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# Mimicking and Inhibiting Urea Hydrolysis in Nonwater Urinals

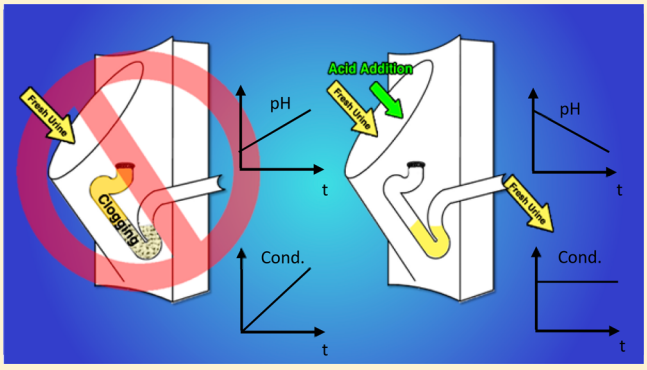
Daniella Saetta*,†,‡ and Treavor H. Boyer‡

†Department of Environmental Engineering Sciences Engineering School of Sustainable Infrastructure & Environment (ESSIE), University of Florida, P.O. Box 116450, Gainesville, Florida 32611-6450, United States

‡School of Sustainable Engineering and the Built Environment (SSEBE), Arizona State University, P.O. Box 873005, Tempe, Arizona 85287-3005, United States

Supporting Information

**ABSTRACT:** Nonwater urinals are critical in the implementation of building-scale water conservation and urine diversion systems. However, because of the composition of urine and the prevalence of the urease enzyme that hydrolyzes urea, minerals readily precipitate in nonwater urinals and pipes. This leads to clogging, malodor, and possible replacement of nonwater urinals with flush urinals. Accordingly, the goal of this research was to provide an improved understanding of the urea hydrolysis process in nonwater urinals to benefit water conservation and phosphate recovery efforts. Acetic acid addition was used in nonwater urinals to inhibit the urea hydrolysis reaction by lowering the pH, thereby making the precipitation of calcium- and magnesium-containing minerals less favorable. Of the acids tested, 2.5 mL of 2500 mequiv/L acetic acid added after every urination event was able to inhibit urea hydrolysis in synthetic urine and real urine as indicated by the pH and conductivity of the effluent urine. Acid addition also allowed for 43% more phosphate recovery via struvite precipitation in the acetic acid addition synthetic urine than the synthetic urine with no acid addition.




## INTRODUCTION

Nonwater urinals, which do not require water flushing, are used in commercial buildings to conserve potable water.[1] However, the urea hydrolysis reaction in urine creates conditions in nonwater urinals that lead to clogging and odors, which are uninviting for users. Furthermore, malfunctional nonwater urinals have been replaced with flush urinals across the U.S. because of clogging, odor, and incompatibility with copper pipes.[2−4] Hence, improving the function of nonwater urinals would reduce maintenance requirements and thereby keep nonwater urinals installed and operational, which would benefit water conservation and create a positive view of water conserving fixtures such as nonwater urinals.[5,6] Urine diversion has been proposed as an alternative wastewater management process that addresses the excess nutrients and pharmaceuticals that enter the environment as a result of inconsistent removal at conventional wastewater treatment facilities.[7,8] Diverting urine by using nonwater urinals and urine-diverting toilets (UDTs) has the ability to conserve potable drinking water and reduce the environmental impact of wastewater treatment by reducing the amount of potable water demand in the sewershed.[9] Life cycle assessments on urine diversion scenarios have indicated that urine diversion has a lower environmental impact and similar economic cost when compared with conventional wastewater treatment.[9,10] Unlike centralized wastewater treatment, where many stakeholders have limited interaction with daily operation, urine diversion breaks the barrier between the user and the wastewater treatment system.[11] Importantly, the sustained operation of a urine diversion system is largely dictated by the performance of the separation and collection components of the system.[5,11−14]

The first component in a building-scale urine diversion system are the nonwater urinals and UDTs, which are used to collect urine at the point of generation.[13,15,16] The urine is conveyed in urine-only piping to a storage tank in the building where it can be processed onsite or transported offsite.[15,17] During conveyance the composition of urine changes because of urea hydrolysis, causing minerals to precipitate in the fixtures and pipes.[12,18] Urea hydrolysis is the chemical reaction catalyzed by the urease enzyme that converts urea into ammonia and bicarbonate as follows:[19]

$$NH_2(CO)NH_2 + 3H_2O \rightarrow 2NH_4^+ + HCO_3^- + OH^-$$

The urease enzyme is produced by a wide range of organisms, from eukaryotes to prokaryotes, and is most active as the bacterial urease in wastewater collection systems.[18,19] The addition of ammonia, bicarbonate, and hydroxide via urea hydrolysis raises the pH of fresh urine from pH 6 to pH 9 and

