one-pass regime that would be used in future characterization and real-world application. Initially, the urine-only column operated at 2.4 times the hydrolysis rate of the activated column. However, after 5 days of one-pass acclimation, the activated column ureolysis rate increased from 166 g total NH_3-N/m^3/day to 3212 g total NH_3-N/m^3/day, indicating adaptation of the filter and the microbial community to the high concentration of urea in urine. After approximately 2 weeks of one-pass acclimation, the experimental phase began.

Tracer Studies

Figure 2 shows a representative tracer graph from the 10 mL/min tracer test. Conductivity data were converted to molar concentration of urine salt to calculate the wash-out function (w(t)), the cumulative age distribution (F(t)), and ultimately average hydraulic residence time (t_{bar}) using Equation 1 through Equation 3. Data was collected until effluent conductivity reached a steady number. Table 3 displays all results of average residence time for each column at each flow rate tested.

$$w(t) = {}^{c_t}/_{c_0}$$

Equation 1 – Wash out function; c_t is urine concentration at time t and c_0 is initial urine

$$F(t) = 1 - w(t)$$

Equation 2 - Exit-age distribution equation

$$t_{bar} = \sum_{0}^{n} [F(t_n) - F(t_{n-1})] \times (t_n - t_{n-1})$$

Equation 3 - Calculation of average residence time from exit-age distribution

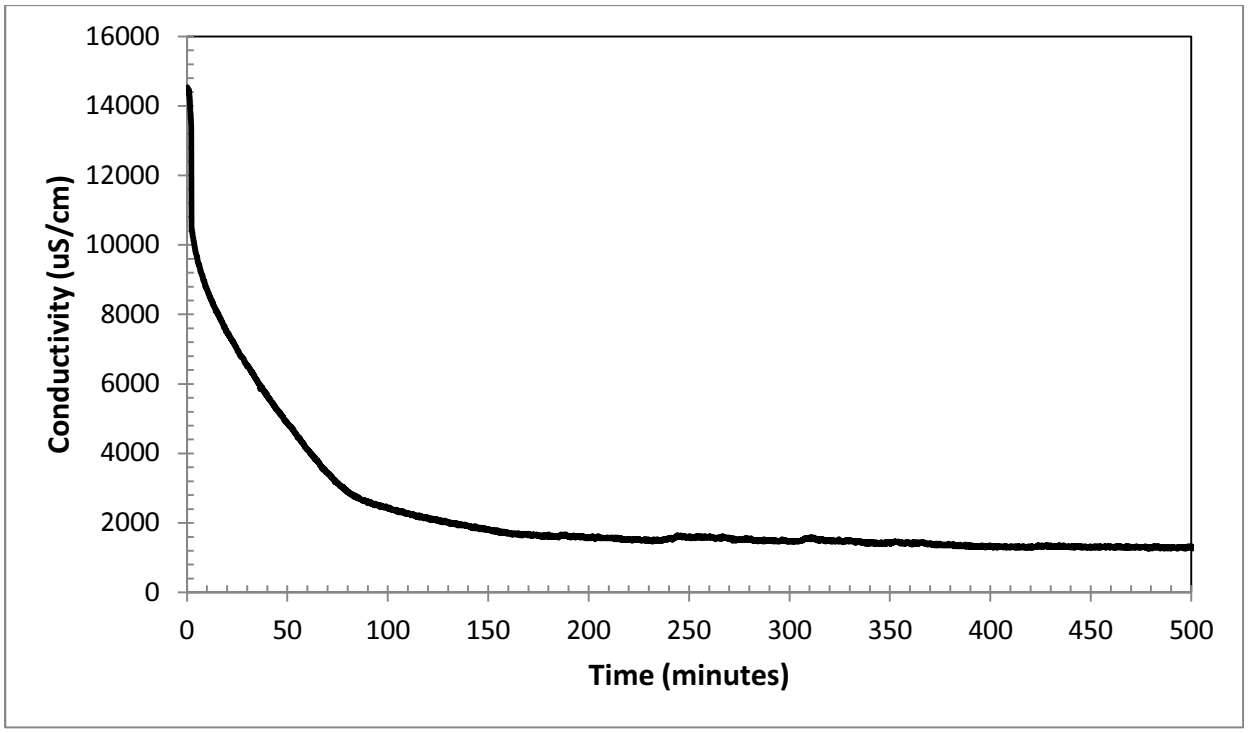

Figure 2. Conductivity over time for wash-out tracer test of activated column at 10 mL/min flow rate

Table 3. Average residence time calculated from wash-out tracer studies at all flow rates for both columns

Flow rate (mL/min)	Activated Column (minutes)	Urine-only column (minutes)
3	44.6	64.0
5	39.3	44.6
10	36.7	30.1
20	9.9	6.4
30	5.9	-

Figure 3 graphically displays the average residence time data from the tracer studies. The urine-only data fits a logarithmic curve, while the activated column shows a lower correlation and a more shallow change in residence time over change in flow rate. Visual inspection and column weight analysis show an increased build-up of solids and biofilm in the activated column, which accounts for the greater residence time at higher flow rates. Due to the biomass build-up, the activated column demonstrates greater liquid hold-up, as described previously by Sant'Anna et al. (1982). At the higher flow rates, biomass build-up (or lack therof) is the determining factor in column hydraulics. However, at lower flow rates, the activated column residence times were lower than the urine-only column, which suggests another limiting factor in column hydraulics. Though this data is not conclusive, one explanation may be that the flow path in the urine-only column is more tortuous, thus providing a less direct exit route for a fluid parcel and more time in the column. Biomass build-up in the activated column may limit the possible flow paths in the column, and the reduced average residence time suggests that this flow path may be more direct than in the urine-only column. Though more in-depth hydraulic analysis is not provided here, data from the tracer tests was used to determine residence time in results discussed below.

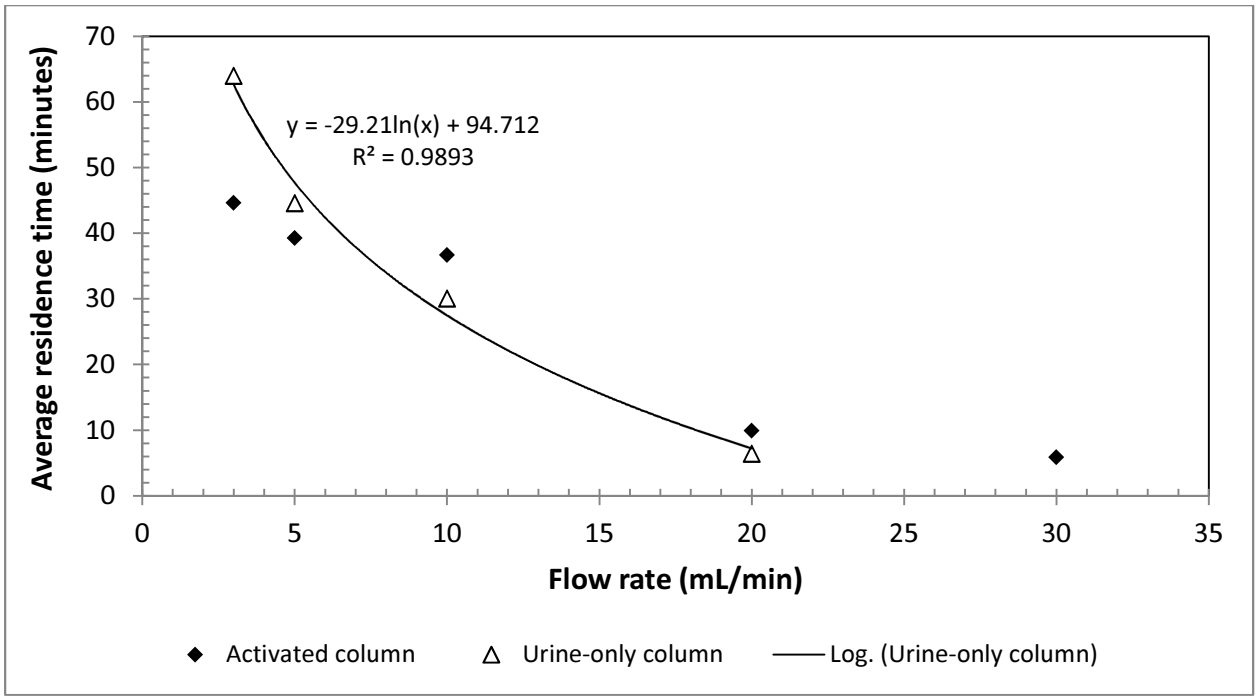

Figure 3. Average residence time derived from tracer data as a function of flow rate for activated and urine-only columns

Experimental Phase

Figure 4 and Figure 5 display ureolysis rates over the experimental timeline for the activated and urine-only columns, respectively. Ureolysis rate is expressed as ammonia production in mg/L (difference between effluent and influent ammonia concentration) normalized over average residence time (minutes). Changes in conditions are denoted by vertical lines. The charts show an initial decrease in production when a new condition began, indicating the acclimation period for the columns. Once a steady output was reached, the clustered points represent data used in column characterization and comparisons. The activated column displays an increase in ureolysis rate with increasing flow rate, reaching a maximum ureolysis rate at 30 mL/min. Throughout the dilution experiments, the ureolysis rate data appears to drop back to the level that initial 10 mL/min tests displayed. On the other hand, the urine column displays a drastic decrease in

ureolysis rate when the flow rate increased to 20 mL/min. The extended period of decreased performance indicates wash-out from the filter, potentially due to the less-mature nature of the biofilm. The increase in ureolysis rate again when the 5/10 dilution experiment began suggests the re-acclimation of the column and re-establishment of the biofilm.

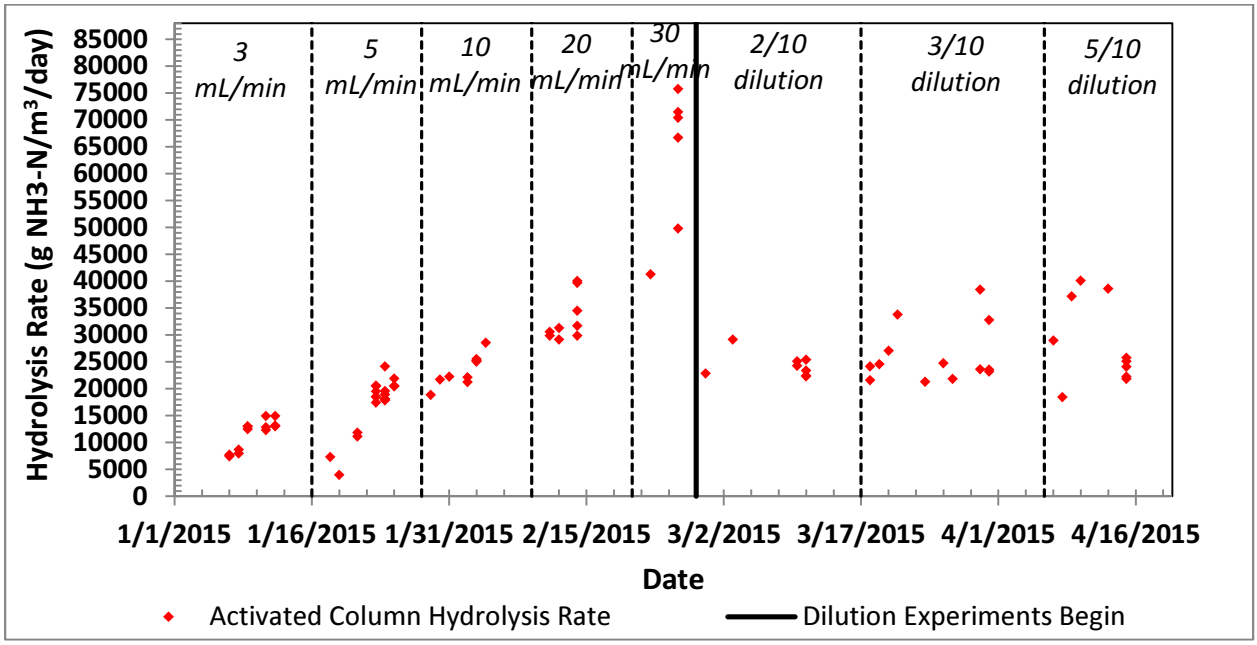

Figure 4. Activated column hydrolysis rate throughout experimental time-frame and at various conditions of flow rate and synthetic fresh urine dilution rates

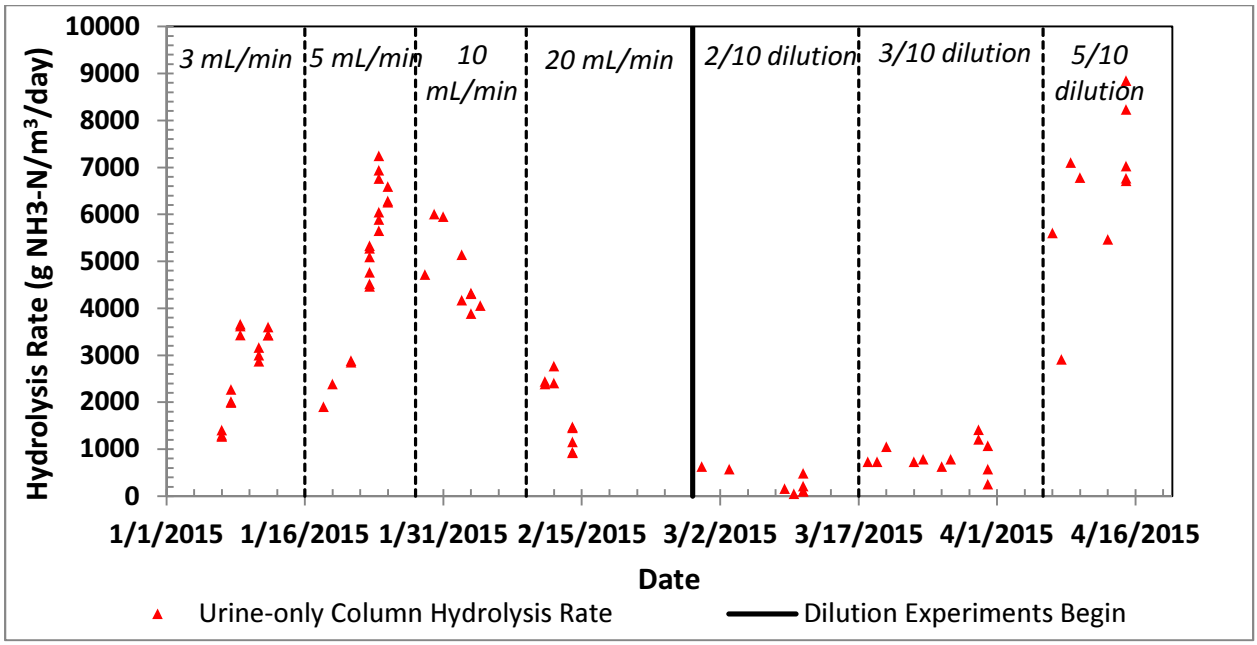

Figure 5. Urine-only column hydrolysis rate throughout experimental time-frame and at various conditions of flow rate and synthetic fresh urine dilution rates

While loading rate, expressed as mg N/m^2/day, depends both on influent flow rate and influent nitrogen concentration, each parameter displayed varying impacts on ureolysis performance in the columns. The kinetic analysis of ureolysis within the columns provides some insight into the relationship between design hydraulic residence time and extent of ureolysis, controlled by the flow rate of urine and shown in Figure 6. Hydraulic residence time is used here to normalize the plot to various reactor designs. In this experiment, the flow rates used were 3, 5, 10, 20, and 30 mL/min, but varying the reactor volume and packing could change the required flow rate. As previously described, build-up of biomass and solids can also impact the residence time. The strong correlation between urea reduction and residence time suggests a steady performance of both columns. The exponential nature suggests that more time will greatly increase extent of ureolysis. Additionally, these approximate fits can be used in initial design of ureolysis biological

filters, based on desired extent of hydrolysis. However, tracer analysis demonstrated the dependence of hydraulic residence time on column characteristics and hydraulic and nitrogen loading rates are much more likely to be used in determining column dimensions. Relationships between urea reduction and loading rates are shown in Figure 7 and Figure 8.

