UREA HYDROLYSIS INHIBITION IN WATERLESS URINALS FOR WATER
CONSERVATION AND NUTRIENT RECOVERY

By

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UREA HYDROLYSIS INHIBITION IN WATERLESS URINALS FOR WATER CONSERVATION AND NUTRIENT RECOVERY

By

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The use of water conserving bathroom fixtures, such as waterless urinals, has been supported for their potential to conserve large volumes of high quality drinking water. Waterless urinals have also been critical in the implementation of urine source separation systems across the world. However, due to urine’s composition and the presence of the urease enzyme that hydrolyzes urea, valuable nutrients readily precipitate in the fixtures and pipes. The goal of this research was to provide an improved understanding of the urea hydrolysis process in waterless urinals so as to benefit water conservation and P recovery efforts. Two acids were used to treat urine in waterless urinals to inhibit the urea hydrolysis reaction by lowering the pH, which makes the precipitation of struvite and hydroxyapatite less favorable. Of the acids tested, 2.5 mL of 2500 meq/L acetic acid added after every urination event was able to inhibit urea hydrolysis in synthetic urine. Treatment also allowed for more phosphorus recovery in subsequent struvite precipitation. When phosphate recovery potentials were compared, struvite precipitation recovered 43% more phosphate in the acetic acid treated urine than the baseline experiment with no treatment. Results from the synthetic urine experiments were used to determine the treatment parameters that were used in a real human urine demonstration. The real human urine
demonstration showed that the acetic acid treatment delayed the urea hydrolysis reaction in the storage tanks.
Urine source separation has been increasingly proposed as a process that addresses the excess nutrients and pharmaceuticals that enter our environment through inconsistent removal at conventional wastewater treatment facilities. Urine alone contributes 50% of the phosphorus and 80% of the nitrogen on a mass basis that enters a wastewater treatment plant, while only contributing to 1% of the volumetric flow.¹ Diverting undiluted urine by using waterless urinals and urine-diverting toilets has the ability to conserve potable drinking water and reducing the environmental impact of wastewater treatment. Life cycle assessments on urine source separation scenarios have concluded that urine diversion has a lower environmental impact when compared with conventional wastewater treatment.²,³ More specifically, implementing urine diversion can save $2.6 \times 10^5 \text{ m}^3 (6.9 \times 10^7 \text{ gal})$ of potable water and $231,000$ per year in a large university setting.³

Unlike centralized wastewater treatment, where the community has very little interaction with its operation, urine source separation breaks the barrier between the user and the wastewater treatment system. User acceptance is crucial in the adoption of decentralized wastewater treatment.⁴ A recent survey by Ishii & Boyer (2016) sought to understand the drivers for personal perceptions of a future with urine source separation. When asked to gauge their support for urine diversion, approximately 84% of university students surveyed expressed support for urine diversion after learning about the benefits of diverting urine from wastewater systems.⁵ However, the longevity and resiliency of a urine diversion operation rest on the strength of the collection and storage system. Without robust technology to support the diversion of urine, the introduction of a urine diversion systems could be in jeopardy.
The first components of a urine source separation operation are the waterless urinals and urine-diverting toilets used to collect undiluted urine from the source of production. Without properly functioning fixtures, the whole system could be at risk of becoming contaminated, diluted before storage, or removed completely. Urine chemistry also changes during conveyance to storage, due to urea hydrolysis, causing valuable nutrients to precipitate in the fixtures and pipes.\textsuperscript{6,7} Urea hydrolysis is the chemical reaction caused by the urease enzyme which converts urea into ammonia and bicarbonate.\textsuperscript{8} The overall reaction is:

$$\text{NH}_2\text{(CO)NH}_2 + 3\text{H}_2\text{O} \xrightarrow{\text{Urease}} 2\text{NH}_4^+ + \text{HCO}_3^- + \text{OH}^-$$

The urease enzyme is produced by a wide range of organisms, from eukaryotes to prokaryotes, and is most active as the bacterial urease in collection systems.\textsuperscript{6,8} The addition of ammonia and bicarbonate by urea hydrolysis raises the pH of fresh urine from pH 6 to pH 9. This becomes problematic in the presence of magnesium and calcium, which are both available in fresh urine, and are precursors to the precipitation of struvite ($\text{NH}_4\text{MgPO}_4\cdot6\text{H}_2\text{O}$) and hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3(\text{OH})$). With the addition of ammonia and the increase in pH, magnesium and calcium become supersaturated and precipitate as struvite and hydroxyapatite, which can have detrimental effects on the function and aesthetics of the waterless urinals and urine-diverting toilets.\textsuperscript{6} Ohki et al. (2009) found that 65% of the scale formation on toilet bowls was due to the precipitation of struvite and calcium phosphate and carbonate, with the remaining 45% of scale being organic-based solids.\textsuperscript{9}

The goal of this research was to provide an improved understanding of the urea hydrolysis process in waterless urinals so as to benefit water conservation and P recovery efforts. The overall approach was to conduct the experiments under realist conditions with real waterless urinals and use frequencies. Results from initial synthetic urine experiments were used to
determine the treatment parameters used with real human urine. The specific objectives of this research were to (i) mimic urea hydrolysis in waterless urinals using synthetic urine and urease addition; (ii) inhibit urea hydrolysis in waterless urinals using synthetic urine, urease addition, and acid addition; (iii) compare P recovery from synthetic urine post-hydrolysis both with and without inhibitor/acid addition; and (iv) demonstrate the results for urea hydrolysis, inhibition, and P recovery using real human urine.
CHAPTER 2
METHODOLOGY

Synthetic Human Urine

Synthetic fresh urine was used in all automated lab experiments. Table 2-1 in the shows the fresh urine recipe used throughout, which was adapted from Wilsenach et al. (2007) and Ronteltap et al. (2007b). Previous studies have used this urine recipe and have modeled the behavior of fresh urine in source separation scenarios. Fresh urine contained urea, sodium, potassium, magnesium, calcium, and chloride and was adjusted to pH 6 with NaOH. Urea hydrolysis was simulated by the addition of jack bean urease powder (Fisher Chemical, EC 3.5.1.5). Urease addition is detailed in the sections below.