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creates supersaturated conditions for precipitation of phosphate minerals, such as struvite ($NH_4MgPO_4 \cdot 6H_2O$) and hydroxyapatite ($Ca_5(PO_4)_3(OH)$).[18,20,21] A study on scale formation on conventional flush toilets bowls found that 65% of the scale was due to the precipitation of struvite, calcium phosphate, and calcium carbonate, with the remaining 45% of scale being organic-based solids.[22] Research on urine diversion systems has indirectly investigated urea hydrolysis by studying the rate, extent, and type of minerals that precipitate in urine.[18,23,24] Previous research that has directly investigated the urea hydrolysis reaction and its inhibition is mostly from soil science and microbiology.[25−27] According to Ray et al., urea hydrolysis inhibitors most commonly found in soil science literature were not able to inhibit urea hydrolysis in urine due to the presence of competing ions.[28] Hence, there is a gap in the literature between the impacts of urea hydrolysis in real-world systems and controlled studies on urea hydrolysis. Using the identified gap in urea hydrolysis research, a novel approach was taken that for the first time sought to study the urea hydrolysis reaction and its inhibition in real nonwater urinals operating under realistic conditions of urination volume and intermittent use. Furthermore, the approach of urea-hydrolysis-in-nonwater-urinals was conducted using synthetic urine and real urine to provide both controlled conditions and realistic insights. The impact of the approach is 2-fold. Understanding urea hydrolysis as it occurs in nonwater urinals will maintain and enhance water conservation in traditional plumbing systems and will enable nutrient recovery in promising urine diversion systems.

The goal of this research was to provide an improved understanding of the urea hydrolysis process in nonwater urinals. The overall approach was to conduct the experiments under realistic conditions using real nonwater urinals and mimicking human use. Results from experiments using synthetic urine were used to determine the conditions for subsequent experiments using real urine. The specific objectives of this research were to (i) mimic urea hydrolysis in nonwater urinals using synthetic urine and urease addition, (ii) inhibit urea hydrolysis in nonwater urinals using synthetic urine, urease addition, and acid addition, (iii) compare phosphate recovery from synthetic urine post-hydrolysis both with and without acid addition, and (iv) demonstrate the results for urea hydrolysis, urea hydrolysis inhibition, and phosphate recovery using real urine.

## ■ MATERIALS AND METHODS

**Materials.** Synthetic urine and jack bean urease (EC 3.5.1.5, Fisher Scientific) were used in all synthetic urine experiments. Real urine was collected from 12 healthy adults for the real urine experiments. Real urine collection was approved as exempt by the University of Florida Institutional Review Board. Kohler Steward Waterless Urinals were used for the entire study. Details on the synthetic urine recipe and composition, real urine collection, nonwater urinal characteristics, analytical methods, and data analysis are described in Supporting Information (SI).

**Mimicking Urea Hydrolysis Using Synthetic Urine.** Three identical synthetic urine experiments were conducted as follows. The first synthetic urine experiment was the urea hydrolysis experiment, in which urea hydrolysis was mimicked in the urinals. The three urinals were connected to two pumps: a high-performance peristaltic pump for delivery of synthetic urine to the urinals and a peristaltic pump for delivery of urease solution to the urinals (Figure S1). The pumps were programed to repeat one urination event over a set period of time. A urination event occurred every 10 min. Urination events consisted of 237 mL of synthetic urine and 2.5 mL of the urease solution concurrently pumped into the urinals for 20 s. Jack bean urease was added in order to catalyze the reaction abiotically. The urination volume was selected based on the average urination volume for healthy males reported in the literature.[29]

A small container was attached to the top of each storage tank to hold 80 mL of effluent urine from the urinal, where effluent urine is used to denote the urine that exits the urinal after every urination event. The 80 mL in the container was flushed out after each urination event by effluent urine from the most recent urination event. A conductivity probe measuring the conductivity every 1 min was held in the container (data only shown for 30 min readings). A small sample of effluent urine was taken from each container every 30 min to measure pH, conductivity (for acid addition experiments only), calcium, and phosphate. The experiment ran for 240 min. Each urinal had its own storage tank where urine was stored after the experiment for subsequent struvite precipitation.

**Inhibiting Urea Hydrolysis Using Synthetic Urine.** Two acids were used to inhibit the urea hydrolysis reaction: acetic acid ($CH_3COOH$, Fisher Chemicals) and citric acid ($C_6H_8O_7$, Fisher Chemicals) based on work by Ray et al.[28] 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 synthetic urine and urease solution (see Figure S1). Acid addition occurred at a frequency of 10 min with a concentration of 2500 mequiv/L acid. The two urea hydrolysis inhibition experiments followed the experimental design described in the Mimicking Urea Hydrolysis Using Synthetic Urine subsection above.

**Phosphate Recovery Using Synthetic Urine.** Because favorable precipitation of struvite occurs at pH 9, urine was stored until it reached pH 9, which was reached in 3 d for the synthetic urine collected from the urea hydrolysis experiment and 10 d for the synthetic urine collected from the urea hydrolysis inhibition (acetic acid) experiment.[23] The storage time was based on pH only and did not consider requirements for building storage. Biological growth was observed in the synthetic urine collected from the urea hydrolysis inhibition (citric acid) experiment and the pH did not exceed pH 7, therefore the experiment was terminated. The biological growth was not identified or tested beyond this point.