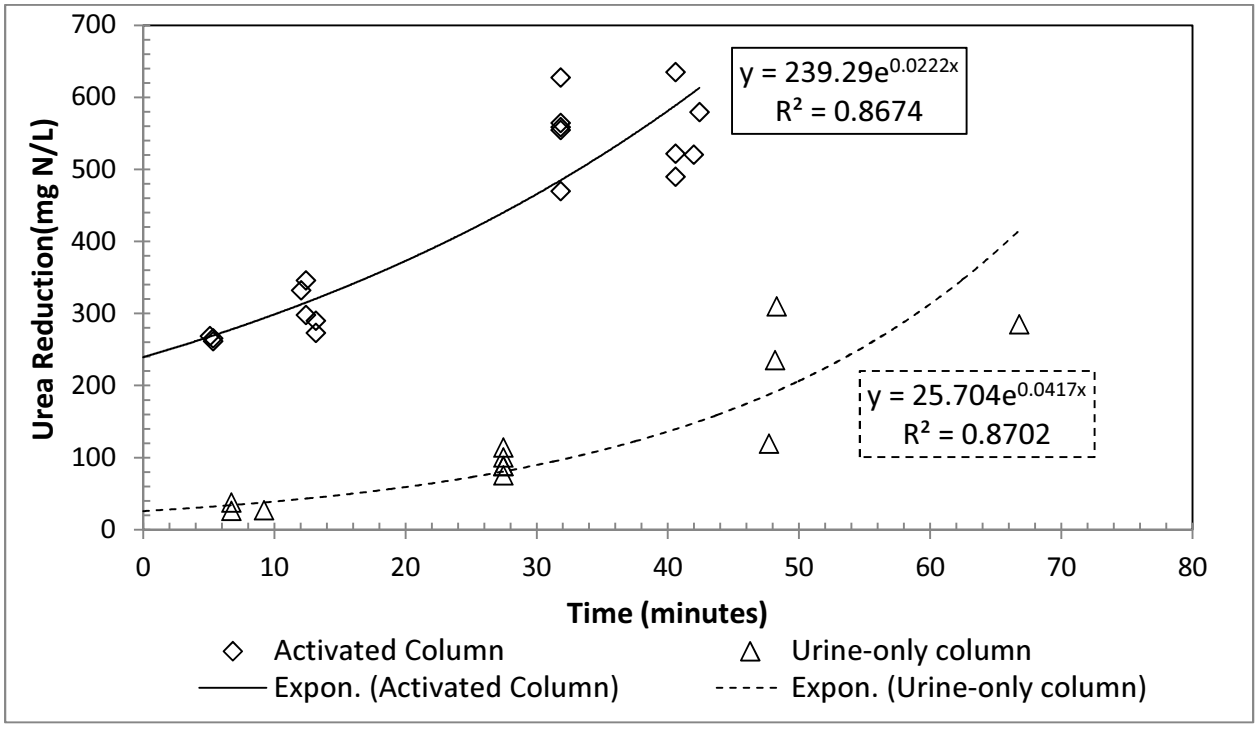

Figure 6. Urea reduction in activated and urine-only columns at experimental residence times with 1/10 diluted synthetic urine, fitted with exponential growth curves

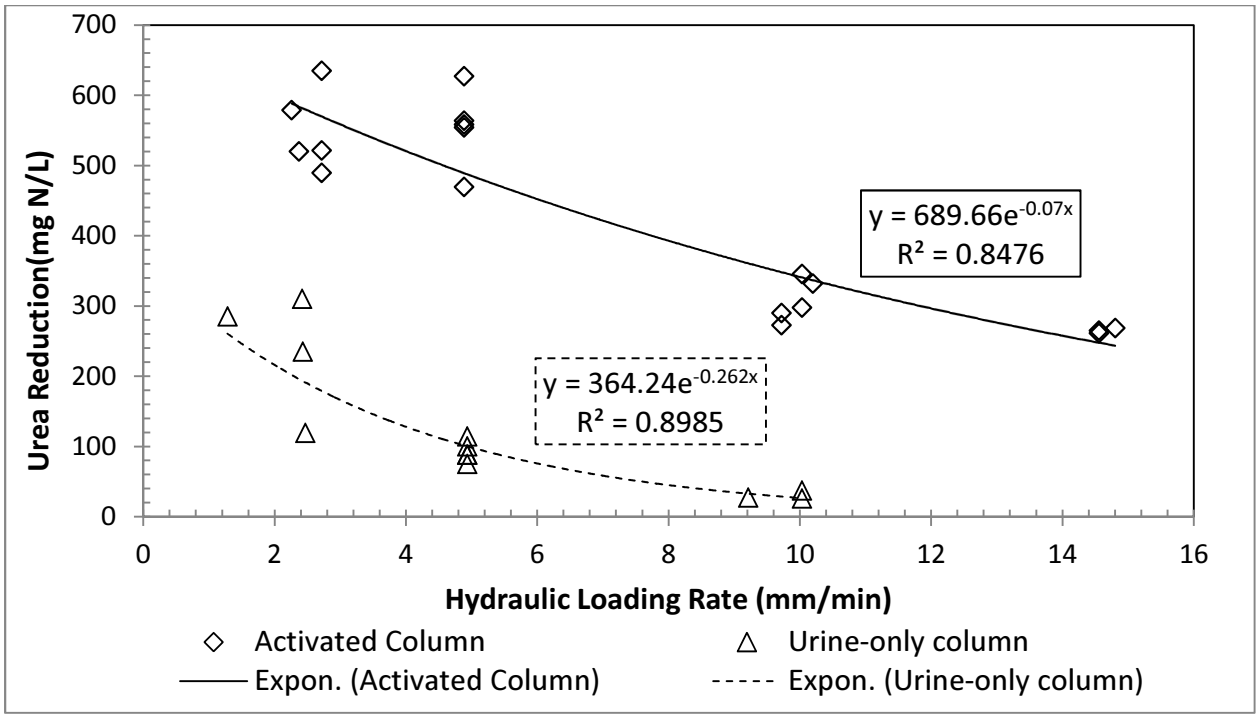

Figure 7. Urea reduction in columns as a function of hydraulic loading rate (Q/A_s) with exponential fits to the data

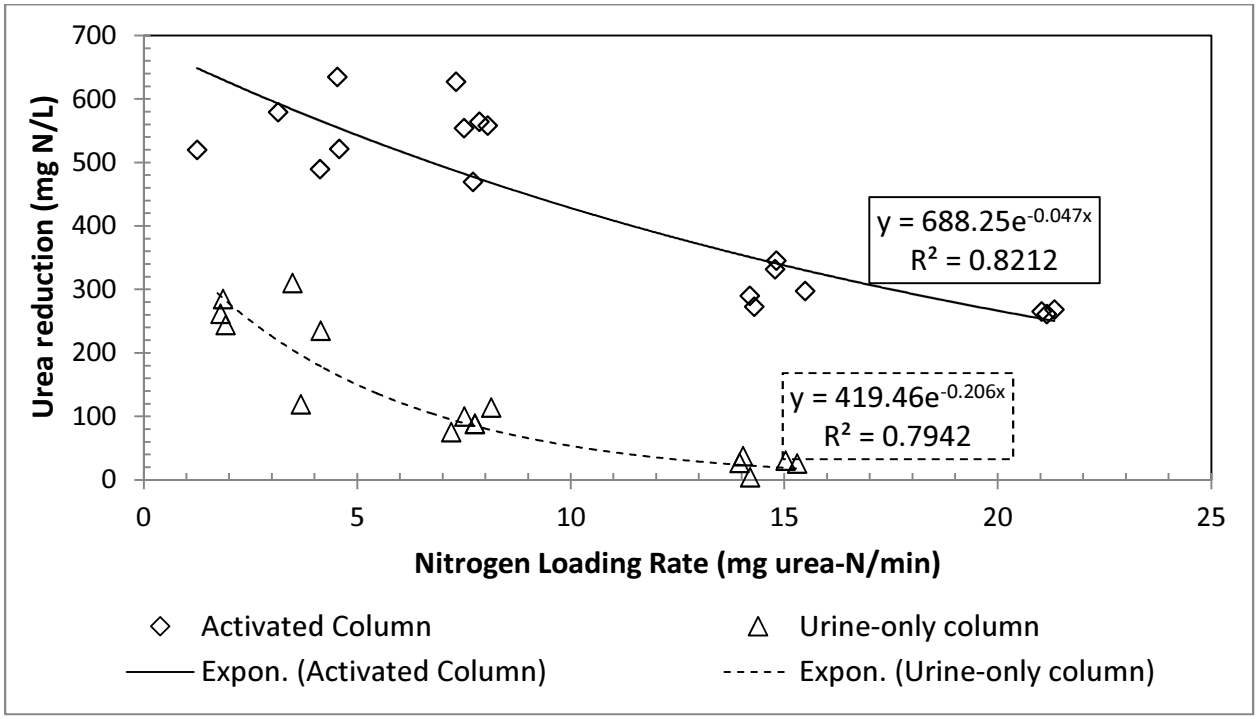

Figure 8. Urea reduction as a function of nitrogen loading rate ($[urea]_{in}*Q$) for urine-only and activated columns fitted to exponential curves

The influence of urine dilution rate on ureolysis is less clear than the relationship between residence time and ureolysis. Figure 9 shows the average hydrolysis rate at various dilution rates of urine at a constant flow rate of 10 mL/min. Since the urine-only column experienced wash-out and decreased performance after the 20 mL/min flow test, data for its dilution test is not shown. Based on a 95 percent confidence interval, this data is not statistically significant, suggesting that ureolysis rate is independent of influent urine concentration.

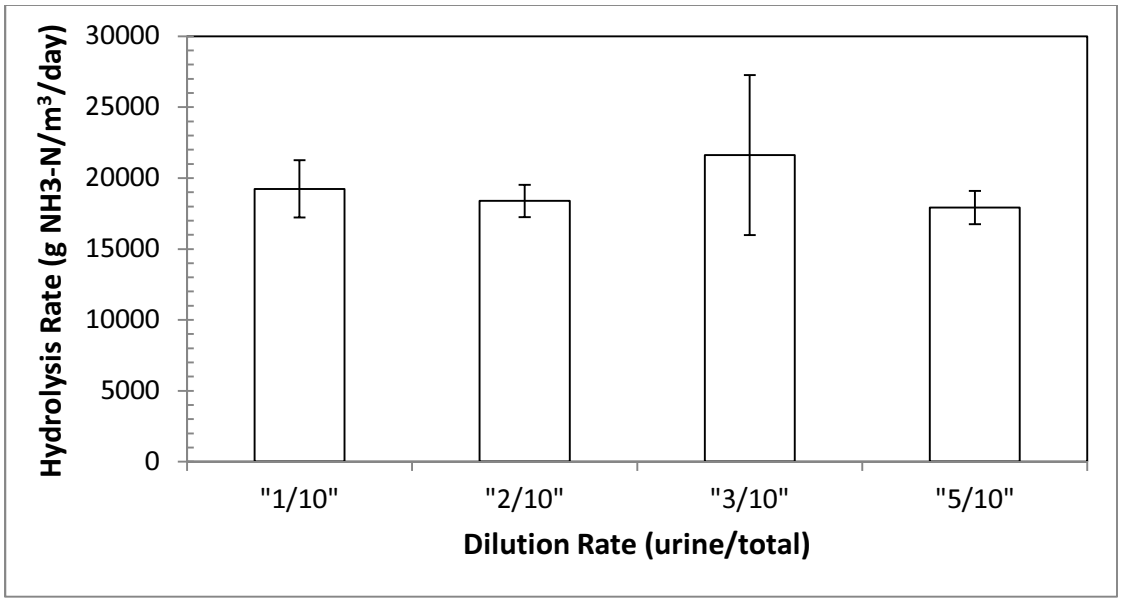

Figure 9. Activated column hydrolysis rates at various dilution rates and constant flow rate of 10 mL per minute

Other variables assessed during ureolysis analysis experiments included pH, DO concentration, and COD concentration, which can all be related to ammonia production. Figure 10 shows the relationship between total and free ammonia concentrations and pH in solution, considering all influent and effluent data from both the urine-only and activated columns. pH increases with increasing total ammonia concentration until the maximum pH is reached of approximately 9.3, demonstrated by the asymptotic behavior approaching the

pKa. The limited amount of free ammonia (NH_3) present at low pH is due to the pKa of ammonia of 9.34 at room temperature. At a pH of 9.34, 50 percent of the total ammonia is present as ammonium ion (NH_4^+), and 50 percent is present as free ammonia (NH_3). Since our experiments never reached the pKa of ammonia, free ammonia never reached 50 percent of total ammonia. Figure 11 shows ammonia production and COD reduction in the activated column as a function of flow rate in the varied flow experiments. Both sets of data appear to follow similar patterns, suggesting some correlation. Figure 12 closes in on this relationship between ammonia production and COD reduction, demonstrating some increase in ammonia production with increasing COD reduction. The weak, yet present correlation suggests a dependence of ureolysis rate on microbial activity. Finally, dissolved oxygen data did not display any clear pattern, but it was consistently consumed within the columns (influent – effluent). This again confirms the presence of aerobic microorganisms in the column that are consuming organics and producing the urease enzyme.