Real Human Urine

Undiluted human urine was collected from twelve healthy adults between 10 am and 4 pm (local time) on work days. Urine was collected inside the female and male bathrooms using graduated plastic bottles and bedpans placed inside the toilet. The donors were asked to log the date, time, and volume of urine collected, and to measure pH and conductivity upon bringing their fresh urine to the demonstration urinal.

Urinals and Storage Tanks

Kohler Steward Waterless Urinals were used for the entire study. Three urinals were used for the automated lab experiments that used synthetic fresh urine and two of the urinals were used for the demonstration experiments that used real fresh urine. The Steward Waterless Urinal has a trapway designed to slowly flush urine without the need of water. A layer of proprietary sealing liquid is used to block odors from entering the bathroom. The sealing liquid is less dense than water, allowing it to float on top of the urine in the trapway, and the trapway is designed to slow the flow of the urine as to avoid the loss of sealing liquid after each urination event. The
sealing liquid was not used for the automated lab experiments, however it was used in the
demonstration experiments due to the use of real human urine. The urinals were cleaned with
deionized (DI) water after each automated lab experiment. The cleaning procedure consisted of
multiple flushes with tap water, DI water, and consumer grade bleach. Effluent urine from each
urinal was stored in 113.6 L (30 gallon) conical bottom medium-density polyethylene tanks (Den
Hartog Industries). A ball valve was attached to the bottom of each tank to empty its contents.

**Automated Lab Experiments: Baseline Synthetic Urine Experiment**

Three identical experiments were conducted in parallel as followed. The three urinals
were connected to two pumps: a high-performance peristaltic pump for delivery of urine to the
urinals and a peristaltic pump for delivery of urease solution to the urinals (Figure 2-1). The
pumps were programmed to repeat one urination events over a set period of time. For the baseline
synthetic urine experiment, in which urea hydrolysis was simulated, a urination event occurred
every 10 min, which consisted of 237 mL of synthetic fresh urine and 2.5 mL of the urease
solution concurrently pumped into the urinals for 20 s. The urease solution was made by
dissolving 12.64 g of urease powder in 250 mL of DI water. The urine volume was selected
based on the average urination void volume for healthy males reported in the literature.12 This
experiment was called the baseline because it was designed to mimic urea hydrolysis. All
subsequent automated lab experiments were compared with the baseline results.

A small container was attached to the top of each storage tank to hold 80 mL of effluent
urine from the urinal. The 80 mL in the container was flushed out after each urination event by
effluent urine from the most recent urination event. In the container, a conductivity probe was
held, which measured the conductivity of the effluent urine every 1 min. A small sample of
effluent urine was taken from each container every 30 min to measure pH, conductivity (for acid
treatment experiments only), calcium, and phosphate concentrations. The experiment ran for 4 h.
Each urinal had its own storage tank where urine was stored after the experiment for subsequent struvite precipitation.

**Automated Lab Experiments: Urea Hydrolysis Inhibition**

Two acids were used to inhibit the urea hydrolysis reaction: acetic acid and citric acid. A third peristaltic pump was used for the acid addition. A small dose of acid (2.5 mL) was pumped into the urinals immediately following the 20 s pumping of urine and urease solution. To understand the effects of acid concentration, three concentrations of acetic acid were tested (2500 meq/L, 5000 meq/L, and 7500 meq/L) in 1 h experiments. The 1 h experiments followed the frequency and timing schedule described above. A concentration of 2500 meq/L was selected as the concentration for the subsequent experiments based on its results (Figure 2-1). Two additional experiments were performed to test the frequency of acid addition while keeping the concentration and the volume of acid consistent (2.5 mL of 2500 meq/L acetic acid). The two additional frequencies that were tested were 20 min and 30 min intervals between acid additions while keeping the urination interval at 10 min (Figure 2-2).

The acid addition frequency of 10 min and a concentration of 2500 meq/L acid were selected. The 4 h acetic acid treatment experiment was run using the experimental design described in the baseline synthetic urine experiment above. The 4 h experiment was repeated using citric acid with the same equivalent concentration, frequency, and volume of acid addition.

**Urine Storage**

For the baseline automated lab experiment, urine was stored in the storage tank for 3 d to allow for the pH to reach 9. A storage time of 3 d was selected because it was expected that the acid treatment experiments would require several days of storage time for pH to increase, therefore both the baseline and the acid addition experiments would have similar storage times. However, the pH of the stored urine determined the length of the storage time and the acetic acid
treatment experiment effluent urine was stored for 10 d before struvite precipitation due to the rate at which the pH increased to 9. The citric acid experiment effluent urine was also stored to allow the pH to increase following the experiment. A similar storage time to the acetic acid experiment was expected, however, biological growth occurred in the stored urine and the pH did not increase to 9 over a 14 d storage time. It was decided that the citric acid treated urine was not at the conditions required for struvite precipitation and the experiment was terminated.