Struvite precipitation was performed using the collected synthetic urine from the experiments with and without acetic acid addition. Magnesium chloride ($MgCl_2 \cdot 6H_2O$, Fisher Chemicals) salt was dosed based on the phosphate concentration in the synthetic urine at the start of each experiment; therefore the magnesium chloride dose was the same for all three storage tanks, even if the phosphate concentrations in the collected 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.[30,31] 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 1. 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


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Table 1. Phosphate Concentrations in Synthetic Urine and Real Urine Corresponding to Fresh, Pre-$MgCl_2$-Addition, Post-$MgCl_2$-Addition, and Filtered Urine for the Synthetic and Real Urine Experiments[a]

| experiment | fresh urine (mmol/L $PO_4$) | pre-$MgCl_2$ addition (mmol/L $PO_4$) | post-$MgCl_2$ addition (mmol/L $PO_4$) | filtered urine (mmol/L $PO_4$) |
|---|---|---|---|---|
| urea hydrolysis synthetic urine | 14.6 | 4.5 ± 2.1 | 0.012 ± 0.004 | 0.006 ± 0.004 |
| urea hydrolysis inhibition (acetic acid) synthetic urine | 16.5 | 12.2 ± 1.9 | 0.060 ± 0.011 | 0.069 ± 0.012 |
| urea hydrolysis real urine 1[b] | | 3.7 ± 0.03 | 3.3 ± 0.0 | 3.5 ± 0.1 |
| urea hydrolysis real urine 2[b] | | 2.8 ± 0.1 | 0.61 ± 0.04 | 0.63 ± 0.03 |
| urea hydrolysis inhibition (acetic acid) real urine 1[b] | | 3.3 ± 0.05 | 2.4 ± 0.05 | 2.1 ± 0.03 |
| urea hydrolysis inhibition (acetic acid) real urine 2[b] | | 2.4 ± 0.01 | 1.1 ± 0.05 | 1.1 ± 0.02 |

[a]Magnesium chloride was added to the stored synthetic and real urine to recover phosphate via struvite precipitation. Samples were grabbed before $MgCl_2$ addition, after $MgCl_2$ addition and mixing, and after urine was filtered. [b]Struvite precipitation was conducted twice in series using the real urine from each experiment. Urine from the first struvite precipitation test was collected and struvite precipitation was repeated in the collected urine.

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 1. The mixed urine was then filtered through a nylon stocking that was attached to the bottom of the conical storage tanks. The nylon stocking allowed for a flexible apparatus when filtering due to the placement of the storage tanks. The ball valve at the bottom of the tank was slowly opened and the settled urine was allowed to flow through the stocking. A sample of the filtered urine, referred to as Filtered Urine in Table 1, was collected at this point. Although phosphate recovery was an objective of this study, studying the characteristics of the precipitated solid was not in the scope of the study.

**Urea Hydrolysis, Inhibition, and Phosphate Recovery Using Real Urine.** One of the urinals used in the synthetic urine experiments was used in the real urine experiments. The urinal was cleaned before it was used. Because of the potential odor from real urine, which was not expected in the synthetic urine experiments, a bottle (89 mL) of Kohler Waterless Urinal Sealing Liquid (containing >80% vegetable oil, > 10% polyamide gel, and >10% proprietary blend of nonhazardous components) was poured into the urinal trap as instructed by the urinal manufacturer.[32] Jack bean urease was not added in the real urine experiments because the biotic form of urease was expected to be present. An 18.9 L opaque, polypropylene container was attached to the urinal outlet with PVC pipe and flexible PVC tubing. Real urine was collected as described in the Supporting Information. Four donors were asked to donate once per day. Because of the complexity required in phosphate concentration analysis, donors were only asked to take pH and conductivity measurements of their urine samples. 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 real urine experiments were run until the storage tank was full.

A second clean urinal from the synthetic urine experiments was used for the real urine urea hydrolysis inhibition (acetic acid) experiment. The urinal setup and collection was identical to that of the urea hydrolysis experiment using real urine, with the addition of a peristaltic pump for the addition of acetic acid after the real urine was poured into the urinal. The concentration of the acetic acid solution was 2500 mequiv/L, matching the concentration used in the synthetic urine experiment. 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 urination volume in the first real urine experiment (301 mL). Urine was collected from 10 am−4 pm local time 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 in series using the real urine collected from the real urine experiments (see Figure S2). Each struvite precipitation test followed the precipitation method described above for the synthetic urine experiments. 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, due to unexpected results, using the filtered and collected urine. Magnesium chloride was dosed twice per storage tank: before the first precipitation test and before the second precipitation test. The dose of magnesium chloride was determined using the phosphate concentration in the real urine collected from the real urine experiments immediately before each struvite precipitation test was performed.

**Analytical Methods.** The pH of synthetic and real urine samples was measured using a Accumet AB15 pH Meter (calibrated to pH 4, 7, and 10 before use). Conductivity was measured using either conductivity probe (Atlas Scientific Conductivity K 1.0 Kit) connected to an Arduino or Thermo Scientific Orion Star A212 Conductivity Meter. 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 $Ca^{2+}$ before use). All samples were filtered prior to phosphate analysis using 0.45 $\mu$m nylon syringe filters (Environmental Express). Phosphate was measured following Standard Method 4500-P ascorbic acid method using a UV absorbance on a U-2900 UV−visible spectrophotometer (Hitachi High Technologies) and 1 cm quartz cuvette at a wavelength of 880 nm.[33] Samples were diluted with DI water at a 1000× dilution factor to fall within the calibration curve of 0−1.2 mg P/L. Conductivity, phosphate, and calcium concentration data were analyzed using analysis of variance (ANOVA). The method and results for the ANOVA tests are described in the SI.