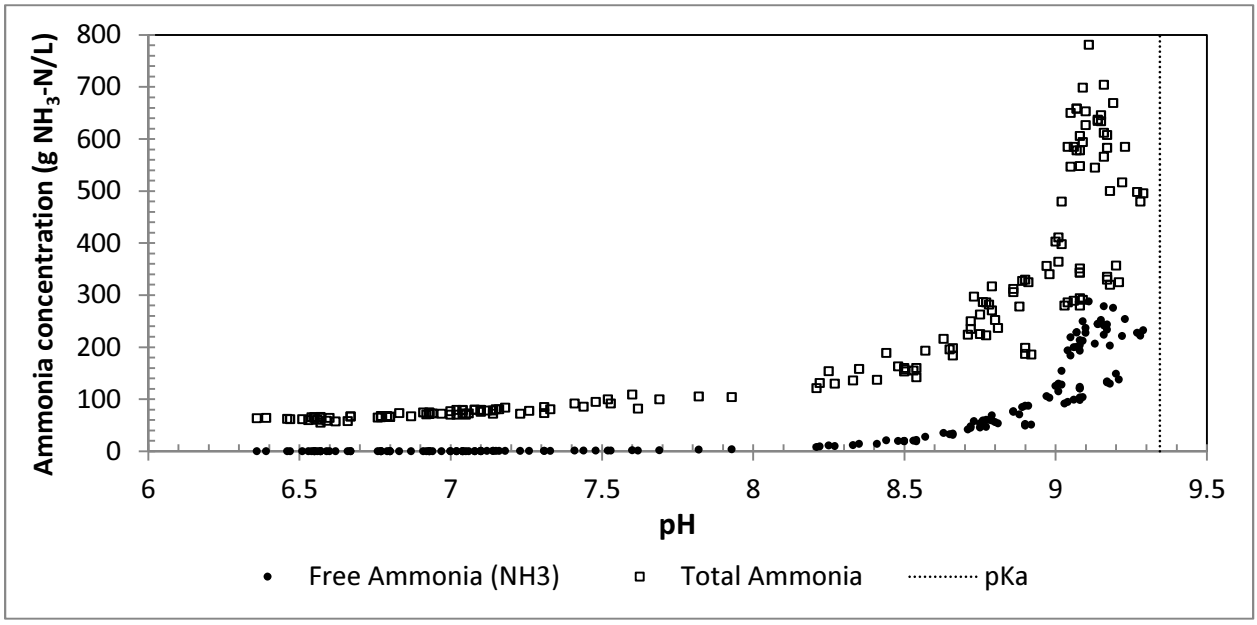

Figure 10. Total ammonia (NH_3/NH_4^+) and free ammonia (NH_3) as a function of pH for influent and effluent data from Urine-only and Activated column flow experiments at 1/10 urine dilution rate

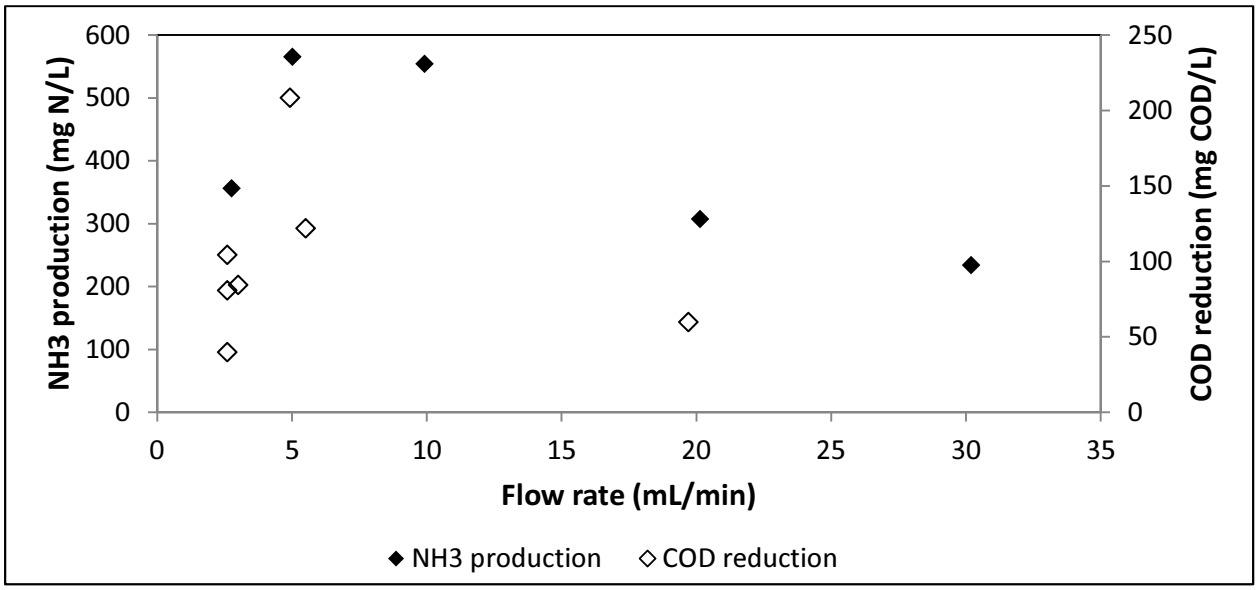

Figure 11. Activated column total ammonia production and COD reduction as a functions of flow rate

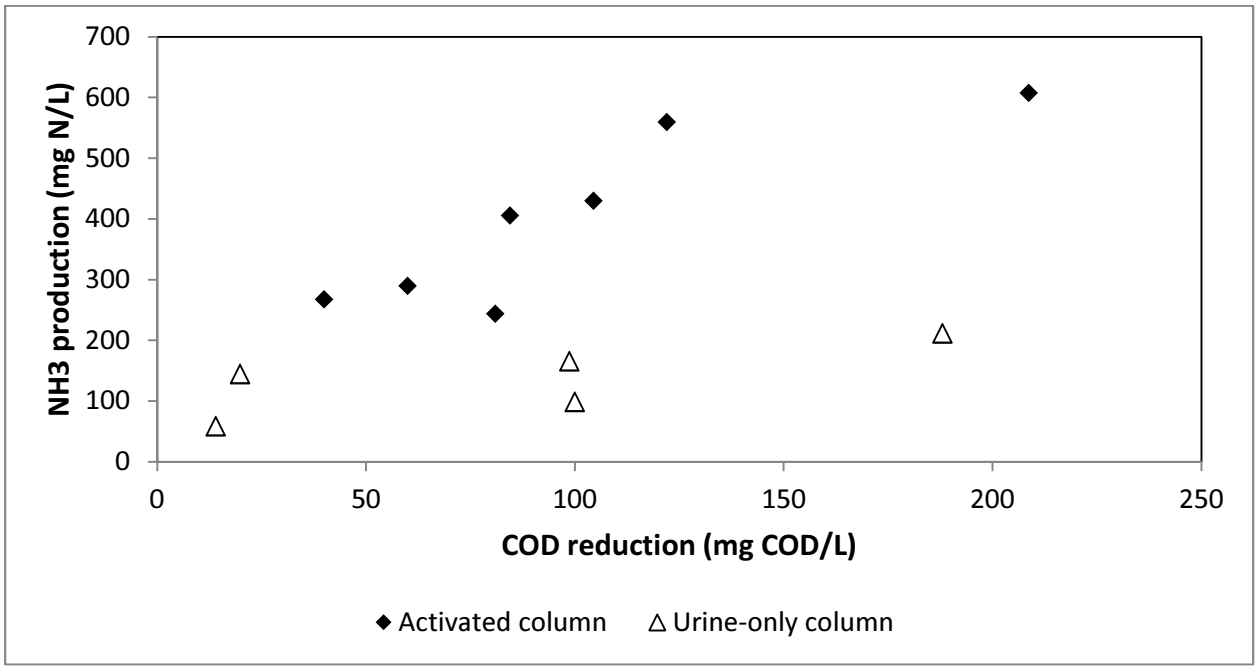

Figure 12. Total ammonia production as a function of COD reduction in the activated and urine-only columns

Comparisons between kinetic behavior of the well-characterized jack bean urease enzyme and ureolysis in the biological columns yield interesting information regarding the relative speed and character of urease produced. Assuming steady state operation of the columns, we can assess urea concentration over time as an indication of enzyme kinetics. The plot of urea concentration over initial concentration as a function of time in Figure 13 demonstrates the decay of urea over time.

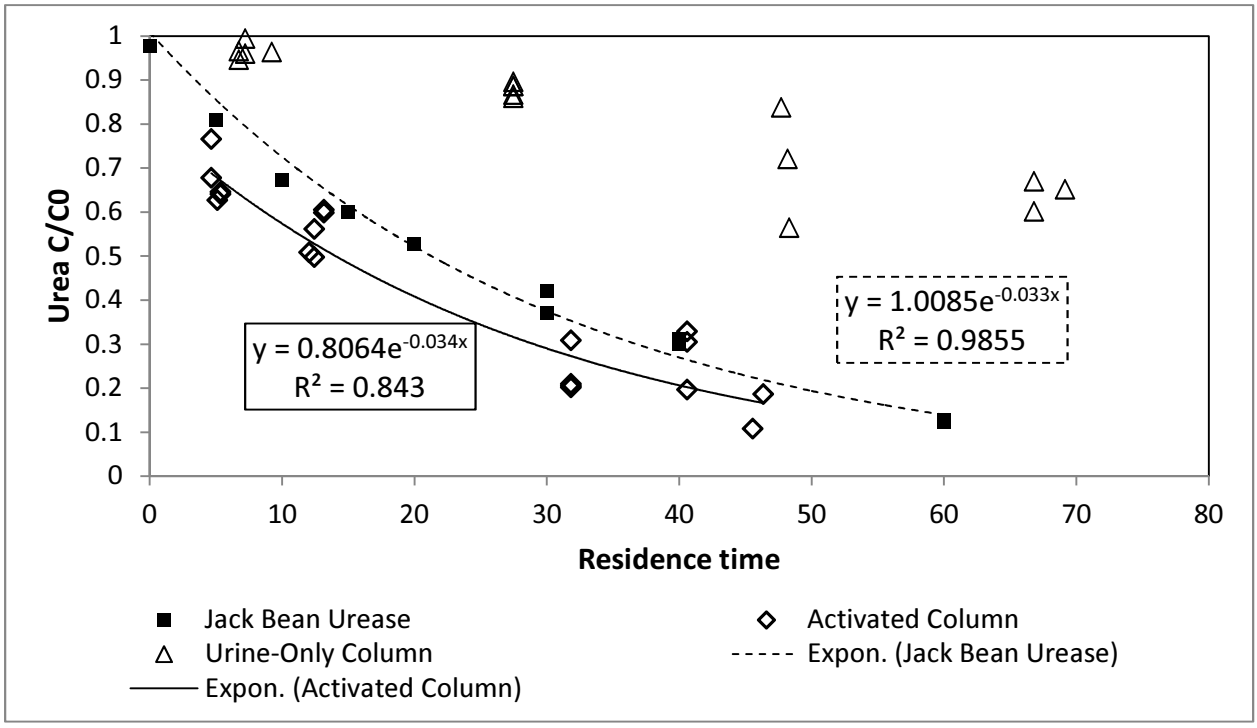

Figure 13. Urea C/C_0 vs. time for kinetics analysis of ureolysis activity. Exponential decay curves demonstrate the first-order behavior of the jack bean urease and the activated column data.

Jack bean urease and the activated column display strong exponential correlation. First-order rate constants for the jack bean enzyme and the activated column are 0.033 min^{-1} and 0.034 min^{-1}, respectively. Lineweaver-burke analysis yields a jack bean urease half-saturation constant (K_m) of 73.0 mM urea, greatly exceeding the literature-reported value of 2.5 mM (Blakeley and Zerner, 1983). This is most likely due to urine's unique

make-up, and in particular to the presence of urease-inhibiting compounds, such as phosphate ions (Blakeley and Zerner, 1983). The same analysis of the activated column data yields a K_m value of 17.5 mM urea, further demonstrating the apparent increased ureolysis rate in the experimental column. While both of the K_m values exceed those of purified jack bean urease described by Blakeley and Zerner (1983), they fall below the upper limit of 100 mM, reported by Mobeley and Hausinger (1989) for all characterized microbial ureases. While first-order reactions exhibit exponential decay, zero-order reactions go through linear decay. The urine-only column did not display a strong exponential relationship, but its behavior may suggest zero-order kinetics. The linear relationship between substrate concentration and time indicates that reaction rate is independent of substrate concentration. This suggests that the reaction is at saturation with respect to urea, which can provide some insight into the amount of urease present in the urine-only column as opposed to the activated column. Since rate of degradation was dependent on the urea concentration, the activated column demonstrates a stronger presence of urease enzyme, while the opposite could be said about the urine-only column.

Precipitates in columns

SEM images of solids collected in the activated column are shown in Figure 14. The crystalline structure suggests the presence of mineral deposits in the column. Additionally, EDS analysis identified that the most abundant elements were oxygen (44.4 percent), magnesium (8.83 percent), phosphorus (23.1 percent) and calcium (22.4 percent). XRD analysis of solids from the activated column confirmed the identity of these solids as struvite. However, in XRD analysis, solids from the urine-only column were unidentifiable, potentially due to presence of organics in the sample.