**Struvite Precipitation**

Struvite precipitation was performed using the stored synthetic urine for the experiments with and without acetic acid addition. Magnesium chloride salt was dosed based on the phosphate concentration in the fresh urine before each experiment began, therefore the magnesium chloride dose was the same for all three storage tanks, even if the phosphate concentrations in the stored urine differed at the time of struvite precipitation. The dose was calculated to match the molar ratio of magnesium to phosphate of 1.1:1 based on struvite precipitation experiments in the literature.$^{13, 14}$ A sample of urine was collected from the storage tank prior to magnesium chloride addition, and is referred to as Pre MgCl$_2$ addition in Table 3-2. The magnesium salt was added to each storage tank and an electric drill with paddle attachment was used to stir the urine for 10 min (1 min of rapid mixing and 9 min of slow mixing). This was performed in the three storage tanks. The mixed urine was allowed to settle for 24 h. A sample of urine was collected from the storage tank after the 24 h settling period, and is referred to as Post MgCl$_2$ addition in Table 3-2. The mixed urine was then filtered through a nylon stocking that was attached to the bottom of the conical storage tanks. The valve was then slowly opened and the mixed urine was allowed to flow through the stocking. A sample of the filtered urine, referred to Filtered Urine in Table 3-2, was collected at this point. The struvite solid collected
inside the stocking was allowed to dry under ambient laboratory conditions for up to 3 d before being weighed and sampled for further analysis.

**Demonstration Experiments Using Real Human Urine**

One of the urinals used in the automated lab experiments was used in the baseline real urine demonstration. The urinal was cleaned before it was used. A bottle of sealing liquid (89 mL) was poured into the urinal trap, as directed by the urinal manufacturer. A 18.9 L plastic container (Coleman Water Carrier) was attached to the urinal with PVC pipe and flexible tubing. Human urine was collected as described in section 2.1.2. above. Four random donors were asked to donate per day. After each donor anonymously logged the urine measurements in a dedicated notebook, they were asked to slowly pour the urine in the urinal doing their best to pour the urine for 15–20 s. The plastic collection bottles were washed with DI water and placed back in the bathrooms for the next donor. The baseline real urine demonstration was run until the storage tank was full.

A second clean urinal from the automated lab experiments was used for the demonstration experiment using real urine and acetic acid treatment. The urinal setup and collection was identical to that of the baseline real urine demonstration, with the addition of a peristaltic pump for the addition of acetic acid after the urine was poured. The concentration of the acid solution was 2500 meq/L, matching the concentration used in the automated lab experiments using synthetic fresh urine with acetic acid addition. The dose of acid, 3.2 mL per urination event, was calculated by using the ratio of synthetic urine to acetic acid (237 mL:2.5 mL) and the average void volume in the baseline real urine demonstration (301 mL). Urine was collected from 10 am–4 pm on Monday–Friday for 3 weeks, except for two Mondays that fell on a national holiday and on a day with a tropical storm warning.
Struvite precipitation was done twice using real human urine collected from the demonstration experiments. The struvite precipitation method described above for the automated lab experiments was used with real human urine from the demonstration experiments. The dose of magnesium chloride was determined using the phosphate concentration in the stored urine collected from the demonstration experiments immediately before the struvite precipitation was performed. The urine was mixed for 10 min and it was allowed to settle for 24 h before filtering. The filtered urine was collected and stored for 3 d before the struvite precipitation method was repeated.

**Analytical Methods**

The pH of synthetic and real fresh urine, Pre MgCl₂ addition urine, Post MgCl₂ addition urine, and filtered urine were measured using a Accumet AB15 pH Meter (calibrated to pH 4, 7, 10 before use). The conductivity for the baseline synthetic urine experiment was measured every 1 min with a conductivity probe (Atlas Scientific Conductivity K 1.0 Kit) which was wired and connected to an Arduino Uno. The code used a three-point calibration curve: dry, low (12,800 µS), and high (80,000 µS). After storage, struvite deposits that formed on the sensing module did not dissolve completely, causing the probes to malfunction when they were being calibrated for the next experiment, therefore the conductivity for the treatment experiments was measured using a Thermo Scientific Orion Star A212 Conductivity Meter (calibrated to 1413 µS/cm, 14,000 µS/cm, and 50,000 µS/cm before use). The calcium concentration in the synthetic urine samples was measured using a Thermo Scientific Orion Dual Star pH/ISE Benchtop with a Thermo Scientific Orion Combination Calcium Electrode (calibrated to 0.5 and 10 mmol/L Ca²⁺ before use). All samples were filtered prior to phosphate analysis using 0.45 µm nylon syringe filters (Environmental Express). Phosphate was measured following Standard Method 4500-P ascorbic acid method using UV absorbance on a U-2900 UV–visible spectrophotometer (Hitachi
High Technologies) and 1 cm quartz cuvette at a wavelength of 880 nm. Samples were diluted with DI water as necessary to fall within the calibration curve of 0–1.2 mg P/L.