## RESULTS AND DISCUSSION

**Mimicking Urea Hydrolysis Using Synthetic Urine.** The pH and conductivity measurements in urine exiting the


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urinals were used as surrogates to determine if the urea hydrolysis reaction was proceeding in the forward direction.[18,28] Ray et al. showed that a direct correlation existed between increasing ammonia concentration and conductivity, therefore ammonia was not measured directly.[28] Figure 1a shows the pH and conductivity of the synthetic urine exiting the nonwater urinals over 240 min for the urea hydrolysis experiment. The pH results are shown individually for each urinal with the pH measurement at 0 min corresponding to synthetic urine solution used for the experiments. The pH steadily increased during the 240 min experiment with all three urinals showing a maximum pH around 8.5−8.6, which is indicative of hydrolyzed urine, from a starting pH of 6.[28] 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 but could have been due to mixing conditions in the urinal discussed in more detail later).

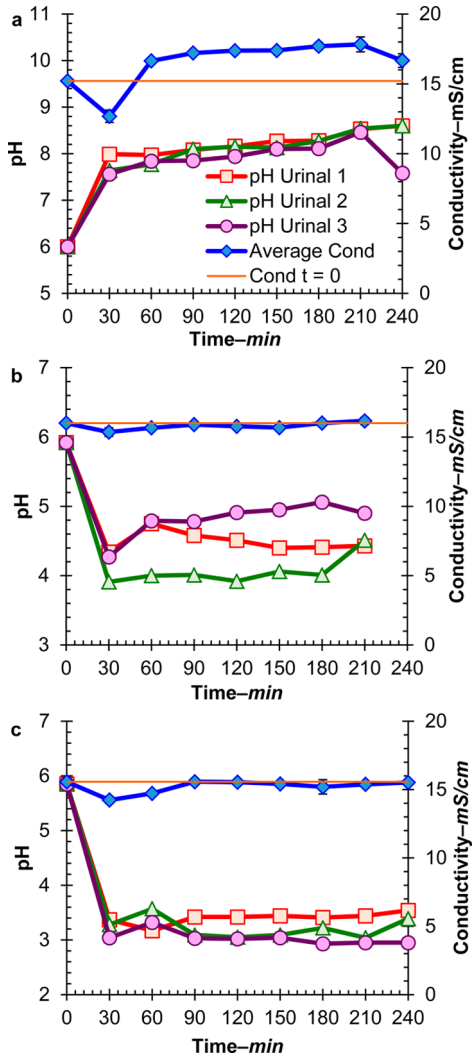


**Figure 1.** pH and conductivity of synthetic urine for (a) urea hydrolysis, (b) urea hydrolysis inhibition (acetic acid), and (c) urea hydrolysis inhibition (citric acid) synthetic urine experiments. pH is shown separately for each urinal and the pH at $t = 0$ was the pH of the initial synthetic urine. Conductivity is shown as the average of the three urinals with error bars showing one standard deviation (contained within symbol). The solid line corresponds to the conductivity at $t = 0$ of the initial synthetic urine. The conductivity was measured every 30 min and the conductivity at $t = 0$ was the conductivity of the initial synthetic urine.

Because the cleaning procedure between experiments resulted in DI water remaining in the urinal trap, 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 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 urine at $t = 0$, which is indicative of urea hydrolysis and formation of charged ammonia and bicarbonate species.[34] For all three urinals, the urine exiting the urinals reached conductivity between 16.7 and 17.4 mS/cm after 240 min. The measurements shown in Figure 1a are consistent with those published in previous studies on urea hydrolysis in real urine,[28,35] and support the abiotic approach used in this work to mimic the bacterial catalyzed urea hydrolysis process observed in nonwater urinals and urine diversion systems.

The results in Figure 2a show that the phosphate concentration in synthetic urine exiting the nonwater urinals was 45 to 73% lower than the phosphate concentration in synthetic urine entering the urinals. The phosphate concentrations decreased from 14.6 to 4.2 mmol/L in the initial synthetic urine in the first 30 min, and remained below 6 mmol/L until the last two measurements at 210 and 240 min. 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 urine (i.e., 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.[18,36,37] This is further explored after considering the calcium data. The large variation in phosphate concentration between the three urinals (e.g., ±3 mmol/L) indicates that there was a possible combination of experimental (i.e., urinal-to-urinal) and analytical (i.e., phosphate measurement) variability during the experiment. However, accuracy controls used in the phosphate method did not exhibit values greater than 10% different to a known concentration. Therefore, urinal-to-urinal variation was the only possible source of variability in phosphate concentrations. It is possible that the intermittent addition of urine to the urinals and the subsequent mixing conditions in the urinals contributed to urinal-to-urinal variability; this idea is explored in a following section. Despite the variability, there was a measurable reduction in the phosphate concentration in urine exiting the nonwater urinals for the urea hydrolysis condition. Although the same synthetic urine recipe was used throughout the experiments, variable initial concentrations of phosphate and calcium were seen. This could be due to spontaneous precipitation of phosphate and calcium during sodium hydroxide (NaOH) addition to raise the pH of the initial synthetic urine to pH 6.