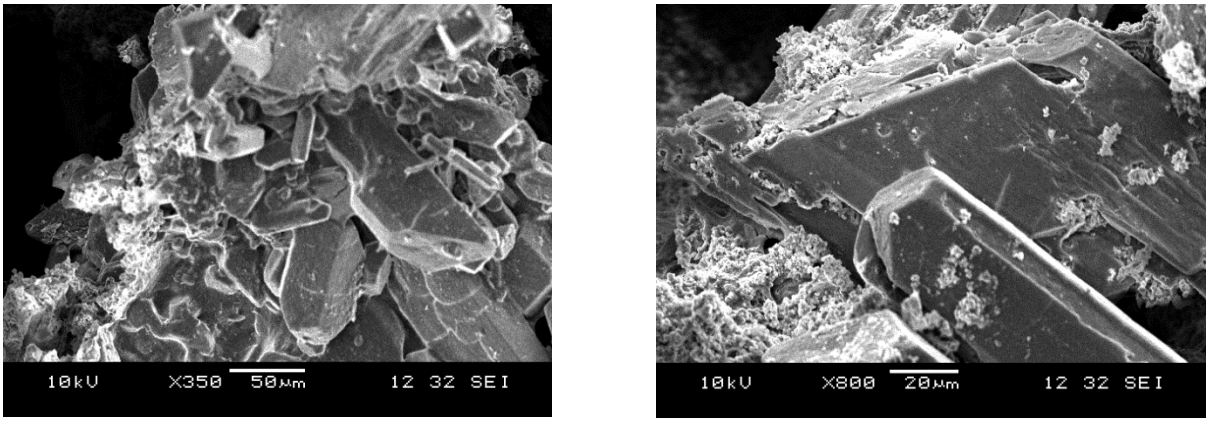

Figure 14. SEM images of solids formed in activated column. Crystalline structure suggests mineral precipitation as opposed to biological fouling.

Pathogen spiking

Figure 15 shows data from the pathogen inactivation experiment at different sampling times. At 10 mL/min, tracer studies estimated average residence times of 30.1 minutes and 37.4 minutes for the urine-only and activated columns, respectively. As a result, data points higher than 45 minutes were assumed to represent steady operation. Data demonstrate a log decrease in *E. faecalis* within the urine-only and activated columns of 0.07 and 0.1, respectively. In terms of disinfection experiments, this number is not significant, demonstrating ineffective removal of pathogens in this column. In the urine-only column, average effluent total ammonia concentration and pH were 283 mg N/L and 7.45, and in the activated column, these values were 466 mg N/L and 8.41. These levels have not been found especially detrimental to pathogenic organisms. Due to time constraints, the columns were not fully acclimated to the urine concentration (3/10) and flow rate, leading to sub-optimum ammonia and pH levels. Better inactivation is expected at the higher ammonia and pH values expected at steady state operation (pH of 9.2 and total ammonia of 806 mg N/L). The other possible disinfection mechanism in the column is

biological competition. However, based on data from Bischel et al. (2014), 4-5 log inactivation of *E. faecalis* is possible via biological processes over a matter of days, and our experiment occurred in minutes. Urine-only and activated samples left out for 18 additional hours at room temperature saw additional 0.09 and 0.2 log reduction, respectively. This, again, is not significant in terms of disinfection.

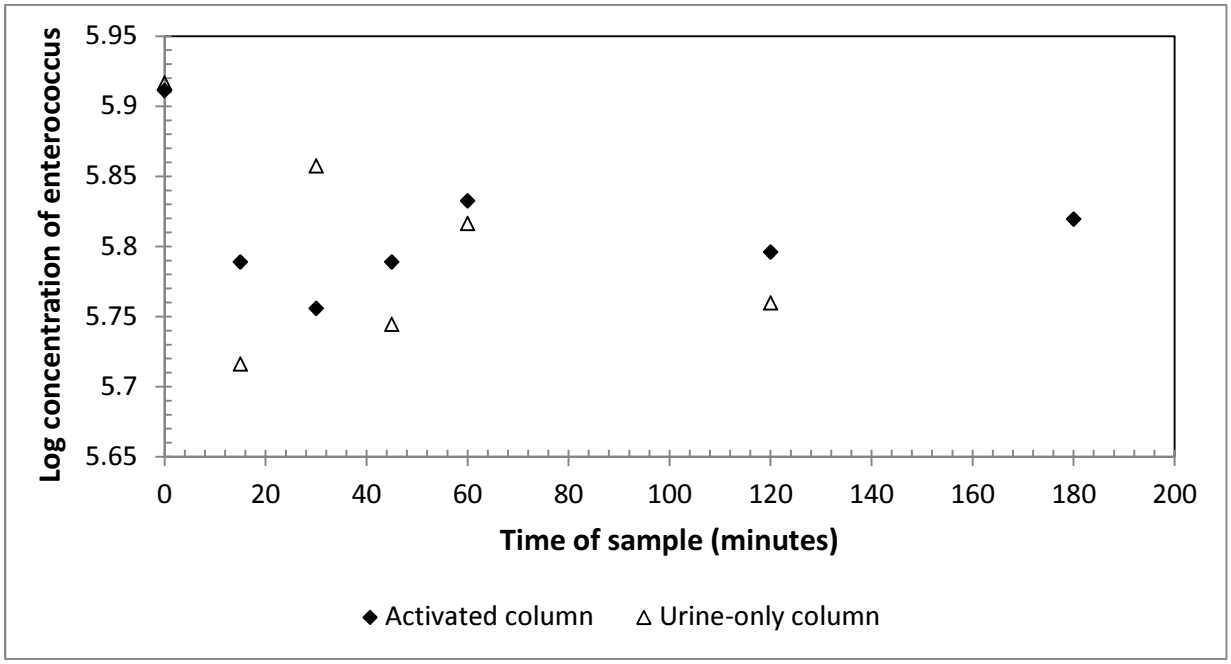

Figure 15. Log concentration of enterococcus as a function of column run time, where t=0 corresponds to the bacteria being spiked in

Characterization of Biofilms

Following sequencing of the DNA and data filtering described above, relative abundance and statistical analyses were carried out. In terms of naming convention, samples with "I" in the name indicate initial samples of carriers from each column, UI being a new carrier and WI being an initial carrier sampled from the WWTP. Samples 1, 2, and 3 represent the sampling periods on 1/9/15, 2/18/15, and 3/24/15, respectively. B and C

are triplicate samples of those described. Figure 16 and Figure 17 display the relative abundance of various orders of bacteria in the activated and urine-only columns, respectively. At the phylum level, the primary bacteria present in the initial carriers from the wastewater treatment plant (used to start the activated column) were Proteobacteria, Bacteroidetes, Chloroflexi, Chlorobi, Acidobacteria, and Planctomycetes, constituting approximately 90 percent of all phyla. Over the course of the column lifetime, the diversity decreased, ultimately revealing Proteobacteria and Firmicutes as 90 percent of the total phyla present. Interestingly, Firmicutes were not initially present in the wastewater carriers, demonstrating a population that may favorably grow on urine as compared to other phyla. When assessing the data at the order level, the change in diversity is further demonstrated. The Gammaproteobacteria, Pseudomonadales, makes up a large portion of the order diversity, particularly at the later sampling dates, but is not found in the initial sample. Other bacteria that develop in the activated column samples include Bdellovibrionales, Nitrosomonadales, and Burkholderiales (Proteobacteria) as well as Clostridiales and MBA08 (Firmicutes). Approximately 68 different orders make up the initial wastewater sample compared to only 31 in the final experimental sample, and the diversity within phylum also decreases. The smaller color bands towards the top of the initial sample columns in Figure 16 demonstrate a wide assortment of Proteobacteria present in the sample, while the later samples possess a much smaller number of Proteobacteria species.

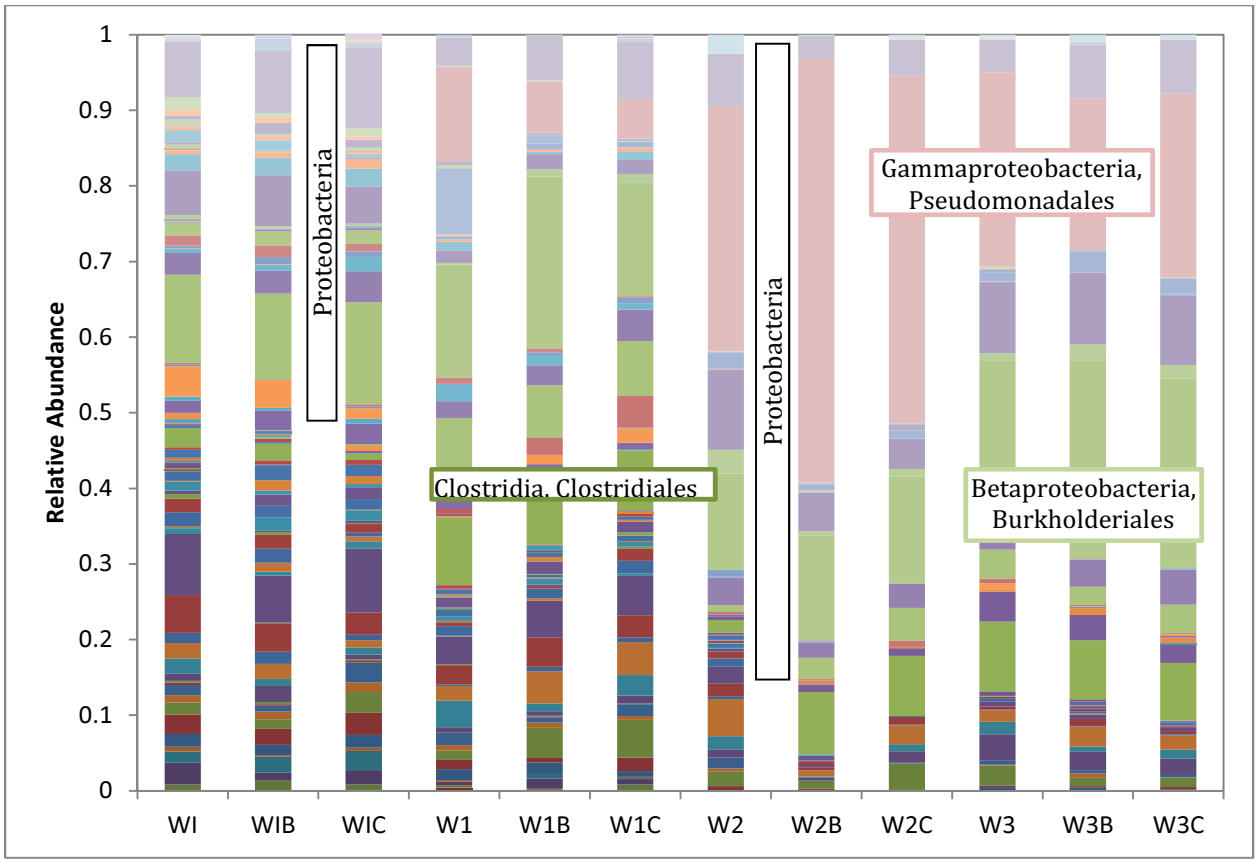

Figure 16. Activated column relative abundance of various bacteria orders. W1: 1/9/15; W2: 2/18/15; W3: 3/24/15. All samples with the same number represent triplicates. Proteobacteria labels demonstrate the bands that represent bacteria of the Proteobacteria phylum, demonstrating the differing diversity over the experimental period. Labels for specific orders indicate the most abundant orders.

The urine-only column demonstrates much less diversity throughout its lifetime. This is first of all confirmed by the predominance of Proteobacteria throughout the experimental period, contributing to anywhere between 70 and 95 percent of the population. When analyzing the phylum data, it is clear that only Proteobacteria in combination with one other phylum, such as Actinobacteria or Bacteroidetes, account for at least 90 percent of the phyla present in the sample. The bar charts for order data in Figure 17 also demonstrate the reduced diversity in the urine-only column. The urine-only data contains less distinct color bars than the activated column, highlighting the predominance

of a few orders of bacteria in the population. The predominant bacteria in the urine-only column include the Alphaproteobacterium, Rhizobiales and Caulobacterales, and the Betaproteobacteria, Burkholderiales. Notably, Burkholderiales had nearly disappeared from the population during the second sampling period, which also took place immediately following the large wash-out of the urine-only column.

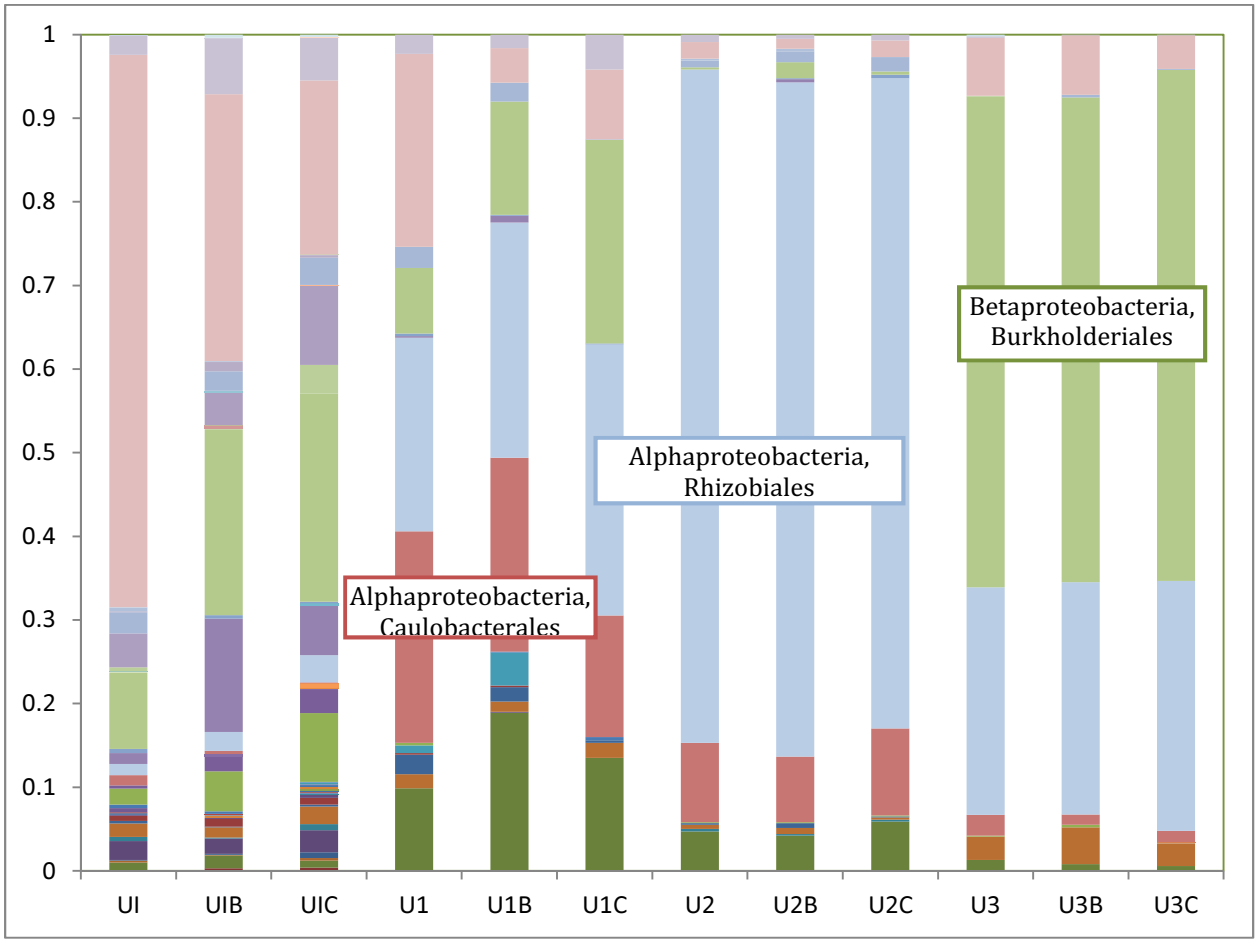

Figure 17. Urine-only column relative abundance of various bacteria orders. U1: 1/9/15; U2: 2/18/15; U3: 3/24/15. Samples with the same number (1, 2, or 3) represent triplicates.