**Data Analysis**

The data were analyzed using the analysis of variance (ANOVA) methodology to determine variance between data sets. One-way ANOVA tests were conducted using StatPlus (AnalystSoft, Walnut, CA) to determine if the means of measurements taken during the automated lab experiments were significantly different (P < 0.05). ANOVA tests were conducted for conductivity, phosphate, and calcium concentrations in effluent synthetic urine. If the ANOVA results showed that a significant difference existed, Tukey’s Honest Significant Difference (HSD) post-hoc test was performed to determine which treatments were significantly different. Percent phosphate recovery was calculated by dividing the concentration of phosphate in the filtered urine samples by the phosphate concentration in the Pre MgCl₂ samples. Total phosphate recovery was calculated by dividing the phosphate concentration in the Pre MgCl₂ samples by the phosphate concentrations in the fresh urine samples and multiplying it by the percent phosphate recovery explained above.
Table 2-1. Synthetic urine composition.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration</th>
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<tr>
<td>CH₂N₂O (urea), mmol/L as N</td>
<td>500</td>
</tr>
<tr>
<td>NaCl, mmol/L</td>
<td>44</td>
</tr>
<tr>
<td>Na₂SO₄, mmol/L</td>
<td>15</td>
</tr>
<tr>
<td>KCl, mmol/L</td>
<td>40</td>
</tr>
<tr>
<td>NaH₂PO₄, mmol/L as P</td>
<td>20</td>
</tr>
<tr>
<td>MgCl₂·6H₂O, mmol/L</td>
<td>4</td>
</tr>
<tr>
<td>CaCl₂·2H₂O, mmol/L</td>
<td>4</td>
</tr>
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Figure 2-1. Experimental design for the automated lab experiments.
Figure 2-2. Conductivity of effluent urine after being treated with 2500 meq/L, 5000 meq/L, and 7500 meq/L acetic acid. Synthetic fresh urine and urease were added every 10 min. Acetic acid was added every 10 min directly after the urine and urease.

Figure 2-3. Conductivity of effluent urine after being treated with acetic acid in 10 min, 20 min, and 30 min intervals. Synthetic fresh urine and urease were added every 10 min.
CHAPTER 3  
RESULTS AND DISCUSSION

Mimicking Urea Hydrolysis

The first objective of this project was to mimic the urea hydrolysis reaction using synthetic fresh urine and jack bean urease solution in real waterless urinals. The pH and conductivity measurements in urine exiting the urinals were used as surrogates to determine if the urea hydrolysis reaction was proceeding in the forward direction. Udert et al. (2003) simulated the urea hydrolysis reaction by tracking the pH of samples of real fresh urine within the first 288 min after urine entered the collection system, and observed that pH increased from approximately 6 in fresh urine to 9 in hydrolyzed urine. Conductivity has also been used to track the urea hydrolysis reaction because the conversion of neutral urea molecules to charged ammonia and bicarbonate species increases the conductivity of the solution. Figure 3-1a shows the pH and conductivity of the urine exiting the waterless urinals over the 4 h baseline automated lab experiment using synthetic fresh urine. The pH results are shown individually for each urinal with the pH measurement at 0 min corresponding to synthetic fresh urine. The pH steadily increased during the 4 h experiment with all three urinals showing a maximum pH around 8.5 to 8.6, which is indicative of hydrolyzed urine, from an initial starting pH of 6. For two of the urinals, the urine exiting the urinals reached its maximum pH of 8.6 at the final time mark of 240 min whereas the third urinal reached a maximum pH of 8.5 before decreasing to pH 7.6 at 240 (the reason for this decrease was not known). The conductivity results are shown as the mean of triplicate samples with error bars showing one standard deviation. Because the cleaning procedure resulted in DI water remaining in the urinal trap and conductivity was recorded every minute, the initial conductivity measurements over the first 30 min showed a dilution effect. Starting at approximately the 30 min mark, the conductivity of urine exiting the urinals was
approximately equal to the conductivity measured in synthetic fresh urine before the start of the experiment (indicated by the horizontal line labeled Cond \( t = 0 \) in the legend). After 30 min, the conductivity of urine exiting the urinals increased above the initial conductivity in synthetic fresh urine, which is indicative of urea hydrolysis and formation of charged ammonia and bicarbonate species. At approximately 170 min until the end of the experiment the conductivity results show greater variability than the earlier results from 30 to 170 min. Precipitation of phosphate minerals (discussed below) might have caused the variability in conductivity measurements. For all three urinals, the urine exiting the urinals reached conductivity between 16.7 and 17.4 mS/cm after 4 h. The measurements shown in Figure 3-1a are consistent with those published in previous studies on urea hydrolysis in real human urine,\(^6,\)\(^17\) and support the abiotic approach pursued in this work to mimic the natural urea hydrolysis process observed in urine diversion systems.

Figure 3-2 shows the concentration of phosphate and Figure 3-3 shows the concentration of calcium measured every 30 min in urine exiting the waterless urinals in the baseline automated lab experiments using synthetic fresh urine. Both phosphate and calcium data show the mean concentration of triplicate samples with error bars showing one standard deviation. The phosphate concentrations decreased from 14.6 mmol/L in synthetic fresh urine to 4.2 mmol/L in the first 30 min, and remained below 6 mmol/L until the last two measurements at 210 and 240 min. Overall, the results in Figure 3-2a show that the phosphate concentration in urine exiting the waterless urinals was 45 to 73% lower than the phosphate concentration in synthetic fresh urine entering the urinals. The precipitation of phosphate minerals is the likely cause of the reduction in phosphate concentration in urine exiting the urinals. Considering the chemical species present in human urine (calcium, magnesium, and phosphate) in combination with urea hydrolysis creates solutions conditions (pH 8 to 9 and high ammonia concentration) that result in
supersaturated conditions for phosphate minerals such as struvite and hydroxyapatite and hence precipitation. This is further explored below after considering the calcium data. The large error bars on some of the phosphate samples (e.g., ±3 mmol/L) indicates that there was a combination of experimental (i.e., urinal-to-urinal) and analytical (i.e., phosphate measurement) variability during the experiment. Considering all analytical measurements made, it is likely that the variability in Figure 3-2a is mostly due to phosphate analysis. Nevertheless, there was a measureable reduction in the phosphate concentration in urine exiting the waterless urinals.