The calcium concentration decreased from 3.1 mmol/L in the initial synthetic urine to below 0.40 mmol/L for all subsequent sampling (Figure 3). Similar to the phosphate results, calcium was being lost from solution between the time synthetic urine entered and exited the urinals. The most likely


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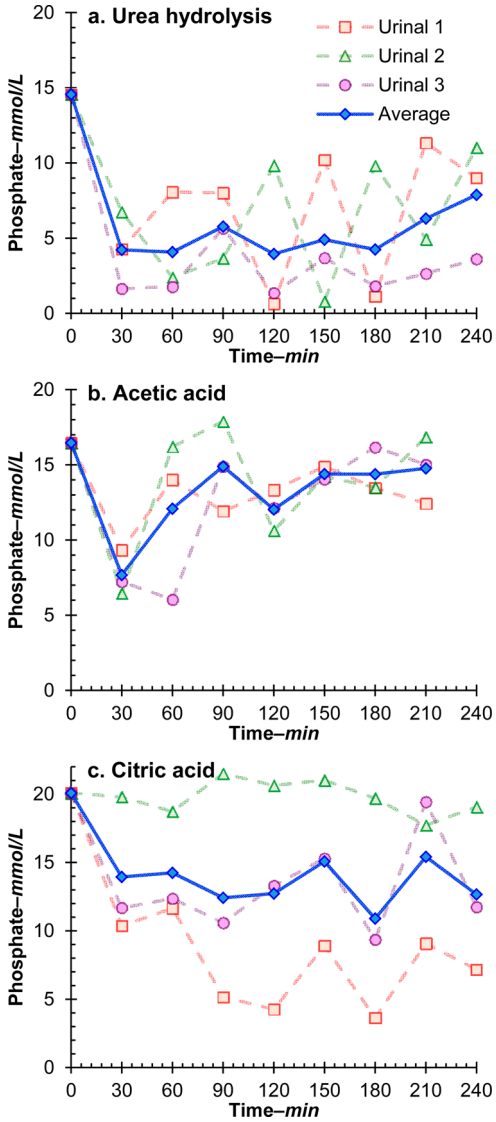


**Figure 2.** Phosphate concentrations in synthetic urine for the (a) urea hydrolysis, (b) urea hydrolysis inhibition (acetic acid), and (c) urea hydrolysis inhibition (citric acid) synthetic urine experiments. Measurements were taken every 30 min. Measurements for each urinal are shown along with the average concentration for the three urinals. Phosphate concentration at $t = 0$ was the concentration in the initial synthetic urine.

explanation is precipitation of calcium phosphate and magnesium phosphate minerals, such as hydroxyapatite and struvite, which remain in the urinal trap. Like it was seen with the phosphate results, the dilution effect due to water remaining in the urinal trap at $t = 0$ was dampened by loss of calcium and phosphate as the dilution effect and precipitation loss occur concurrently. That was not seen in the conductivity results, as the conductivity was increasing over time while the dilution effect decreases conductivity.

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 ($Ca_5(PO_4)_3(OH)$) precipitation and loss of magnesium was due solely to struvite ($NH_4MgPO_4{\cdot}6H_2O$) precipitation.[20,23] Using a reduction in calcium concentration of 2.8 mmol/L from Figure 3, the corresponding loss of phosphate is 1.7

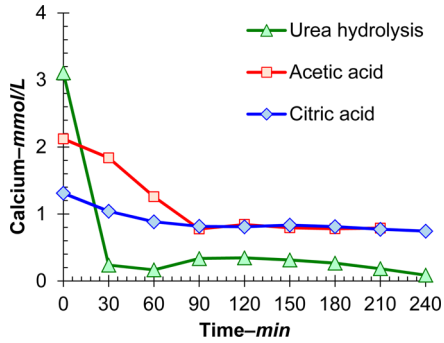


**Figure 3.** Calcium concentrations in synthetic urine for the urea hydrolysis, urea hydrolysis inhibition (acetic acid), and urea hydrolysis inhibition (citric acid) synthetic urine experiments. Measurements were taken every 30 min and are shown as the average concentration for the three urinals with error bars showing one standard deviation (contained within symbol). Calcium concentration at $t = 0$ was the concentration in the initial synthetic urine.

mmol/L. Using 4 mmol/L of magnesium in synthetic 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 urine (14.6 mmol/L, Figure 2a), the estimated phosphate concentration in urine exiting the urinals due to precipitation of calcium as hydroxyapatite and magnesium as struvite would be 8.9 mmol/L of phosphate, which is within 12% difference of the final phosphate measurement in Figure 2a. Although precipitation of calcium carbonate and other phosphate minerals in possible, the calculations illustrate the measured reductions in phosphate and calcium are generally explained by precipitation of struvite and hydroxyapatite, which is well documented in the literature.[23,36,38]

