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Accelerating urea hydrolysis in fresh urine by modifying operating conditions of a sequencing batch reactor

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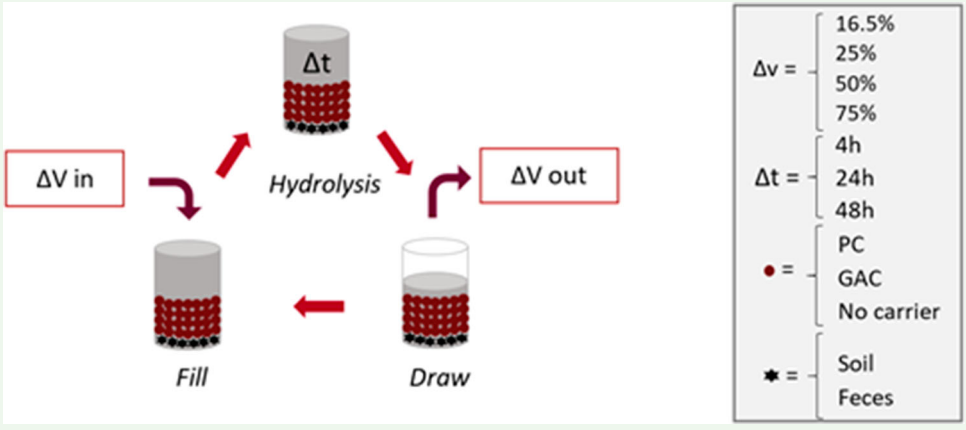
ABSTRACT

A number of existing and emerging technologies can recover nitrogen from urine. A preliminary step in many nitrogen recovery processes is hydrolyzing urea to ammonium, a biologically-mediated process that can take days to weeks without intervention. The ability to achieve urea hydrolysis quickly and reliably would increase the feasibility of decentralized nitrogen recovery, especially where space and treatment time are constrained. The goal of this research was to determine whether urea hydrolysis could be accelerated by providing an inoculum containing microorganisms likely to have urease activity (feces or soil), providing a carrier to support attached growth (plastic carriers, granular activated carbon, or no carrier), and modifying the hydraulic retention time (HRT; 1.3, 2, and 4 days) and feeding frequency ($\Delta t = 4$, 24 h). Inoculated reactors achieved significantly more urea hydrolysis, and reactors inoculated with soil were able to sustain higher urea hydrolysis rates over time than those inoculated with feces. The mean zero-order rate constants (mM/hr) for reactors with a soil inoculum (15.1) were about three times higher than that of reactors with an inoculum of feces (4.9). A reactor with GAC and an inoculum of soil fed daily with fresh urine achieved greater than 90% hydrolysis with an HRT of 2 days; results suggest the HRT could be reduced to 16 h without reducing performance. No significant benefit was provided by increasing the frequency of feedings for the same HRT, likely because urease enzymes were saturated and operating at maximum hydrolysis rates during most of the reaction period.



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1. Introduction

Recovering nutrients like nitrogen and phosphorus from undiluted waste streams such as urine provides multiple benefits. First, reducing the amount of nutrients discharged to the environment decreases the potential for eutrophication, including the conditions that lead to harmful algal blooms [1]. Second, recovering nutrients as fertilizer could also reduce the need to mine phosphorus from finite reserves [2] or utilize the energy-intensive Haber-Bosch process to produce synthetic ammonium fertilizer [3,4]. One strategic concentrated waste stream for decentralized nutrient recovery is

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urine, which contains 75–80% of the nitrogen and 50–55% of the phosphorus found in domestic wastewater in 1% of the volume [5]. Building-scale urine separation and treatment has advantages such as lower energy demand and more selective contaminant removal compared to centralized treatment [6,7].