The calcium concentration decreased from 3.1 mmol/L in synthetic fresh urine to 0.24 mmol/L in the first 30 min, and then remained below 0.40 mmol/L for all subsequent samples. Similar to the phosphate results, calcium was being lost from solution between the synthetic fresh urine entering and exiting the urinals. As described in the previous paragraph, the most likely explanation is precipitation of calcium phosphate and magnesium phosphate minerals, such as hydroxyapatite and struvite, that remain in the urinal trap.

Although the concentration of magnesium in urine was not measured in this study, the amount of magnesium that was precipitated and lost in the urinal can be estimated by assuming that loss of calcium was due solely to hydroxyapatite (HAP, Ca$_5$(PO$_4$)$_3$(OH)) precipitation and loss of magnesium was due solely to struvite (NH$_4$MgPO$_4$$\cdot$6H$_2$O) precipitation. Using a reduction in calcium concentration of 2.8 mmol/L from Figure 3-3, the corresponding loss of phosphate is 1.7 mmol/L. Assuming 4 mmol/L of magnesium in synthetic fresh urine (based on urine recipe) and complete loss of magnesium as struvite, 4 mmol/L of phosphate would be removed from solution. Using the measured initial concentration of phosphate in synthetic fresh urine (14.6 mmol/L, Fig. 3a), the estimated phosphate concentration in urine exiting the urinals
due to precipitation of calcium as hydroxyapatite and magnesium as struvite would be 8.92 mmol/L of phosphate, which is in fair agreement with the results in Figure 3-2.

**Inhibiting Urea Hydrolysis**

The second objective of this research was to inhibit the urea hydrolysis reaction by in situ chemical addition to urine in the waterless urinals. Based on other work by the authors Ray et al (2016), acid addition was found to be effective technique to inhibit urea hydrolysis. Ultimately, acetic acid and citric acid were selected because of their effectiveness in inhibiting the urea hydrolysis reaction and their use as active ingredients in consumer cleaning products. Figure 3-1b and 3-1c show the results for the pH and conductivity of the urine exiting the waterless urinals after being treated with acetic acid or citric acid, respectively, after each urination event. The results are shown in a similar manner as Figure 3-1a where pH measurements are shown separately for each urinal and conductivity measurements are the mean of triplicate samples. The conductivity measurements plotted in Figure 3-1b and 3-1c were for samples taken every 30 minutes due to the change in the instrument used to measure conductivity. In Figure 3-1b and 3-1c, the first point at t = 0 min was the measured conductivity of the fresh urine, while in Figure 3-1a, the conductivity measurements between t = 0 and t = 30 min were the conductivity measurements of water slowly mixing with urine entering the urinal trap. Both acid addition treatments lowered the pH of the urine exiting the urinals. With the acetic acid addition, the pH decreased from pH 5.9 in synthetic fresh urine to pH 3.9 to 5.1 in urine exiting the urinals with pH remaining relatively consistent for each urinal. Similarly, with the citric acid addition, the pH decreased from pH 5.8 in synthetic fresh urine to pH 3.0 to 3.6 in urine exiting the urinal with consistent pH values for each urinal. The lower pH of urine treated with citric acid than acetic acid was because citric acid has three carboxyl functional groups that deprotonate at a pKa of 3.13, while acetic acid only has one carboxyl functional group and a pKa of 4.76. For the
addition of acetic acid or citric acid, the conductivity of urine exiting the urinals remained essentially unchanged (<5% variation for acetic acid, <6% variation for citric acid) from the conductivity of fresh urine entering the urinals. Together, the pH and conductivity results indicate that the urea hydrolysis reaction in synthetic fresh urine was inhibited for the sequence of concurrent urine and urease addition immediately followed by acid addition.

The phosphate concentrations in urine exiting the waterless urinals for acetic acid and citric acid addition is shown in Figure 3-2. The phosphate concentration in synthetic fresh urine used for the acetic acid addition experiment was 16.5 mmol/L. The phosphate concentrations decreased below 9 mmol/L in the first measurement taken after 30 min, however, all subsequent measurements were between 12–14 mmol/L. The phosphate concentration in the synthetic fresh urine used for the citric acid addition experiment was 20.1 mmol/L. The mean phosphate concentration from samples collected from 30 min through 240 min was 13.4 mmol/L but the variation from urinal-to-urinal was high with standard deviation > 5 mmol/L. For example, the minimum phosphate concentration measurement taken from urinal 2 was 17.7 mmol/L at 210 min, while the minimum phosphate concentrations for urinal 1 and urinal 3 were 3.65 mmol/L and 9.36 mmol/L at 180 min. Unlike the pH and conductivity results detailed above, where the pH had the smallest range of measurements when compared to the other two experiments, the phosphate concentrations were not consistent from urinal to urinal.