**Inhibiting Urea Hydrolysis Using Synthetic Urine.** The conductivity measurements plotted in Figure 1b and 1c were for samples taken every 30 min due to the change in the instrument used to measure conductivity. In Figure 1b and 1c, the first point at $t = 0$ min was the conductivity measured in the synthetic urine. Both acid additions lowered the pH of the urine exiting the urinals. With the acetic acid addition, the pH decreased from pH 5.9 in the initial synthetic urine to pH 3.9–5.1 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 the initial synthetic urine to pH 3.0–3.6 in urine exiting the urinal with consistent pH values for each urinal. The lower pH of urine with citric acid addition than acetic acid addition was because citric acid is a stronger acid, as indicated by its $pK_a$ values.[39] For the addition of acetic acid or citric acid, the conductivity of urine exiting the urinals remained essentially unchanged from the conductivity of synthetic urine entering the urinals (<5% difference for acetic acid addition, <6% difference for citric acid addition). Together, the pH and conductivity results indicate that the urea hydrolysis reaction in synthetic urine was not proceeding in the forward direction for the sequence of concurrent urine and urease addition immediately followed by acid addition.

The phosphate concentration in urine exiting the nonwater urinals for acetic acid and citric acid addition is shown in Figure 2b and 2c. The phosphate concentration in synthetic urine used for the acetic acid addition experiment was 16.5 mmol/L. The phosphate concentration decreased below 9 mmol/L in the first measurement taken after 30 min due to dilution by DI water


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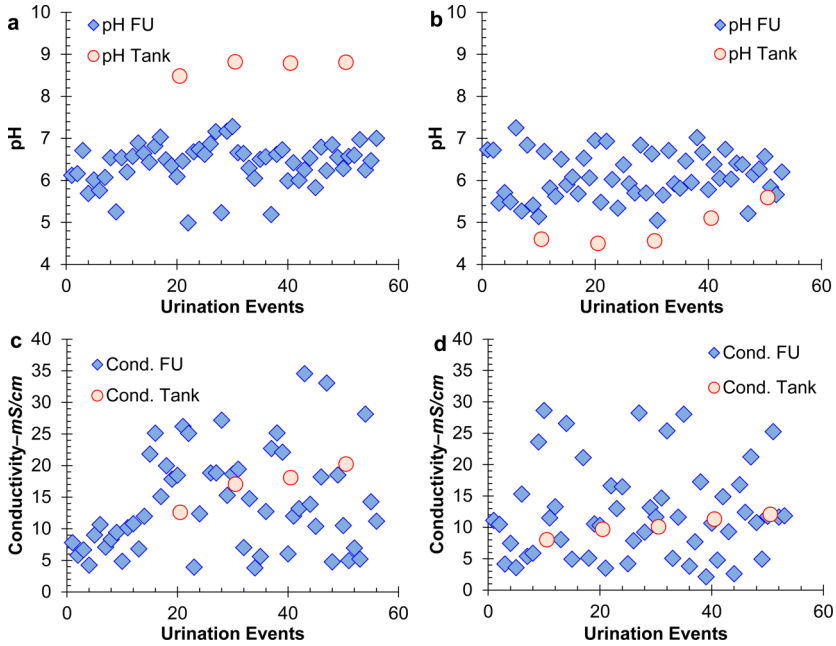


**Figure 4.** pH and conductivity of real fresh urine (FU) samples and composite storage tank samples for urea hydrolysis (a, c) and urea hydrolysis inhibition (acetic acid) (b, d) real urine experiments. The pH of each urination event is shown as a single point.

remaining in the urinal trap, and all subsequent measurements were between 12−14 mmol/L. When citric acid was used, 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. Once again, accuracy controls were within 10% difference to a known phosphate concentration and indicate that the high variation did not stem from analytical error.

The urea hydrolysis inhibition experiments showed similar results to each other in terms of calcium concentration in urine exiting the nonwater urinals (Figure 3). 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 citric acid addition was 1.3 mmol/L and decreased to 0.7 to 0.8 mmol/L, a 38% reduction. Both acetic acid addition and citric acid addition resulted in more calcium remaining in solution on a percentage basis and absolute concentration compared with the urea hydrolysis experiment. Together the phosphate and calcium results indicate that less precipitation (presumably calcium and magnesium phosphates) occurred in the urea hydrolysis inhibition experiments than the urea hydrolysis experiment using synthetic urine. The pH and conductivity results for the urea hydrolysis inhibition experiments imply that urea hydrolysis was inhibited; however, there was still a measurable reduction in phosphate and calcium concentrations. One possible explanation for phosphate and calcium losses, and variability between urinals, that were observed is incomplete mixing within the urinal trap. It is unknown if the urine flow through the urinal behaved more like a plug flow or completely mixed flow reactor, as the hydraulics of the system were not analyzed. Being so, it was possible for zones within the urinal trap to undergo urea hydrolysis to some extent before exiting the urinal.

**Phosphate Recovery Using Synthetic Urine.** The urea hydrolysis and urea hydrolysis inhibition (acetic acid) precipitation experiments were both able to recover more than 99.5% of the phosphate present in the collected synthetic urine (Table 1). The urea hydrolysis inhibition (acetic acid) experiment followed by struvite precipitation recovered 74% of the total phosphate present in the initial synthetic urine compared with 31% in the urea hydrolysis experiment. This was due to the presence of more phosphate in the stored synthetic urine with acetic acid addition because it inhibited the precipitation of phosphate inside the urinals. This shows the benefit of inhibiting the urea hydrolysis reaction with acetic acid, as it allowed for a greater amount of phosphate to be recovered via struvite precipitation in synthetic urine. Although there have been numerous studies published on optimizing struvite precipitation by changing the parameters of the precipitation (e.g., magnesium source, temperature, pH),[31,40,41] the results reported herein are the first to show that acid addition at the point of collection is able to increase nutrient recovery potential in synthetic urine.