Morisita-Horn Similarity Index and a relative abundance heatmap were used to assess the differences between samples and the appropriateness of averaging samples for other analysis. The Morisita-Horn Similarity Index compares samples to themselves and all

other samples, and displays an index closer to 1 when the samples are more identical. Figure 18 shows all of the Morisita-Horn calculations, in which boxes that are darker demonstrate higher similarity, with black being identical. The Morsita-Horn Similarity Index demonstrates high similarity between replicates in both the urine-only and activated columns. A relative abundance heatmap was constructed, which numerically and pictorially displayed the families present in each sample. The heatmap demonstrated similarity between replicates as well. Based on the similarity between replicates, the samples from each condition were averaged for subsequent analysis.

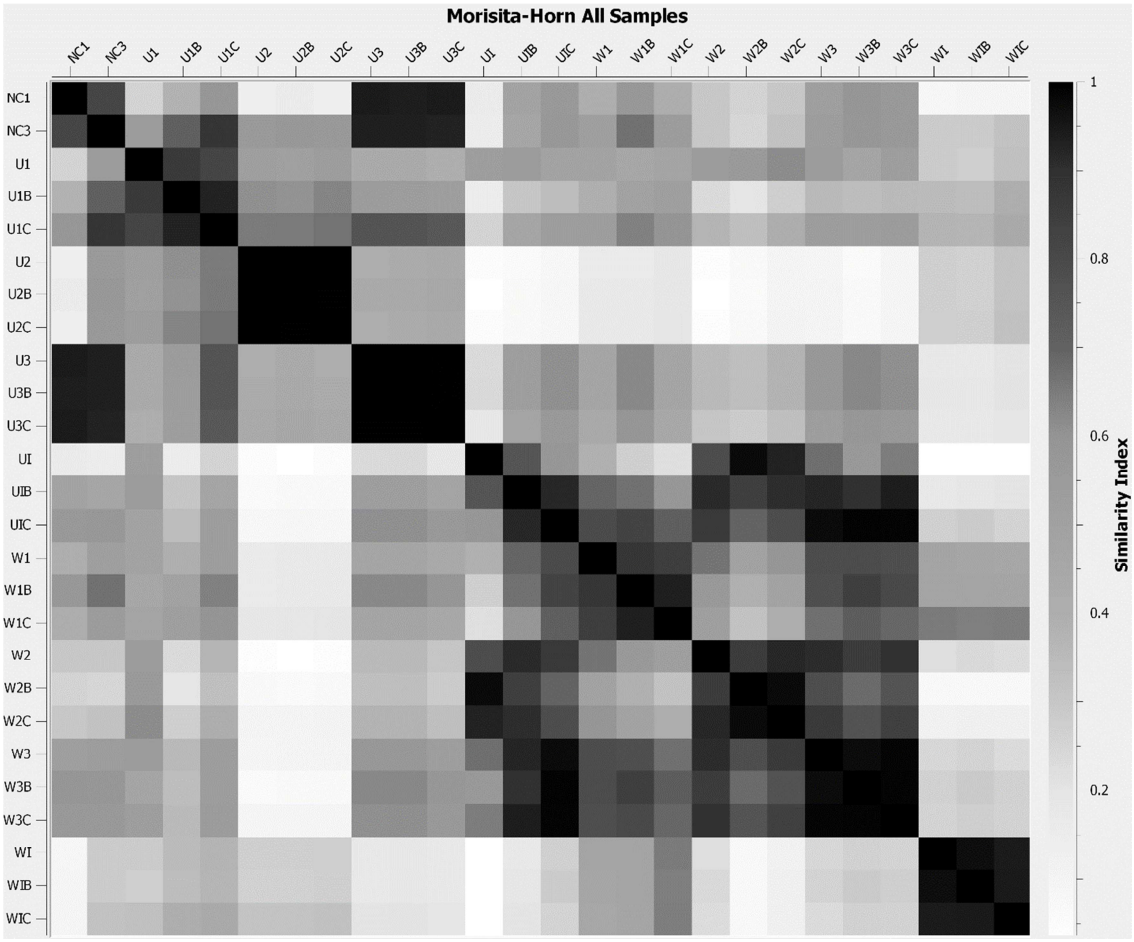

Figure 18. Morisita-Horn Similarity Index for all samples from the Urine-Only (U) and Activated (W) columns. Also included are 2 of the negative controls (NC).

Figure 19 shows an overall comparison of microbial communities in each column in the form of a heatmap. This is an average of all sampling times, and it demonstrates a predominance of Rhizobiales and Burkholderiales in the urine-only column as compared with the activated column. This suggests that these organisms are able to survive in a harsh urine environment, in which the high pH and ammonia concentration can decrease the rate of carbon metabolism. This chart highlights the increased diversity in the activated column, with no one organism fully dominating.

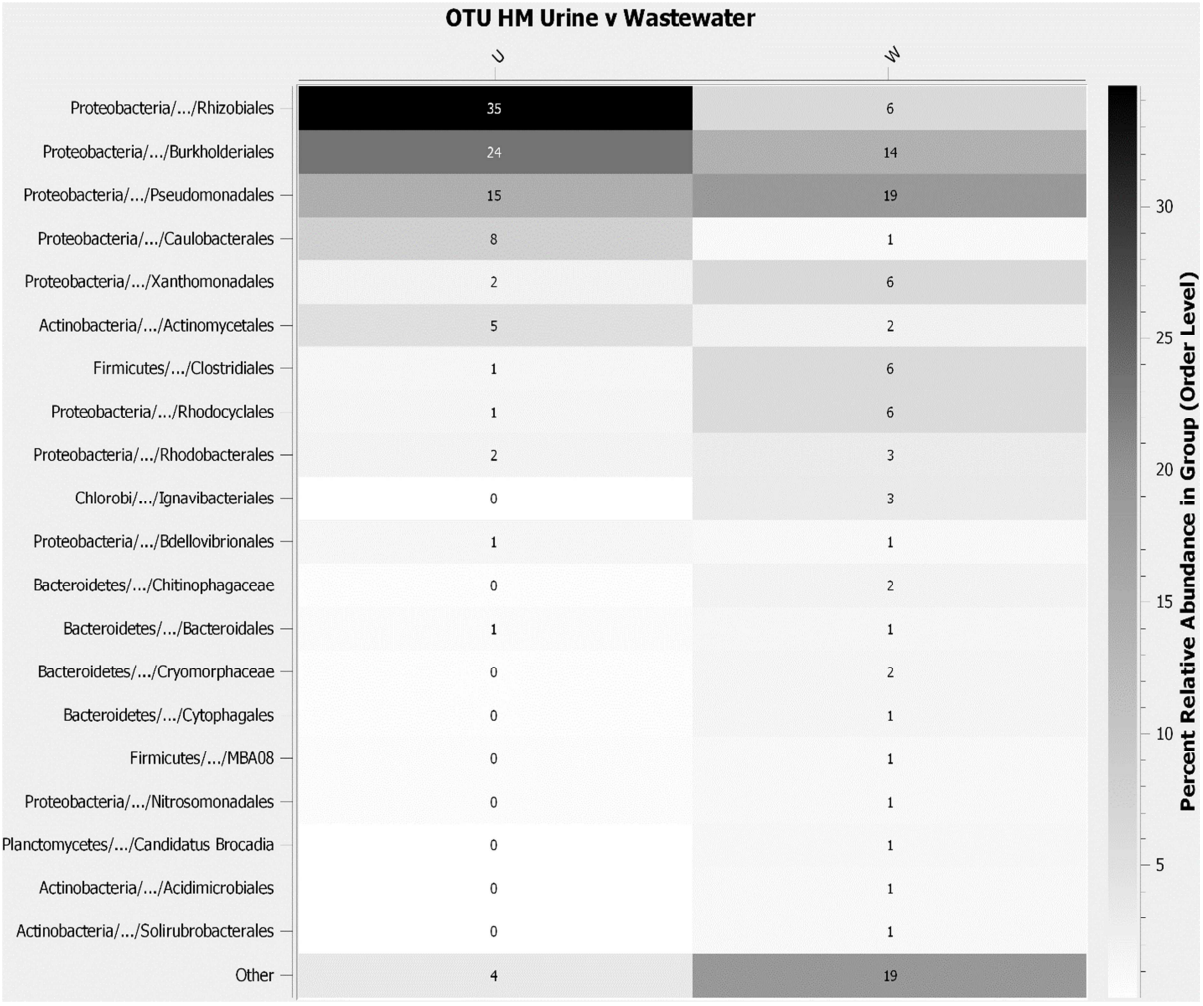

Figure 19. Operational Taxonomic Unit Heatmap at the family level for all urine (U) and all activated (W) samples averaged together.

Figure 20 is an image from the urine-only column, in which small, circular bacteria can be observed. Further results were unable to be obtained due to a microscope malfunction.

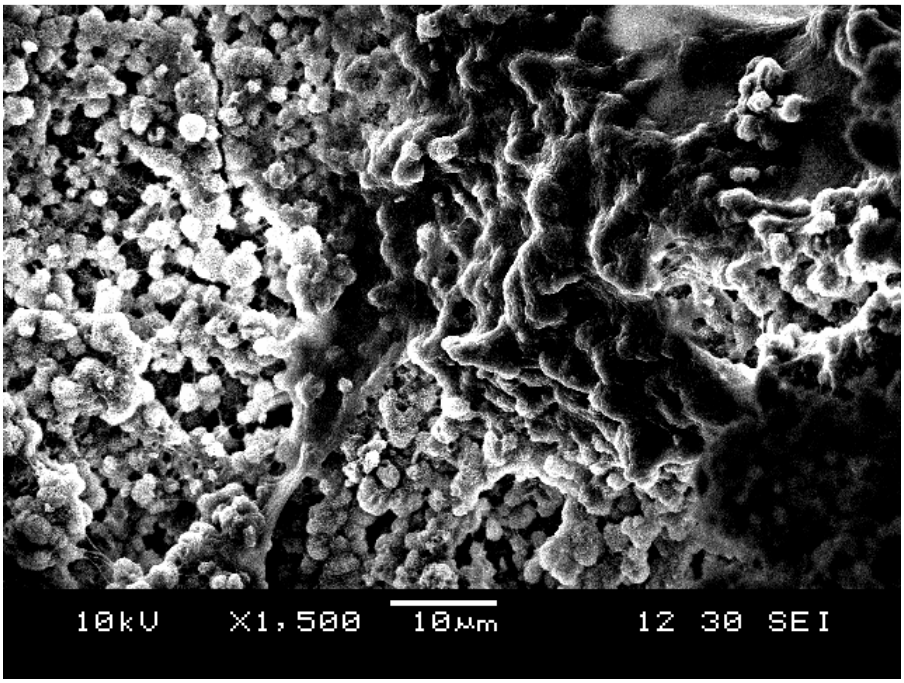

Figure 20. SEM image of urine-only biofilm sample. Small, circular objects suggest presence of bacteria.

Discussion

The effective conversion of urea to NH_3/NH_4^+ demonstrates the presence of urease enzyme in the trickling filter systems. Over the entire experimental period and based on averages from each experimental conditions, the maximum ureolysis rates in the columns were 7520 g N/m^3/day and 66850 g N/m^3/day for the urine-only and activated columns, respectively. Previous experiments by Udert et al. (2003) modeled and quantified the ureolysis occurring in EAWAG's urine-diverting system. Experiments were conducted by collecting a sample of biofilm from transport pipes and monitoring conversion of urea in a

well-mixed reactor with the biofilm sample and urine. Theoretically, the extent of ureolysis in a well-mixed batch reactor exceeds that of a continuous flow system, such as a pipe or our trickling filters. The maximum rate in the urine-only column is within the range of 4610 g N/m^3/day and 9860 g N/m^3/day, reported in experiments done by Udert et al. (2003). However, ureolysis rate in the activated column exceed these values by an order of magnitude. The differences in our values and those reported confirm a strong presence of urease-producing organisms in our system and demonstrate the variability in urease activity in urine-collecting systems. In addition, it is well-known that urease activity varies from species to species, creating large variation in the rate of ureolysis with any given community of organisms (Mobeley and Hausinger, 1989).

Correlations between urea reduction (NH_3/NH_4^+ production) and residence time, hydraulic loading rate, and urea-nitrogen loading rate can be used in preliminary design of biological ureolysis columns in practice. Based on the molar ammonium-to-phosphate ratio in struvite of 1:1, 0.4 mg ammonia-nitrogen per mg phosphate is required for precipitation of all phosphate in the form of struvite. The fitted design curves for both columns and all design values are shown in Table 4, and these equations are used in sample calculations for required residence time and loading rates shown in Table 5. These relationships were derived from this experiment and must only be used in preliminary estimates, as every system may be different based on the urine feed stream and environmental factors. In addition, further tests could verify the applicability of these correlations, as they are only based on one column and flow regime. Hydraulic loading rate is the flow rate (Q) divided by the cross-sectional area (A_s) of the column, and urea loading rate is the influent urea concentration multiplied by influent flow rate. These theoretical calculations are based on

the assumption that all urea reduction results in the same amount of total ammonia-nitrogen production. It is also important to consider free ammonia (NH_3) volatilization, as the proportion of total ammonia present as NH_3 increases with increasing pH. As shown previously in Figure 10, free ammonia concentration reached a maximum of 47 percent of total ammonia concentration in the experiments presented. While this indicates the potential for nitrogen loss to ammonia volatilization, our data do not show significant changes in total nitrogen from influent to effluent, confirming that free ammonia volatilization in this system is negligible. Additionally, as the onset of struvite precipitation has been observed at a pH of approximately 7.2 (Udert et al., 2003), with increasing precipitation above that pH, projected pH can be determined by Figure 10 to confirm that both the ammonia and pH requirements for struvite formation are met. Finally, negative values appearing in Table 5 indicate that this level of urea reduction is beyond the maximum possible urea reduction in the column, suggesting a performance threshold in the case of the urine-only column.