In terms of calcium concentration in urine exiting the waterless urinals (Figure 3-3), the acetic acid and citric acid addition experiments showed similar results. The initial calcium concentration for the acetic acid addition experiment was 2.1 mmol/L and decreased to 0.8 mmol, a reduction of 62%. The initial calcium concentration for the citric acid addition experiment was 1.3 mmol/L and decreased to 0.7 to 0.8 mmol/L, a reduction of 38%. Both acetic
acid addition and citric acid addition resulted in more calcium remaining in solution on a percentage basis and absolute concentration. Together the phosphate and calcium results indicate that less precipitation (presumably calcium and magnesium phosphates) occurred in the synthetic urine experiments with acid addition than without acid addition. However, the pH and conductivity results for the synthetic urine experiments with acid addition imply that urea hydrolysis was completely inhibited so losses of calcium and phosphate could have occurred in the tubing leading to the urinals. At pH 6, HAP and struvite can precipitate at small amounts due to supersaturation of calcium and magnesium. It could be conceivable that HAP and struvite precipitated in the tubing prior to entering the urinals and it could be the losses seen in Figure 3-2 and 3-3 even though urea hydrolysis was inhibited. Overall, comparison of experiments using synthetic fresh urine with and without acid addition show that addition of acetic acid or citric acid after urine and urease was able to halt or inhibit urea hydrolysis and in turn limit precipitation of phosphate minerals.

ANOVA tests were conducted for the conductivity, phosphate, and calcium concentrations and results are shown in Table 3-1. The results showed that there exists a significant difference between at least two of the three treatments (baseline, acetic acid treatment, and citric acid treatment) for conductivity and phosphate concentrations, however there was no significant difference in calcium concentrations between any of the three treatments. For conductivity, the Tukey’s HSD post-hoc tests showed that there was a significant difference between the baseline and the citric acid treatment experiment, but there was no significant difference between any other pair of treatments. For phosphate concentrations, the Tukey’s HSD post-hoc tests showed that there was significant difference in concentrations between the baseline and the acetic acid treatment experiment and also between the baseline and
the citric acid treatment experiment. However, there was no significant difference in phosphate concentrations between the acetic acid and citric acid treatment experiments.

Demonstration of Urea Hydrolysis, Urea Hydrolysis Inhibition, and Phosphorus Recovery Using Real Human Urine

The fourth objective of this project was to demonstrate the results found using synthetic fresh urine in the automated lab experiments with real human urine. Figure 3-4 shows the pH and conductivity of the fresh human urine samples and the tank composite measurements as a function of urination event for the baseline and acetic acid addition experiments. The pH of each urination event is shown as a single point as a function of urination events. The pH of the fresh urine donations fell within the same range (pH 5–7) between the two experiments, which is the reported range for fresh human urine from Putnam (1971). A composite sample taken from the storage tank was used to determine the extent of urea hydrolysis. The first pH tank measurement taken in the baseline experiment was 8.5 after 20 urination events, compared with the same measurement in the acetic acid experiment being pH 4.5. The final pH measurement taken of the stored urine after acetic acid treatment was 5.6, compared with 8.8 in the baseline experiment. The acetic acid treatment inhibited the pH of the urine in the tank from increasing past the pH of the fresh urine samples.

Unlike the pH measurements of the fresh urine, the conductivity of fresh urine is highly dependent on time of donation, hydration, and diet unique to each donor. In the data collected for the real human urine experiments, void volume was the best determinant of conductivity in fresh urine samples. The conductivity measurement was higher in low void volume samples and lower in samples with a high void volume. Figure 3-4c and 3-4d shows the results for conductivity in a similar manner as the pH results described above. The conductivity measurements of the fresh urine samples fell between a range of 3.80–34.5 mS/cm (mean 14.1 ±
7.90 mS/cm) and 2.13–28.6 mS/cm (mean 12.2 ± 7.32 mS/cm) for the baseline and acetic acid treatment experiments respectively. Nonetheless, the conductivity measurements of the urine storage tank showed that the conductivity increased for the baseline experiment, and increased at a reduced rate for the acetic acid treatment experiment. After 20 urination events, the conductivity of the composite sample was 12.6 mS/cm and 9.69 mS/cm for the baseline and acetic acid treatment experiments respectively. The final conductivity measurement in the tank for the baseline experiment was 20.2 mS/cm, compared with 12.1 mS/cm for the acetic acid treatment experiment, which is an increase of 38% in the baseline experiment and 20% in the acetic acid treatment experiment. A conclusion can be drawn that urea hydrolysis was inhibited with the acetic acid treatment in the storage tanks based on these results and the results shown in the synthetic urine experiments. Drawing a conclusion on the urea hydrolysis inhibition inside the urinal trapway was more difficult to form because measurements of the effluent urine were not taken. The composite tank samples were the only way to track the urea hydrolysis reaction. Nevertheless, it was evident that the pH and conductivity were lower in the storage tank that received acetic acid treatment than in the storage tank that received no treatment.

Phosphorus recovery, via struvite precipitation, was performed with the stored human urine to compare the phosphate recovery potentials for the baseline and acetic acid treatment demonstration experiments. The results from the struvite precipitation tests can be seen in Table 3-3. The struvite precipitation method was repeated for each batch of urine after lower phosphorus recovery efficiencies were calculated than in the synthetic urine experiments and previous literature. It is noteworthy that the phosphate concentration of the fresh urine samples was not measured and therefore total phosphate recovery was impossible to calculate. However, we can still compare the Pre MgCl₂ concentrations and the final concentrations. The first test
only recovered 11% and 27% of the phosphate available for the baseline and acetic acid experiments respectively. On the second baseline test, 78% of the available phosphate was recovered, equaling 83% of the phosphate available if the amount recovered in the first test was summed to the amount recovered in the second test. Similarly, the amount of phosphate recovered increased on the second test for the acetic acid treated urine. The amount recovered in the second test was 54%, equaling to 67% of the available phosphate. The lower phosphate recovery percentages found in the real urine experiments when compared with the results from the synthetic urine experiments can be attributed to the greater complexity of the real urine that was not present in the synthetic urine. More specifically, the synthetic fresh urine did not contain endogenous metabolites such as creatine, lysine, L-cysteine, and taurine.\textsuperscript{21} Published literature has shown that metabolites can have inhibiting effects when precipitating struvite, as the organic compounds bond with the forming struvite crystals and inhibit the growth of the crystals.\textsuperscript{22,23} The difference in phosphate recovery between the baseline and the acetic acid experiment can be due to the addition of an organic acid that has the potential to hinder the crystallization of the precipitates.