**Urea Hydrolysis, Inhibition, and Phosphate Recovery Using Real Urine.** The pH of each urination event (Figure 4a and 4b) is shown as a single point. The pH of the real urine samples fell within the same range (i.e., pH 5−7) between the two experiments, which is the reported range for real urine documented in the literature.[23,42] The pH of real urine when mimicking urea hydrolysis was pH 8.5 after 20 events, which corresponds to 5 d of experiment time. During the same time, the pH was 4.5 in urea hydrolysis inhibition (acetic acid) urine. After 3 weeks, the urine collected from the urea hydrolysis experiment had a pH 8.8, compared with pH 5.6 in the real urine collected from the urea hydrolysis inhibition (acetic acid) experiment. The acetic acid addition prevented the pH of the urine in the tank to rise above the pH of the fresh real urine samples, presumably by limiting the extent of urea hydrolysis.

Unlike the pH measurements of the fresh real urine, the conductivity of real urine is highly dependent on time of donation, hydration, and diet unique to each donor.[43] In the data collected from the real urine experiments, urination volume was the best determinant of conductivity in the fresh


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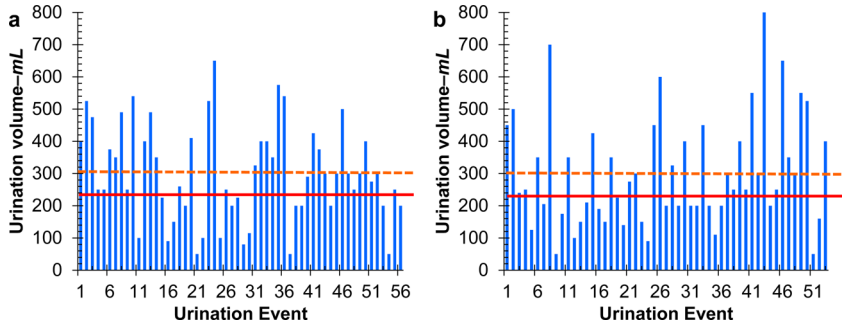


**Figure 5.** Volume of real urination events for the (a) urea hydrolysis and (b) urea hydrolysis inhibition (acetic acid) real urine experiments. The dashed line shows the average urination volume for each real urine experiment. The solid line shows the urination volume used in the synthetic urine experiments.

real urine samples (Figure S2). The conductivity value was higher in low urination volume samples and lower in samples with a high urination volume. Figure 4c and 4d shows the results for conductivity in a similar manner as the pH results. The conductivity values of the real urine samples were in the range of 3.80−34.5 mS/cm (mean 14.1 ± 7.9 mS/cm) and 2.13−28.6 mS/cm (mean 12.2 ± 7.3 mS/cm) for the urea hydrolysis and urea hydrolysis inhibition (acetic acid) experiments, respectively. Despite the range of conductivity in real urine, the conductivity measurements in the urine storage tank showed that the conductivity increased over the course of the urea hydrolysis experiment, whereas the conductivity in the urine storage tank increased to a lesser extent over the course of the urea hydrolysis inhibition (acetic acid) experiment. After 20 urination events (corresponding to 5 d), the conductivity of the composite sample was 12.6 mS/cm and 9.69 mS/cm for the urea hydrolysis and urea hydrolysis inhibition (acetic acid) experiments, respectively. The final conductivity measurement in the tank for the urea hydrolysis experiment was 20.2 mS/cm compared with 12.1 mS/cm for the urea hydrolysis inhibition (acetic acid) experiment. This corresponded to a 38% and 20% increase in conductivity, respectively. Conductivity and pH results show that urea hydrolysis occurred when using real urine without an external addition of jack bean urease, confirming the assumption that urease was present in the environment surrounding the urinal. Hence, the results for pH and conductivity suggest that urea hydrolysis was inhibited in the storage tank due to acetic acid addition in the urinal immediately following real urine delivery, which are similar results to those observed in the experiments using synthetic urine.

The average urination volume (±one standard deviation) for the urea hydrolysis experiment was 301 ± 148 mL and the average urination volume for the urea hydrolysis inhibition (acetic acid) experiment was 301 ± 169 mL (Figure 5). Urination volume is directly related to fluid intake and 24-h urinary frequency.[29] The upper dashed horizontal line depicts the average urination volume for each real urine experiment and the lower horizontal line depicts the urination volume used in the synthetic urine experiments.