Table 4. Model equations derived previously (Figure 6, Figure 7, Figure 8) from data, showing urea reduction (y) as a function of the time or loading rate.

X-value	Urine-only	Activated
Hydraulic Residence Time (min)	$y = 25.704e^{0.0417x}$	$y = 239.29e^{0.0222x}$
Hydraulic Loading Rate (mm/min)	$y = 364.24e^{-0.262x}$	$y = 689.66e^{-0.07x}$
Urea Loading Rate	$y = 419.46e^{-0.206x}$	$y = 688.25e^{-0.047x}$

Table 5. Sample calculation results for design of a ureolysis column to meet minimum NH3 provision for struvite precipitation. Negative numbers can be considered zero for practical purposes

Dilution of urine	Phosphate concentration (mg PO_4/L)	Required ammonium concentration (mg NH_4^+-N/L)	Hydraulic Residence Time (min)	Hydraulic Loading Rate (mm/min)	Urea Loading Rate (mg N/min)
Activated					
1/10	230	92	-59.1	33.7	50.4
2/10	459	184	-27.9	24.0	35.6
3/10	689	276	-9.6	18.2	27.0
5/10	1148	460	13.4	10.9	16.2
undiluted	2296	919	44.6	1.0	1.4
Urine Only					
1/10	230	92	22.0	6.6	9.1
2/10	459	184	38.7	4.0	5.7
3/10	689	276	48.4	2.4	3.8
5/10	1148	460	60.6	0.5	1.3
undiluted	2296	919	77.3	-2.2	-2.1

The activated column appears to have a considerable amount of urease present in the column, evidenced by the first-order behavior and concentration-dependence of the ureolysis. While the dilution experiments did not further prove the dependence of ureolysis in the activated column on urea concentration, it can be speculated from kinetic analysis

that higher concentrations of urine at additional flow rates would yield a higher reaction rate.

While data from the dilution experiment did not show much variation in ureolysis rate, one impact from increasing concentration of urine was an increase in solids build-up, particularly with the activated column. As the pH and ammonium concentration approached that necessary for struvite precipitation, more struvite formed in the lower regions of the column, which caused 3 clogging episodes. These episodes greatly impacted the flow regime through the column and the residence time in the column. Additionally, the need to clean the column in response to clogging episodes may have led to biofilm dislodging from the carriers. Quantifying the effect of solid build-up on hydrolysis rate was not completed in this experiment, but future work can track the mass of solids in the column and its effect on average residence time and ureolysis rate. This is also an important operational issue to be aware of in practice. In addition, the formation of solids was partially a result of experimental setup. We periodically recycled the urine in the effluent reservoir during acclimation to a new condition. The resulting influent reservoir had higher levels of ammonium and a higher pH, leading to more precipitation potential. However, this condition is less likely to occur in practice, as the column will be fed fresh urine.

While data demonstrate consistency in ureolysis rates, the impact of intermittent flow was not assessed. Since toilets are not used constantly, the continuous feed of urine used in this experiment is not realistic. The greatest effects of intermittent flow would be the drying out of biofilms and the wash-out of biofilm as a result of high flow surges of

urine. As demonstrated by the resilience of the activated column at flow rates up to 30 mL/min and the subsequent failure of the urine-only column at 20 mL/min, biofilm maturity and strength are important considerations in the case of high flow surges. Both of these impacts could be mitigated by controlling the flow to the column after the toilet is used for urination. Design of a flow diffuser and small reservoir or use of a pump could help allow a slow trickle of urine to the column over an extended period of time after the urination takes place, but this would cause additional ureolysis prior to the column influent. This will not only keep the flow rates down, but it will ensure a more continuous feed of fluid to the biofilms, reducing the risk of biofilms drying out or being washed out.

Conclusions

This research demonstrates the possibility of engineered ureolysis of urine within a biologically-active column. Engineering the process of ureolysis can help to reduce losses of valuable nutrients, by enabling the capture of spontaneously-precipitated phosphate minerals that are typically lost in storage systems and by controlling nitrogen losses to free ammonia volatilization. Additionally, capture of all phosphate prior to land application of urine can reduce clogging incidences, particularly in drip irrigation systems.

We demonstrated the strong correlation between ureolysis rates and hydraulic loading rate in a trickling filter, allowing for preliminary design of an engineered ureolysis filter. Based on maximum loading rates and required residence times, column volume, cross-sectional area, and flow distribution design can be selected.

In addition, this research provided a first genetic analysis of bacteria that may utilize fresh urine as a growth substrate. This information can be used in the future

development of urine treatment and nutrient recovery technologies utilizing fresh urine. While urine's unique make up is generally considered non-ideal for microbial growth, this research indicates which species thrive under conditions of high salt concentration, urea, and COD.

Engineered ureolysis demonstrates potential of biological treatment as a supplementary tool for on-site urine treatment and recovery of nutrients. While this technology will not enhance nutrient recovery on its own, the principles discussed here will provide a launching point for innovation and improved process for applications such as rapid ammonia stripping with sulfuric acid to recover ammonium sulfate, and phosphate precipitation. Ultimately, this and other technology developments may improve user satisfaction and desire to recover nutrients, providing economic incentive for having a toilet and, ultimately, increased sanitation coverage throughout the world.

Chapter III: Phosphate Recovery Column

Introduction

Food security and sanitation provision are two major focuses of the United Nations initiative for the Sustainable Development Goals. Under these umbrellas, resource recovery and recycling has been stressed in initial proposals (United Nations, 2014). Wastewater has a history of receiving attention for its waste-to-resource potential, with agricultural use as one possibility. Heffer and Prud'homme (2014) estimated that by 2018, world fertilizer demand would reach 200 metric tons (Mt), comprised of 120 Mt nitrogen, 46.2 Mt phosphorus, and 34.2 Mt potassium. Despite hopeful outlooks for increasing supply of nutrients for agriculture (Heffer and Prud'Homme, 2014), resource extraction has been the cause of numerous international conflicts and extensive environmental damage. In addition, even with an available supply of fertilizer, it is often too expensive and out of reach for poor communities, jeopardizing food security.

Potential for Nutrient Recovery from Urine

Human waste contains a large amount of nutrients that are commonly used in agriculture fertilization, including nitrogen, phosphorus, potassium, calcium, and magnesium. In particular, urine is a concentrated nutrient solution, contributing approximately 80 percent of nitrogen, 50 percent of phosphorus, and 90 percent of potassium found in human waste (Larsen et al., 2001). Mihelcic et al. (2011) studied the global phosphorus potential from human urine and feces based on 2009 and 2050 values and projections of population and diet. Based on 2009 data, phosphorus available from urine was approximately 1.68 million metric tons, representing 11 percent of global phosphorus demand. This potential appears great, but logistical issues must be addressed,

such as the shift of populations from rural to urban. Mihelcic et al. describe the need to create links between the urban population and rural farmers.

Struvite Precipitation

Struvite (NH_4MgPO_4) precipitation has been used in wastewater treatment plants as a method of controlling undesirable precipitates from forming and clogging pipes. However, the same technique has been utilized as a method of nutrient recovery in traditional wastewater treatment plants and in source-separated urine. Zsofia Ganrot (2005) investigated the combination of precipitation and adsorption for efficient nutrient recovery. Magnesium oxide was added to urine to encourage struvite (NH_4MgPO_4) precipitation, recovering 98-100 percent of phosphorus, 22-64 percent of potassium, 2-5.6 percent of calcium, and 25 percent of nitrogen. The recovery of elements other than those found in struvite indicates the formation of additional fertilizer minerals. Additionally, Ganrot demonstrated that mineral adsorption in combination with struvite precipitation recovered between 64 and 80 percent of nitrogen in laboratory tests, with optimum N and P recovery with 0.1 grams of MgO and 15 to 30 grams of zeolite added per liter of stored and diluted urine.

Grau et al. (2012) developed a fully automated struvite reactor in the field at eThekwini, demonstrating the potential to recover up to 93 percent of total phosphorus in the form of struvite. This reactor used turbidity and conductivity as proxies for phosphate concentration, as demonstrated by Wylie (2009), Maurer and Gujer (1995), and Etter et al. (2011). Magnesium was added at a ratio of 1.1:1 (mol Mg: mol P), and the solution was mixed in a stirred batch reactor.

In addition to the need for large batch reactors, one limitation to struvite precipitation as a method of recovery is the lack of sustainable magnesium sources. Lee et al. (2003) demonstrated struvite precipitation using bittern, a concentrated by-product from salt production. The study analyzed three sources of magnesium, including magnesium chloride ($MgCl_2$-H_2O), seawater (1,200 mg/L Mg^{2+} and 400 mg/L Ca^{2+}), and bittern (32,000 mg/L Mg^{2+} and 8,000 mg/L Ca^{2+}). Through a jar test, bittern was found to recover 76 percent of phosphate, as compared to 75 percent for $MgCl_2$ and 81 percent for seawater. Sakthivel et al. (2012) also demonstrated the potential use of wood ash as a low-cost magnesium source for struvite precipitation. Removal of phosphate from initial urine after 0.5 hours, 1.5 hours, and 4 hours was 87 percent, 97 percent, and 99 percent, respectively. Additionally, the study concluded that a dosage ratio of 1.5 mol Mg/mol P could be used for struvite precipitation using wood ash (Sakthivel et al., 2012).

Phosphorus Sorption to Biochar

Biochar is a high-surface area product made of organic waste materials pyrolyzed at high-temperatures. While it is typically used to remove hydrophobic organic compounds, some studies have investigated the adsorption of charged compounds, such as phosphate ($PO4^{3-}$). Chintala et al. (2014) investigated phosphorus adsorption to biochars from 3 different feed stocks. The maximum phosphorus uptake with corn stover biochar was approximately 3.4 mg P/kg char, and the Ponderosa pine wood residue biochar displayed an order of magnitude lower uptake. Pre-treating biochar is one option for increasing phosphorus uptake, as demonstrated by Fang et al. (2014), who produced magnesium and calcium-supplemented biochar at 600˚C and observed Langmuir maximum P adsorption of 327 mg/g.

Research Approach and Objectives

This study examined 4 solid magnesium sources as well as compost to assess the viability of in-line, continuous phosphate recovery from ureolyzed urine. Media were characterized for their nutrient content and their ability to transfer urine phosphorus from solution to the solid phase. The major objectives of this research included:

1. Assess magnesium sources of varying physical properties for their affinity for phosphate recovery in a continuous-flow scenario
2. Determine factors to be considered in design of a phosphate recovery filter

Materials and Methods

Column Design

Columns were constructed with 1-inch glass columns from Ace Glass and PVC fittings. The columns were connected to neoprene and vinyl tubing that was replaced regularly throughout testing. Neoprene peristaltic pumps were used and connected to flow regulators to enable adjustment of flow rate. The pump flow rate was checked before and after testing, as flow rates tended to shift over time. Fine fiberglass mesh was used at the effluent of the column to prevent solids from exiting in the effluent.

Solid Media

Media were chosen based on the need for transport through the column and on quantity and characteristics of magnesium sources. Magnesium sulfate, soil, dolomite stone, and magnesium-treated biochar acted as supplemental magnesium sources, and compost was used as a filtration media. Characteristics of media used in experiments are described in Table 6.

Table 6. Characteristics and sources of various solid media used in phosphorus recovery filter assessments

Media	Formula	Source	Characteristics
Magnesium Sulfate (Epsom Salt)	$MgSO_4$-$7H_2O$	Local gardening store	Granular substance 9.5 percent Mg 12.5 percent S Easily soluble
Soil	-	Guinda, CA; area common for serpentine soils	Sieved to passing no. 12 standard sieve
Dolomite	$CaMg(CO_3)_2$	Found in nature, but purchased from PetStore.com – Estes dolomite used in household aquariums with a high-gloss resin on the outside	Less soluble, though K_{sp} is difficult to characterize
Mg-supplemented biochar	-	Bamboo feedstock soaked in $MgCl_2$ solution (described below)	Sieved to 12x40 standard sieve size
Compost	-	Boulder County Landfill composting facility – made up of organic waste including food waste, green waste, and paper products	Sieved to 12x40 standard sieve size

Biochar Production

Magnesium oxide enriched biochar (MgO-B) was produced with bamboo and $MgCl_2$ mixture as described by Fang et al. (2014). Bamboo was cut into small pieces and soaked in 52 g/L $MgCl_2$ solution at 1 to 3 mass to volume ratio for 2 hours. The mixture was then dried in a furnace at 103˚C overnight. The bamboo was then pyrolyzed in an oxygen-free environment and held at 900˚C for 2 hours. Following production, biochar was ground to 12 X 40 for uniformity and to enable flow in the column.

Synthetic Urine Preparation

Synthetic urine was prepared based on the recipe for stored urine from Amstutz et al. (2012) in autoclaved containers. The ingredients are listed in Table 7 and were added to ultra-pure water in the order listed. All chemicals were lab-grade, and each ingredient was allowed to completely dissolve prior to addition of the subsequent ingredients.

Table 7. Stored Synthetic Urine Recipe (Amstutz et al., 2012)

Compound	Concentration (g/L)
Na_2SO_4 anhydrous	2.30
NaH_2PO_4 anhydrous	2.10
NaCl	3.60
KCl	4.20
NH_4Ac	9.60
25% NH_4OH solution (mL/L)	13.0
NH_4HCO_3	21.40

Batch Experiments

Batch experiments with biochar were conducted to assess the maximum phosphorus recovery and to determine its viability in column experiments. These were conducted by mixing biochar with synthetic urine in 50 mL centrifuge tubes at doses between 6.25 and 25 g/L. Samples were then placed in a tumbler, which allowed for complete mixing of the samples for approximately 24 hours. Samples were then filtered with 0.45 µm vacuum filters, and the filtrate analyzed for phosphorus and pH to assess phosphorus uptake.