A difference in concentrations was also seen between the synthetic urine and the real urine struvite precipitation results. A higher concentration of phosphate was expected in the acetic acid treated real urine before struvite precipitation, however that was not the case. The urine collected in the baseline experiment had a concentration of 3.7 mmol/L PO$_4$, which was 0.3 mmol/L PO$_4$ higher than the concentration in the acetic acid treated urine. Comparatively, the acetic acid synthetic urine from the automated experiments had a concentration of phosphate 63% higher than the baseline synthetic urine. The similarity in phosphate concentrations in the real urine storage tanks could be attributed to phosphate loss due to biological uptake of
phosphate. The researchers observed unknown microorganisms in the acetic acid urine storage tank that were not present in the baseline urine storage tank. As will be discussed below, the treatment with acetic acid added considerable amounts of COD to the human urine. The combination of high COD, available phosphate, and pH could have formed favorable conditions for the growth of microorganisms.

The future applications and implications of the technique to inhibit urea hydrolysis described above is explored in the following paragraphs. In public restrooms with high frequency of use, such as an airport, a small, automated dispenser with an infrared sensor would release a small doses of acid on a timer or by sensing the use of each urinal. This would reduce the amount of maintenance needed because the dispenser would only need to be refilled on a periodic basis, while the acid treats the fresh urine entering each urinal. However, as seen in the real urine demonstration, there could be impacts of applying the same dose or concentration to all urination events, as each event can vary in volume and can lead to the over application of acid (Figure 3-5 and Table 3-4), which can have economic and environmental impacts. The addition of acetic acid would also add a considerable amount of COD into the stored urine. Fresh urine has a COD between 6.3–10.6 g/L, which is ten times more concentrated than high strength domestic wastewater (0.8 g/L COD). Based on the additions described above, the acetic acid addition would add approximately 1.70 g/L of additional COD because 1 g of acetic acid accounts for 1.07 g of COD, which enhances the strength of an already high strength wastewater. The high concentrations of COD could be problematic for further treatment. However, the added COD could be beneficial if the process described above were to be coupled with a microbial fuel cell (MFC) for energy generation and COD reduction. MFCs have been shown to reduce the COD concentrations in waste streams with high COD, such as swine waste.
Overall, inhibiting the precipitation of calcium and magnesium crystals can reduce the amount of scale formation that leads to clogging and odor problems. Reducing the maintenance needed will keep waterless urinals installed, which can have large water conservation impacts and will aid in counteracting the reputation that was established due to poorly designed fixtures. Potable water savings alone can be a rewarding motivation for the installation of functional waterless urinals. For instance, the installation of waterless urinals in the Frankfurt Airport saved 4.2 million liters of potable water per year.\textsuperscript{28}

The second benefit comes when waterless urinals are coupled with nutrient recovery from urine. Inhibiting the urea hydrolysis reaction can prevent the precipitation of value nutrients, such as struvite and hydroxyapatite. Keeping their constituents in solution can increase the efficiency of nutrient recovery in the desired treatment location. The results above showed that approximately 40\% more phosphate was recovered when urea hydrolysis was inhibited in the urinal. Because the focus of this work was to target the urea hydrolysis reaction in fixtures that do not use flushing water, it is highly possible that the results could be translated to bathroom fixtures with similar characteristics. Urine-diverting toilets use similar plumbing mechanisms as waterless urinals to flush urine without the use of potable water and are usually coupled with waterless urinals in real world urine source separation systems.
Figure 3-1. pH and conductivity of effluent urine for (a) baseline, (b) acetic acid, and (c) citric acid treatment automated lab experiments. pH is expressed for each urinal, which pH at t = 0 being the pH of fresh urine. Conductivity is expressed as the average of the three urinals with error bars showing one standard deviation. For the baseline, conductivity was measured every 1 min and points between t = 0 and 30 min were the dilution effect. For acetic and citric treatment, the conductivity was measured every 30 min and the conductivity at t = 0 was the conductivity of fresh urine.
Figure 3-2. Phosphate concentrations for the baseline, acetic acid, and citric acid automated lab experiments. Measurements were taken every 30 min and are expressed as the average concentration for the three urinals with error bars showing one standard deviation. The phosphate concentration at $t = 0$ was the concentration of fresh urine.
Figure 3-3. Calcium concentrations for the baseline, acetic acid, and citric acid automated lab experiments. Measurements were taken every 30 min and are expressed as the average concentration for the three urinals with error bars showing one standard deviation (coefficient of variance > 0.4 for all points). The calcium concentration at t = 0 was the concentration of fresh urine.

Table 3-1. ANOVA one-tailed test (95% confidence interval) results for conductivity, phosphate, and calcium concentrations for measurements taken during the automated lab experiments (B is baseline, A is acetic acid treatment, and C is citric acid treatment). Tukey’s HSD post-hoc tests were used when ANOVA tests resulted in there being a significant difference. c. Tukey’s HSD critical value for conductivity = 1.717. Tukey's HSD critical value for phosphate = 3.452. Comparison is significantly different if the difference of means is greater than the critical value.