In the real urine experiments, the urine collected from the urea hydrolysis experiment had 19% higher phosphate concentration then the urea hydrolysis inhibition (acetic acid) experiment. In contrast, in the synthetic urine experiments, the urine collected from the urea hydrolysis experiment had 63% lower phosphate concentration then the urea hydrolysis inhibition (acetic acid) experiment. The initial phosphate concentration in the urine donations was not measured, which could explain the higher phosphate concentration in real urine without acid addition than real urine with acid addition. A small amount of phosphate losses could also be attributed to biological activity in the tanks. Phosphorus is a vital nutrient needed for bacterial growth, as it makes up about 50% of the inorganic composition of prokaryotic cells.[44] Biological growth was visible in the acetic acid real urine storage tank and it was not visible in the urea hydrolysis storage tank. Acetic acid addition became an additional source of organic substrate (measured as chemical oxygen demand, COD) for biological growth. The combination of high COD, available phosphorus and nitrogen, and neutral pH could have created favorable conditions for the growth of microorganisms.[44] Further testing into the interaction between nutrient concentration and biological growth must be conducted to determine the effect of acetic acid addition on real urine composition, as well as its effects on treatment processes downstream from urine collection.

Phosphate recovery via struvite precipitation was performed using the stored urine from the real urine experiments (see Table 1 and Figure S2). Phosphate concentration in the initial fresh urine samples was not measured due to the experimental design of urine donation, and therefore the total phosphate recovery could not be calculated. However, the phosphate concentration in the pre-$MgCl_2$ and post-$MgCl_2$ samples could be compared. The first precipitation test only recovered 11% and 27% of the phosphate in the stored real urine for the urea hydrolysis and urea hydrolysis inhibition (acetic acid) experiments, respectively. The recovery results from the first precipitation test were lower than those in the synthetic urine experiments and previous literature. Previous literature has shown phosphate recovery >90% when precipitating struvite from real urine with $MgCl_2$ addition.[45] For this reason, the filtered urine was collected and struvite precipitation was repeated using the same urine that had already gone through the precipitation process one time. When the stored real urine from the urea hydrolysis experiment was precipitated a second time, 78% of the phosphate remaining in the stored real urine after the first round of struvite precipitation was recovered, equal to 83% of the phosphate 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 in the second test for the stored real urine from the urea hydrolysis inhibition (acetic acid) experiment. The amount recovered in the second test was 54%, equal to 67% of the available phosphate.


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The lower phosphate recovery percentages found in the real urine experiments when compared with the results from the synthetic urine experiments is likely attributed to the greater complexity in the composition of the real urine. More specifically, the synthetic urine recipe did not contain endogenous metabolites that can act as organic complexing agents, such as citrate, creatine, lysine, L-cysteine, and taurine.[46] 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.[38,47] In the medical literature, Prywer et al. found that increased levels of citrate in urine had inhibitory effects on the formation of struvite kidney stones because it forms magnesium complexes with the excess $Mg^{2+}$ in solution.[48] In urine diversion literature, the influence of organic complexing agents has been largely neglected because they degrade during extended storage of urine.[23] However, simulations of urine diversion systems and struvite precipitation potentials showed that "high differences occurred" in precipitation when urine contained organic complexing agents while undergoing urea hydrolysis.[23] Due to the 3 week collection and storage time that was employed in the real urine experiments in this work and the unexpected struvite precipitation results, it could be assumed that the urine was not completely hydrolyzed and the organic acids were not completely degraded, even though the stored real urine was at the characteristic pH 9. During hydrolysis, the ammonia concentration increases, creating favorable conditions for the precipitation of struvite. If the extent of hydrolysis was limited, the concentration of ammonia would not create the supersaturated conditions needed for complete precipitation of struvite. Similarly, the difference in phosphate recovery between the two real urine experiments could be due to the presence of acetic acid that has the potential to complex with $Mg^{2+}$ and $NH_4^+$ similar to the endogenous metabolites.[38]

The strategy taken in this study sought to illuminate the changes that urine undergoes inside nonwater urinals using synthetic and real urine conditions. Although variability was introduced by the experimental design, working with real nonwater urinals was the only way to investigate urea hydrolysis in urinals. The results show that phosphate and calcium are lost between the time that urine enters and exists the urinals. However, the fate of these components is largely variable and remains mostly unknown. To further illuminate the fate of precipitation in nonwater urinals, it is recommended to conduct a hydraulic tracer test of flows inside nonwater urinals. This can answer whether urinal-to-urinal variation was due to heterogeneous mixing in the urinal traps. Additionally, a wider range of analytes should be measured in real-time, including magnesium, potassium, and phosphate speciation (i.e., total phosphorus and dissolved orthophosphate concentrations to find the particulate phosphorus concentration by difference of the two concentrations). This can add strength to the hypothesis that struvite and hydroxyapatite are precipitating and clogging nonwater urinals.

**Future Application.** In public restrooms with nonwater urinals and high frequency of use, the findings of this study suggest the addition of a small dose of acetic acid into nonwater urinals after every use. This can be achieved by implementing an automated dispenser with each nonwater urinal. However, as seen in the real urine experiments, there could be challenges in applying the desired dose or concentration of acid to each urination event as each event can vary in both volume and composition.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b03571.

Information regarding the synthetic urine recipe, real urine collection, urinals and storage tanks used, and data analysis, as well as additional supporting tables and figures (PDF)

## ■ AUTHOR INFORMATION


### Corresponding Author

*Tel.: 1-480-727-2945. E-mail: dsaetta@asu.edu.

### ORCID

Daniella Saetta: 0000-0003-3107-1154

Treavor H. Boyer: 0000-0003-0818-5604


### Notes

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