Column Experiments

Phosphorus recovery in a continuous-flow setup was analyzed by pumping synthetic urine at 5 mL/min through the packed columns. Each column was designed based on a combination of magnesium content and column size limitations. Tilley et al. (2008) suggested that struvite precipitation favorably occurred when magnesium was present in excess, at a molar ratio of 3:1 (Mg:P). Potential for slow dissolution of magnesium in the continuous flow setup was taken into consideration. As a result, ideal magnesium provision in these experiments was 5 times the magnesium required for 1 liter of urine. However, density and concentration of magnesium in the soil restricted the addition of this amount of magnesium, and it was designed based on the media packing regime shown in Figure 21. Solid media were used for flow distribution and filtration media to help capture precipitates formed in the column. Since compost contained very little magnesium and provided more porosity than soil, it was selected as the filtration media. Column design included a pre-magnesium layer for flow distribution and a post-magnesium layer for capturing precipitates. Masses of each magnesium source and filter media are described in

Table 8. It is important to note that while magnesium added with the soil filter does not meet the suggested 3:1 (Mg:P) molar ratio for struvite precipitation, it does still exceed the minimum requirement for struvite formation of 1:1 (Mg:P).

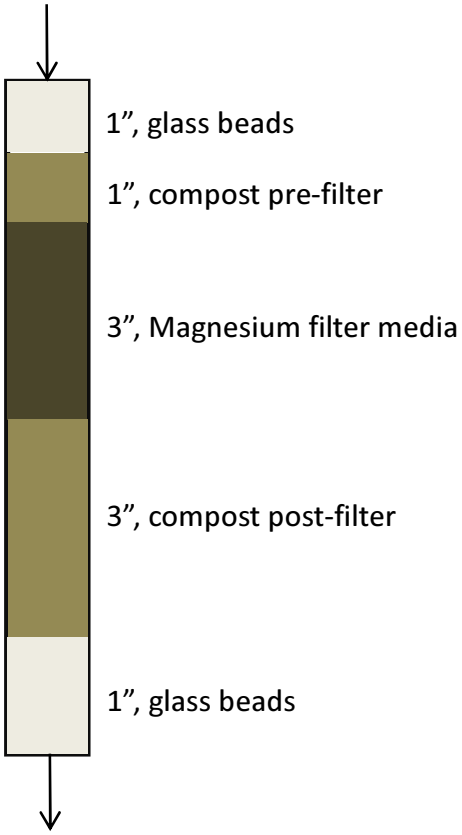

Figure 21. Column depths in column recovery experiments

Table 8. Magnesium media added for each source column test and Mg:P ratio based on magnesium added and P concentration in urine

Magnesium source	Mass of media added (grams)	Initial Mg:P molar ratio in magnesium filter (mol Mg/mol P)
Magnesium Sulfate	20.9	5.0
Soil	44.2	1.25
Dolomite Stone	15.1	5.0

Sampling

Solid samples were collected and well-mixed prior to analysis to achieve representative samples. Compost solid samples were ground to smaller sizes to avoid the

bias of large particles. Liquid samples were collected in sterile 40 mL centrifuge tubes and stored at 4˚C until analysis. Different experiments required different sampling frequencies, but most samples spanned approximately 2 minute duration and were collected directly from the effluent.

Characterization and Analysis

Column media were characterized with inductively coupled plasma mass spectrometry (ICP-MS), scanning electron microscope (SEM) imaging, electron dispersive spectroscopy (EDS), and x-ray dispersive technology (XRD).

Analyses of NH_3-N, TKN, COD, and PO4 were accomplished with HACH (Loveland, CO) TNTplus chemical kits. Samples were diluted with ultra-pure water to ensure a readable value. The vials were read using the DR 3900 or DR 2800 Benchtop spectrophotometer.

Results

Solid Media Characterization

Magnesium Sulfate

ICP-MS data are presented in Table 9 for solid samples used in the magnesium sulfate column test. “Compost-supplemented” refers to the middle section of the column where the $MgSO_4$ was added, and “post-supplemented” refers to the compost filter following the portion supplemented with $MgSO_4$. As demonstrated by the noticeable increase in nutrient content from initial compost to post-supplemented compost, the post-filter served to capture nutrients that dissolved in the supplemented section but were not

yet precipitated until further down the column. On the other hand, the higher phosphorus and magnesium content in the supplemented section versus the post-supplemented section suggests that the majority of struvite was precipitated immediately when the urine came in contact with the $MgSO_4$. This data clearly demonstrates an increase in nutrient content of compost as a result of added magnesium and the filtration of urine.

Table 9. Compost and MgSO4 column run solid nutrient analysis results

Element	Compost - Initial	Compost – supplemented ($MgSO_4$)	Compost – post-supplemented ($MgSO_4$)
P (mg/kg)	1.97	$1.27x10^5$	4520
Mg (mg/kg)	7.28	11330	4940
Ca (mg/kg)	24	12010	11750
Na (mg/kg)	188	5050	6100
K (mg/kg)	394	7290	7920

Soil

Table 10 presents ICP-MS results for solids used in the soil phosphorus recovery column. The high initial magnesium concentration in the soil decreased from 11,000 to 9,000, indicating approximately 20 percent dissolution and wash-out of magnesium from soil. However, the post-soil compost filter appears to have retained some of the washed-out magnesium, as the compost magnesium concentration increased by one thousand times. The additional increase in phosphorus and calcium in the post-soil compost suggests the retention of struvite as well as calcium phosphate precipitates. Ca, Na, and K exhibit similar

behavior, with the elements washed out of the soil and transported to the post-soil compost.

Table 10. Soil column run solid nutrient analysis results

Element	Soil – Initial	Compost - Initial	Soil – urine filter	Compost – post-soil filter
P (mg/kg)	1270	1.97	1115	19260
Mg (mg/kg)	11190	7.28	9030	6210
Ca (mg/kg)	14400	24	11710	62970
Na (mg/kg)	30790	188	1470	1250
K (mg/kg)	31290	394	16730	14180

Mg-Biochar

Table 11 shows ICP-MS results for 600˚C biochars produced both with (MgO-B) and without ("bamboo biochar") $MgCl_2$ pre-treatment. During pre-treatment, 92 grams of magnesium was added to the soaking solution and an initial mass of 581 grams of bamboo. After pyrolysis, 162 grams of char were produced, a yield of 28 percent. Based on this increase in magnesium as a result of pre-treatment, only 0.051 percent of magnesium was added to the bamboo and retained during the pyrolysis process. This also explains the results from the biochar batch study, shown in Table 12. Due to the low success of magnesium treatment and low uptake of both biochars, biochar was not used as a column media option.

Table 11. Bamboo biochar nutrient analysis for biochar produced with and without magnesium chloride pre-treatment

Element	Bamboo Biochar	MgO-B
P (mg/kg)	1270	1995
Mg (mg/kg)	2710	3000
Ca (mg/kg)	660	698
Na (mg/kg)	46	50
K (mg/kg)	24570	27560

Table 12. Biochar batch test results in the form of mean (±SD)

Char	Dose (g/L)	Percent P removal	Uptake (mg P/g char)
Bamboo Biochar	25	-14 (±5%)	-2.46 (±0.88)
	12.5	7 (±6%)	2.34 (±2.1)
	7	4 (±2%)	2.35 (±1.1)
MgO-B	25	8 (±7%)	1.42 (±1.2)
	12.5	4 (±1%)	1.48 (±0.34)
	7	4 (±10%)	2.74 (±6.6)

Column Experiments

Elemental data demonstrates some phosphorus recovery in column media, but data over time suggest quick wash-out of magnesium. Figure 22 displays percent phosphorus recovery and magnesium concentration in effluent over the extent of the magnesium sulfate experiment. Percent phosphorus recovered from synthetic urine remained above 90 percent for approximately 3 hours, at which point phosphorus recovery decreased rapidly.

At the same time, magnesium concentration in effluent of column displayed a surge of magnesium and then a decrease to 0 mg/L over the next 3 hours. The decrease in phosphorus recovery and magnesium concentration approaching zero suggest magnesium limitation of phosphorus recovery in the column.

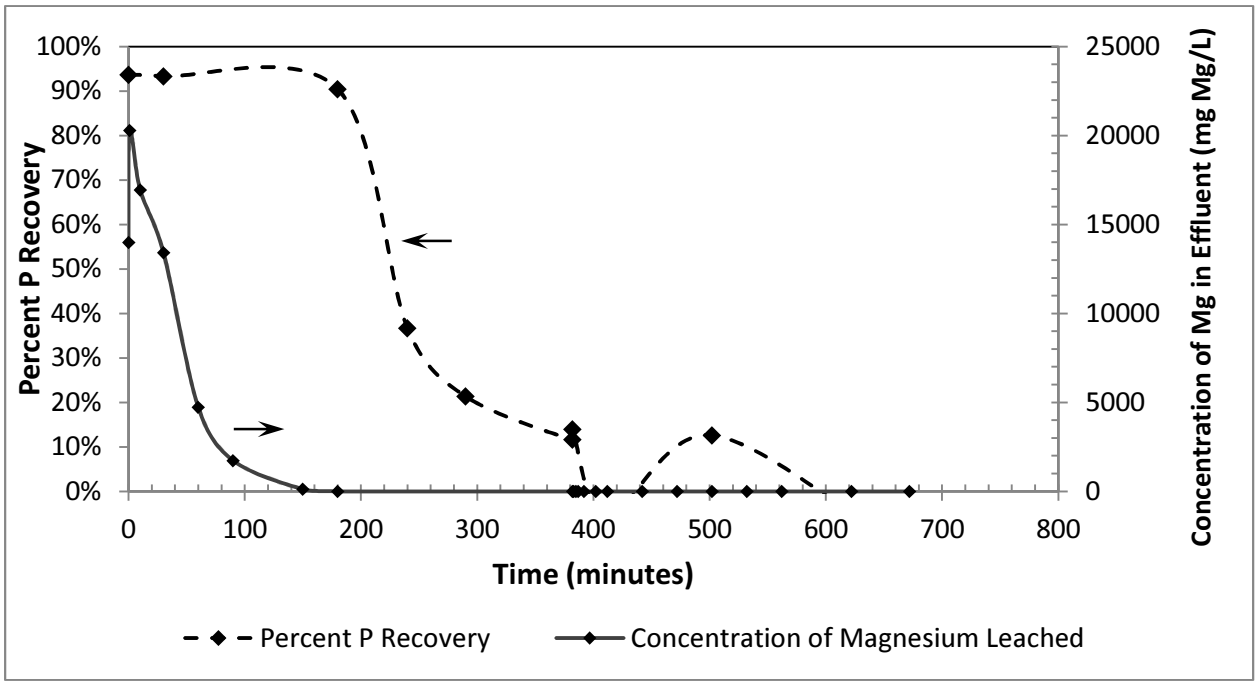

Figure 22. Percent phosphorus recovery and magnesium leached in effluent over time when using magnesium sulfate in struvite recovery column

Figure 23 shows the same percent phosphorus recovery data for both the soil and dolomite stone experiments, compared to magnesium sulfate. The soil displays similar behavior to the magnesium sulfate, but with the onset of decrease in recovery at 5 minutes and leveling out at 20 percent after 30 minutes. This was also observed during experiment, as white material appeared in the column immediately as urine ran through it. In addition, effluent samples from time 0 to 30 minutes displayed formation of solid precipitates immediately upon sampling. The dolomite stone demonstrated some recovery of phosphorus in the column, but this remained steady at approximately 10 percent throughout the experiment. Data for each material for mass of phosphorus delivered and captured in the column for 1 L of urine are shown in

Table 13. Additionally, this data shows the moles of phosphorus captured per mol of magnesium initially present in each column experiment. Phosphorus capture for the dolomite stone is an order of magnitude lower than the other two sources, indicating the limited availability of dolomite magnesium for precipitation due to low solubility.

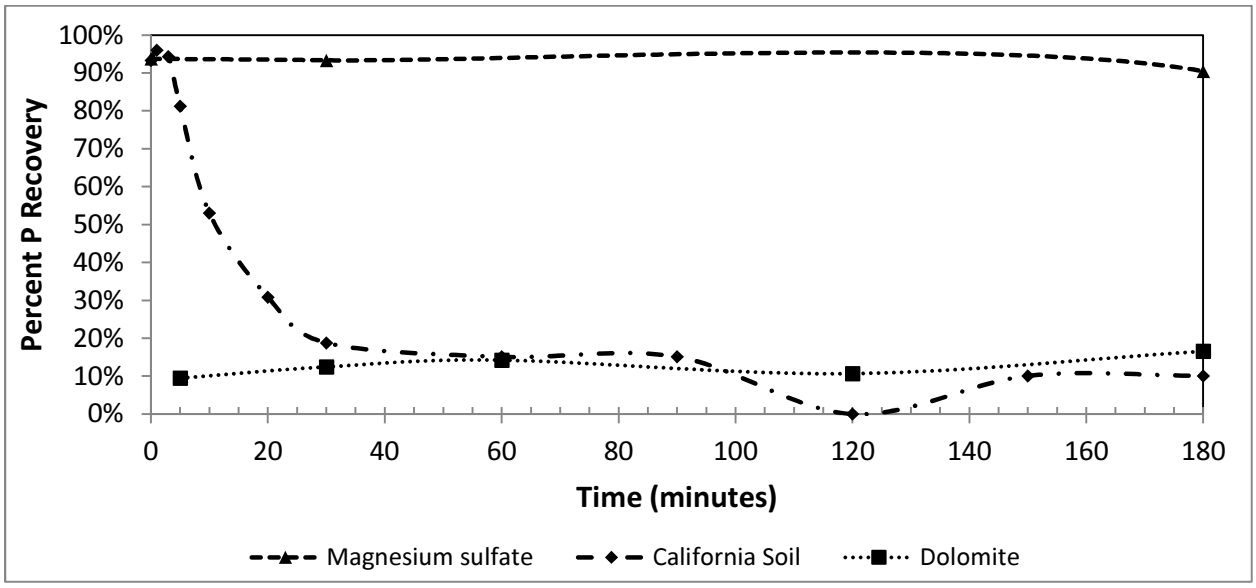

Figure 23. Percent continuous phosphorus recovery over first 3 hours of column experiments with magnesium sulfate, soil, and dolomite stone

Table 13. Mass of phosphorus supplied to and captured in columns supplemented with each source and fed 1 liter of synthetic hydrolyzed urine

Magnesium Source	Mass P delivered in 1 L urine (mg P)	Mass of P captured from 1 L urine (mg P)	Mol P captured per Mol Mg present in media (mol P/mol Mg)
Magnesium Sulfate	475	420	0.17
California Soil	486	74	0.12
Dolomite Stone	551	67	0.027

Discussion

Overall phosphorus recovery with each source

Experiments demonstrated the impact of magnesium source on effective continuous flow phosphate recovery. While magnesium sulfate demonstrated the largest overall phosphate recovery within the experimental period, dolomite stone shows promise to recover a fraction of phosphate over a longer period of time. Future studies can quantify the potential long-term use of a dolomite stone column to determine how long it will last until exhausted. It is not entirely accurate to compare the soil column performance to the others, due to the lower magnesium content in the media available for phosphate interaction. However, the reason for only 0.12 mole phosphorus capture per mole of magnesium initially present was a result of rapid magnesium leaching in the effluent. This provides interesting insight into the interaction between soil and urine, as data indicates that this magnesium withstood the storm event that happened immediately prior to soil sampling from the environment. Magnesium release from the soil could be a result of the

basic pH and interactions with surface groups and/or ion exchange of positively charged ions with surface magnesium. Based on data in Table 10, the positively-charged ions potassium, calcium, sodium, and magnesium were all released from the soil as a result of urine application. This suggests that ammonium (NH_4^+) ions may have been responsible for ion exchange in the soil, but further research should be conducted to better characterize the interaction between soil and ureolyzed urine.