<table>
<thead>
<tr>
<th>Test</th>
<th>ANOVA Results</th>
<th>Tukey's HSD Post-hoc test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity</td>
<td>F(2, 23) = 3.116, p = 0.043</td>
<td>B to A diff = 0.711</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B to C diff = 1.284</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A to C diff = 0.573</td>
</tr>
<tr>
<td>Phosphate</td>
<td>F(2, 23) = 19.604, p = 1.07E-05</td>
<td>B to A diff = 7.123</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B to C diff = 7.953</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A to C diff = 0.830</td>
</tr>
<tr>
<td>Calcium</td>
<td>F(2, 23) = 1.772, p = 0.192</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Table 3-2. Phosphate concentrations of fresh, Pre MgCl\(_2\), Post MgCl\(_2\), and filtered urine for the baseline and acetic acid automated lab experiments. Magnesium chloride was added to the stored synthetic urine to recover phosphate by struvite precipitation.

<table>
<thead>
<tr>
<th>Test</th>
<th>Fresh Urine mmol/L PO(_4)</th>
<th>Pre MgCl(_2) Addition mmol/L PO(_4)</th>
<th>Post MgCl(_2) Addition mmol/L PO(_4)</th>
<th>Filtered Urine mmol/L PO(_4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea Hydrolysis</td>
<td>14.6</td>
<td>4.5 ± 2.1</td>
<td>0.012 ± 0.004</td>
<td>0.006 ± 0.004</td>
</tr>
<tr>
<td>Acetic Acid Addition</td>
<td>16.5</td>
<td>12.2 ± 1.9</td>
<td>0.060 ± 0.011</td>
<td>0.069 ± 0.012</td>
</tr>
</tbody>
</table>

Figure 3-4. pH and conductivity of fresh urine samples and composite storage tank samples for baseline (a, c) and acetic acid treatment (b, d) demonstration experiments.
Table 3-3. Phosphate concentrations of Pre MgCl$_2$, Post MgCl$_2$, and filtered urine for the baseline (B) and acetic acid treatment (AA) demonstration experiments. Magnesium chloride was added to the stored human urine to recover phosphate by struvite precipitation. The method was repeated in order to increase the percent of phosphate recovered.

<table>
<thead>
<tr>
<th>Test</th>
<th>Pre MgCl$_2$ Addition mmol/L PO$_4$</th>
<th>Post MgCl$_2$ Addition mmol/L PO$_4$</th>
<th>Filtered Urine mmol/L PO$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>B test 1</td>
<td>3.7 ± 0.03</td>
<td>3.3 ± 0.0</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>B test 2</td>
<td>2.8 ± 0.1</td>
<td>0.61 ± 0.04</td>
<td>0.63 ± 0.03</td>
</tr>
<tr>
<td>AA test 1</td>
<td>3.3 ± 0.05</td>
<td>2.4 ± 0.05</td>
<td>2.1 ± 0.03</td>
</tr>
<tr>
<td>AA test 2</td>
<td>2.4 ± 0.01</td>
<td>1.1 ± 0.05</td>
<td>1.1 ± 0.02</td>
</tr>
</tbody>
</table>

Figure 3-5. Volume of urination events for the (a) baseline and (b) acetic acid treatment demonstration experiments. The orange line shows the average void volume for each demonstration experiment. The red line shows the void volume used in the automated lab experiments.

Table 3-4. Total volume collected, average urination volume, and total volume of acid added for the baseline and acetic acid treatment demonstration experiments.

<table>
<thead>
<tr>
<th>Test</th>
<th>Total volume collected, L</th>
<th>Average urination volume, mL</th>
<th>Total volume acid added, mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>16.83</td>
<td>301 ± 148</td>
<td>–</td>
</tr>
<tr>
<td>Acetic acid addition</td>
<td>15.97</td>
<td>301 ± 169</td>
<td>169.6</td>
</tr>
</tbody>
</table>
Urea hydrolysis was mimicked in waterless urinals using synthetic urine and realistic bathroom conditions, specifically urine void volume and urine frequency. Acetic and citric acid treatment inhibited the urea hydrolysis reaction in situ by lowering the pH of the urine in the trapway. Conductivity, phosphate, and calcium concentrations were higher in the effluent urine when treated with the acids, further proving that urea hydrolysis was inhibited and subsequent precipitation reactions were reduced. Total phosphate recovery, via struvite precipitation, was higher in the synthetic urine treated with acetic acid than in the urine that received no treatment. Urea hydrolysis was mimicked in the waterless urinals using real human urine. When using real human urine, acetic acid treatment inhibited the urea hydrolysis reaction in the storage tanks, but future work is needed to better understand the inhibition that occurred in the waterless urinals.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Daniella Saetta earned a Bachelor of Science degree in environmental engineering sciences from the University of Florida in December 2014. In the summer of 2012, she began working under the guidance of Treavor Boyer as an undergraduate research assistant. Her undergraduate research project entailed performing a life cycle assessment of a small, coastal drinking water treatment plant facing saltwater intrusion in Cedar Key, FL. The results from that study were published in the Journal of American Water Works Association and won the 2016 Engineering & Construction Division Best Paper Award awarded by the American Water Works Association. Her interest in pursuing a graduate degree stemmed from her pleasant experience as an undergraduate researcher. She began pursuing her Master of Science degree in January 2015. Upon graduation in December 2016, Daniella will begin pursuing a PhD in environmental engineering at Arizona State University.