Phosphorus recovery with the MgB-O was lower than predicted based on the Fang et al. (2014) study using a concentrated swine wastewater with similar pH and high concentrations of ions. The biochar was produced identically to the method described in that paper, aside from the feedstock choice. Bamboo and corncob biochars most likely have different surface groups, which would influence the potential surface reactions for phosphate sorption. In addition, a study by Yao et al. (2012) of biochars made from 4 feedstocks under varying conditions demonstrated that use of bamboo biochar actually caused phosphate leaching in solution. To re-evaluate potential adsorption of phosphate from urine, Mg-biochar should be reproduced, beginning with corncob and potentially with other feedstocks besides bamboo.

The magnesium sources tested in this preliminary study can be further characterized, as well as other sources tested in the same continuous-flow regime. Grinding dolomite stone has potential to make magnesium and calcium more accessible, thus increasing the availability for phosphate precipitation. Additionally, wood ash has previously been tested as a potential magnesium source for struvite precipitation (Fang et al., 2014). In the event that Mg-biochar is successfully produced and demonstrating

increased phosphorus uptake, bittern, a concentrated waste solution produced in salt production, may be tested as a soaking solution for Mg-biochar preparation. This would utilize a waste stream and may be more cost-effective in regions where salt is regularly produced (Etter et al., 2011).

Phosphate-Recovery Columns at Laboratory Scale and Beyond

Laboratory design of continuous-flow phosphate recovery columns can be improved by considering the time required between magnesium dissolution and struvite formation. While struvite formation kinetics has been studied, the absence of complete mixing brings in the question the dissolution rate in a column. Analysis of this process could lead to improved design of lab columns, providing a greater depth of media below the magnesium filter to provide reaction time and to capture precipitated minerals before the effluent. This was demonstrated in the formation of minerals in the effluent samples collected, particularly in the soil experiment. Had the compost post-filter been deeper to provide greater residence time, these minerals may have formed in the column rather than in the sample tubes. The media could then be removed and used as a nutrient-supplemented soil additive. In these experiments, we did not have issues with clogging in the compost columns, but we did have clogging in the soil column. As long as the media has enough porosity, clogging can be minimized, though it is still an operational issue to be aware of in column design and operation, ensuring that media is removed for use before clogging becomes an issue.

Design of a phosphate recovery column at a household scale must consider volume of urine produced as well as concentration of phosphorus present. Additionally, properties

of the magnesium source will dictate phosphorus recovery over time. Readily-dissolved magnesium sources appeared insufficient for extended operation of a phosphate recovery filter, because a large portion of magnesium washes out immediately, leaving little to no magnesium left in the column for the remaining urine entering the column. As this research has not fully characterized the process of continuous-flow phosphate recovery, specifics of scaling up are not provided. In addition, financial viability of a household filter is questionable and very context specific. In Nepal, for instance, Etter et al. (2011) demonstrated that 4,000 liters per day of urine production yielded 32,000 Nepalese rupees per year in struvite production, equivalent to approximately 320 USD. On a household scale, urine production per day is closer to 4 liters, based on a 4-person household. Based on the struvite value of 41 Nepalese rupees per kg struvite reported by Etter et al. (2011), annual potential income from struvite sales is 2 USD at the household level. These calculations do not take into account the cost of an additional magnesium source, which is required for full struvite precipitation. Thus, household application of this technology is not viable at this point. Recovery of additional urine nutrients would be required to increase the economic incentive for a family to recover its own urine for economic gain. On the other hand, scaling up of phosphate recovery technologies can increase the economic viability of the process, which could provide opportunities for microenterprises, which may buy household-collected urine at a low price, process it at centralized plant, and sell the recovered struvite product in the fertilizer market.

Conclusions

While phosphorus recovery appears possible in a continuous flow setup, issues of longevity exist due to magnesium wash-out. Readily soluble magnesium sources, such as

magnesium sulfate, though appropriate for batch reactors, prove insufficient for continuous flow operation. Additionally, sources on the opposite side of the spectrum, such as dolomite stone, also prove insufficient, due to their limited dissolution and availability.

Use of soil for struvite recovery has some potential in regions of magnesium-rich soil, as the magnesium appears readily available. Future research can characterize the interaction between soil and urine and phosphate recovery.

While this study provides preliminary assessment, various avenues of research into in-line nutrient recovery from urine are open and can add to the practicality of urine nutrient recovery in both the developing and developed world contexts.

Chapter IV: Synthesis: Improved source-separated urine management and nutrient recovery during continuous-flow applications

Future Research

Given this preliminary work on engineered ureolysis, many opportunities for further research exist. While preliminary studies quantified ureolysis rates in the engineered column, further research should design experiments to allow for monitoring and analysis at varying depths within the columns, to better model the ureolysis process and microbial activity. Furthermore, future characterization should monitor additional characteristics, such as changes in media weight, biological activity, and urease concentration within the column to get a better picture of the mechanism at play. Further analysis could be carried out regarding hydraulic behavior in the columns. By performing similar tracer studies as those described in Chapter 2 and monitoring biofilm growth and solid build-up in the columns, conclusions could be drawn about the impact of these parameters on residence time. Since all tracer studies were conducted after the flow tests, experiments described here could address the impact of time and filter development on residence time. To make results more applicable to a real system, increased flow rates and intermittent flow should be tested. The average urinating flow rate is 1200 mL/min, which greatly exceeds flow rates tested here (Haylen et al., 1989). The median volume of urine is 195 mL in a single urination event. Based on the desired hydraulic loading rate described above, the columns can be redesigned with a higher cross-sectional area to accommodate for the higher flow rates. For intermittent flow, pumps can be programmed to deliver surges periodically at flow rates based on the previously described study, simulating urination events and extended dry periods. This will assess the robustness of the system.

Finally, as this was the first metagenomics analysis of microbial communities grown on human urine, analysis and further biological identification can be carried out.

Research on phosphorus recovery from wastewater streams is abundant, with the major focus on struvite reactors. However, based on results in this thesis, a number of aspects warrant more research. First, interactions between soil and urine should be better characterized, both in terms of nutrient removal from urine and the impact of urine on ion mobility in soil. Second, given recent reported success with phosphate adsorption onto pre-treated biochar, biochar preparation processes should be refined and then tested as an alternative method of phosphorus recovery. Finally, further economic analysis should be developed to assess the benefit of household scale phosphorus and nitrogen recovery. Business models can be developed around urine nutrient recovery for agriculture if further research and data is presented on the benefits of such models.

Implications for Engineering in Developing Communities

Though urine holds promise as a fertilizer, user attitudes and reuse capacity remain barriers to implementation and effective reuse. These attitudes are context-specific, and are often influenced by cultural and religious beliefs and practices. For example, the Quran mentions specific rules around urination and defecation as well as contact with human waste (Hydri, 1990). When communities are not invested, human waste recovery projects can end in epic failure.

In addition to user attitudes, operational barriers include the need for and transportation of large volumes of liquid in order to reuse urine. Storage requires a large tank on site. Six months of storage, based on a family of four and production of 1 liter per

person per day requires 720 liters of storage, or approximately a 200 gallon tank. In densely-populated urban areas, space is a limiting factor in urine storage. Though rural communities have space available for tanks, the cost of storage tanks can be limiting for poor communities. Once stored, a number of options exist for urine reuse, including direct reuse and nutrient recovery.

The engineered ureolysis filter described in this thesis may be used as a supplement to other urine reuse technologies. For instance, using it as described in Chapter 2, in conjunction with a phosphate precipitation reactor not only maximizes phosphorus recovery but may also decrease operational issues, such as clogged drip lines in direct reuse of liquid urine. Using sub-surface drip irrigation can reduce nitrogen losses to ammonia volatilization, and initial removal and recovery of phosphate precipitates will improve the efficiency of this process (Zandee, 2012). In the case of nutrient recovery technologies, controlled ureolysis can provide process control for other biological and chemical processes, allowing for optimization of influent NH_3/NH_4^+ concentration and pH.

Design of a ureolysis filter at a household scale must consider the number of people using the toilet and ideally incorporate flow regulation in the influent. As demonstrated in this research, the activated column was robust to changes in flow rate and urine concentration. This research did not, however, test all possible conditions that these filters would experience, such as intermittent surges of urine as opposed to continuous flow. Another limitation of these filters is the operational issue of solids accumulation. Increasing the urine concentration in the influent led to numerous clogging episodes in the activated column. Given the stigma around human waste, handling clogging episodes in practice will

be difficult and undesirable. Furthermore, the highest urine concentration in this research was 1:1 (urine:water), and in dry urine-diverting toilets, this concentration may be much higher, if rinsing is not common practice. Based on experiences in this research, clogging issues will only increase in these situations. This effect should be better quantified in biological columns run solely with fresh urine as the feed, which has not reached a point where spontaneous precipitation is favorable. Further research can demonstrate the factors influencing clogging of the columns and methods to mitigate this issue.

Recovery of phosphorus from urine at a household scale is not financially advantageous in terms of fertilizer sales. This was described in Chapter 3, as one family's urine could yield 94 grams of struvite per year if all phosphorus in urine was captured. This only includes phosphorus present after spontaneous precipitation in the storage tank. 94 grams of struvite translates to a monetary value of ~$2 USD and does not factor in the purchase of an external magnesium source. With these considerations, phosphorus recovery from human urine should be viewed in light of savings rather than income.

Urine diversion in general can provide fertilizer savings both at a household and community scale. Preliminary calculations on fertilizer purchase savings were carried out for a family of 6, based on average fresh urine concentration values reported by Udert et al. (2003). Values used in the calculations include an average urine production of 1 liter per person per day, total nitrogen concentration of 7600 mg N/L and phosphorus concentration of 463 mg P/L. Nitrogen is valued at $0.73/kg N (Knorr, 2015) and phosphorus at $0.28/kg P (Knorr, 2015). These assumptions yield nitrogen savings of $6.06 per year and phosphorus savings $0.14 per year, based on 50 percent nutrient

recovery. This corresponds to approximately 1 percent of the lowest median household annual income worldwide (Phelps and Crabtree, 2013). The 8.3 kg nitrogen recovered annually can fertilize 400 square meters based on a conservative requirement for corn production from Scharf et al. (2005). The 0.51 kg of phosphorus can fertilize 150 square meters based on fertilizer use applications recommended by the California Department of Food and Agriculture ("Corn Fertilization Guidelines", 2015). On a per person basis, this leads to 67 square meters per person based on nitrogen, and 25 square meters per person based on phosphorus, which can be scaled up to a community garden based on community toilet usage. These estimates are conservative and naturally-present nitrogen and phosphorus will decrease the required fertilizer application rate.

While both technologies assessed here have many limitations, they have been developed under the premise of the proposed goals 2 and 6 of the new SDGs. These goals call for actions to "end hunger, achieve food security and improved nutrition, and promote sustainable agriculture" and to "ensure availability and sustainable management of water and sanitation for all" (United Nations, 2014). When placed next to each other, the interdisciplinary nature of these goals is brought to light. Urine source-separation contributes to solving a multi-faceted problem. Pursuing this in all contexts requires the expertise of engineers, anthropologists, psychologists, economists, chemists, farmers, and more.

Chapter V: Conclusions

As mentioned in Chapter 1, the main hypotheses addressed by this thesis are as follows:

1. Urine can be a substrate for biological growth, and specific microorganisms can thrive on its supply of nutrients, high salt concentration, and pH.
2. Ureolysis can be engineered by cultivating microorganisms such as those described in hypothesis 1 and utilizing the kinetics of ureolysis in the system.
3. Solid magnesium-supplemented filters can act as an alternative to well-mixed batch reactors for initial and potential longer term effective struvite recovery from source-separated urine.
4. Different magnesium sources will exhibit varying struvite recovery potential based on solubility and availability of magnesium to the solution.

The results and conclusions from each hypothesis are listed below.

1. This thesis effectively demonstrated the start-up and operation of a biological filter with urine as the sole substrate. Evidence of microbial community development and urease enzyme activity confirmed hypothesis 1 and was supported by microbial community analysis.
2. Potential for engineered ureolysis was demonstrated by drawing correlations between design parameters, such as flow rate, nitrogen loading rate, and residence time. With consistent total ammonia production rates reported across conditions, this process was characterized kinetically, with rate constants and insights into the limiting factors in ureolysis.
3. Solid magnesium supplemented filters were employed to recover a small portion of phosphorus in urine, but these filters did not perform on par with well-mixed

struvite reactors. The continuous flow nature of these columns led to magnesium leaching and loss of struvite recovery potential over time.

4. The magnesium sources tested demonstrated different behavior based on physical properties, with the magnesium sulfate representing a readily soluble magnesium source and the dolomite representing a slow-release, nearly insoluble source. While magnesium sulfate recovered large percentages of phosphorus up front, its performance declined rapidly. On the other hand, dolomite steadily recovered 20 percent of phosphorus in the influent, demonstrating a source with lower recovery but potential for longevity in a continuous-flow system.

In addition to the above-mentioned hypotheses addressed, this thesis drew many other conclusions about the practicality of continuous flow management of source-separated urine nutrient recovery such as complications associated with struvite build-up in urine diverting systems and the potential economic savings associated with recovery of phosphorus and nitrogen from urine. The author also postulated a number of ideas for improvement of experimental design and effective urine resource recovery in the developing world context.

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