Prior to recovering nitrogen from urine, many processes require urea to be hydrolyzed to ammonium [8]. Approximately 85% of the total nitrogen content in fresh urine consists of urea [9]. Technologies that typically require a preliminary urea hydrolysis step include gas stripping [10,11], ion exchange [12,13], struvite precipitation [14,15], partial nitrification/distillation [16], and (bio)electrochemical methods [17,18]. The urea hydrolysis reaction ($CO(NH_2)_2 + 3H_2O \rightarrow 2NH_4^+ + HCO_3^- + OH^-$), catalyzed by the urease enzyme, can be a lengthy process; in one study in which fresh urine was stored without the addition of stored urine, hydrolysis was reported to take over 41 days [19]. The duration of urea hydrolysis was reduced to only four days when fresh urine was added to stored urine [20]. Achieving urea hydrolysis quickly and reliably could increase the feasibility of nitrogen recovery, especially in decentralized contexts where space and treatment time are constrained. For example, a compact urea hydrolysis reactor could treat fresh urine underneath a source-separating toilet, or in the basement of a building, prior to treating the ammonia-rich effluent with one of the nitrogen recovery technologies listed above.

A range of strategies have been investigated to accelerate urea hydrolysis. Elevated temperatures and stirring can accelerate urea hydrolysis, but require the input of energy [18,20,21]. Inoculation appears to be promising, but more research is needed on inoculants that can sustain high urease activity over time. While an inoculum of Jack Bean urease was shown to accelerate urea hydrolysis to a couple of hours [22], it is expensive and would have to be continuously added. Alternatively, stored urine that had already undergone hydrolysis worked as an inoculum, but hydrolysis required multiple days (note that these experiments were not intended to achieve rapid hydrolysis) [21]. In a different investigation, combining 20% stale urine with 80% fresh urine increased the extent of urea hydrolysis, but HRT was not determined [20]. Mixing together similar volumes of fresh urine and feces (v/v = 1.3:1) was shown to achieve near full urea hydrolysis after 4 h at 23°C [23]; however, the high volume of feces used in the study is not ideal for downstream nutrient recovery. An inoculum of mixed anaerobic ureolytic sludge was reported to achieve hydrolysis with 68% efficiency in a stirred sequencing batch reactor (SBR) fed 11 g N/L/d with an HRT of 0.45 days at 28 °C under anaerobic conditions [8]. In the research presented herein, feces was chosen for further study as an inoculum, as well as soil, given that urease-positive bacteria are ubiquitous in the environment [21,22,24]. Both of these inocula are inexpensive and widely available [23].

No prior studies have investigated the ability of carriers to accelerate urea hydrolysis, although urea hydrolysis has been reported to occur due to biofilms that grow on urine collection pipes [9,21]. Carriers have a long history of being used for attached growth in wastewater treatment [25]. Carriers such as plastic or granular activated carbon are inexpensive and could provide additional surface area for urease-positive bacteria to attach and grow, thus providing more urease to facilitate the conversion of urea to ammonia.

The overall goal of this study was to investigate whether urea hydrolysis could be accelerated in a simple, low-energy (no mixing), passive SBR by varying three different operating conditions: (i) providing an inoculum, (ii) providing carriers, and (iii) modifying the draw volume and frequency. First, we hypothesized that providing an inoculum (soil or feces) of organisms likely to possess the urease enzyme would accelerate hydrolysis. Second, we hypothesized that growth of microbial communities that contain urease-positive bacteria could be enhanced by providing carriers (plastic or granular activated carbon) with greater surface area for attached growth [26]. Third, we hypothesized that a SBR could achieve near full hydrolysis with a daily draw percentage of 50%.

2. Materials and methods

2.1 Operational conditions

SBRs with no mixing were utilized to investigate the acceleration of urea hydrolysis (Figure 1). Fresh urine was collected from 25 male and female volunteers between 20 and 45 years old at the University of California, Berkeley in a Fisherbrand Commode Specimen Collection System (Committee for Protection of Human Subjects, Protocol 2019-04-12054). The collected unhydrolyzed urine was homogenized in a sterile, closed container and stored at 5°C for a maximum of three days. The low temperature prevented urea hydrolysis during storage. Initially, 250 mL of fresh urine was added to each SBR. Each SBR was an autoclaved 250-mL glass bottle (Corning Pyrex) with a screw cap placed inside a fume hood at ambient temperature as shown in Figure S1. After hydrolysis occurred over varying time steps (Δt = 4, 24 h, 48 h), a percentage of the urine was removed (ΔV = 17%, 25%, 50%, 75%) (i.e. the 'draw'

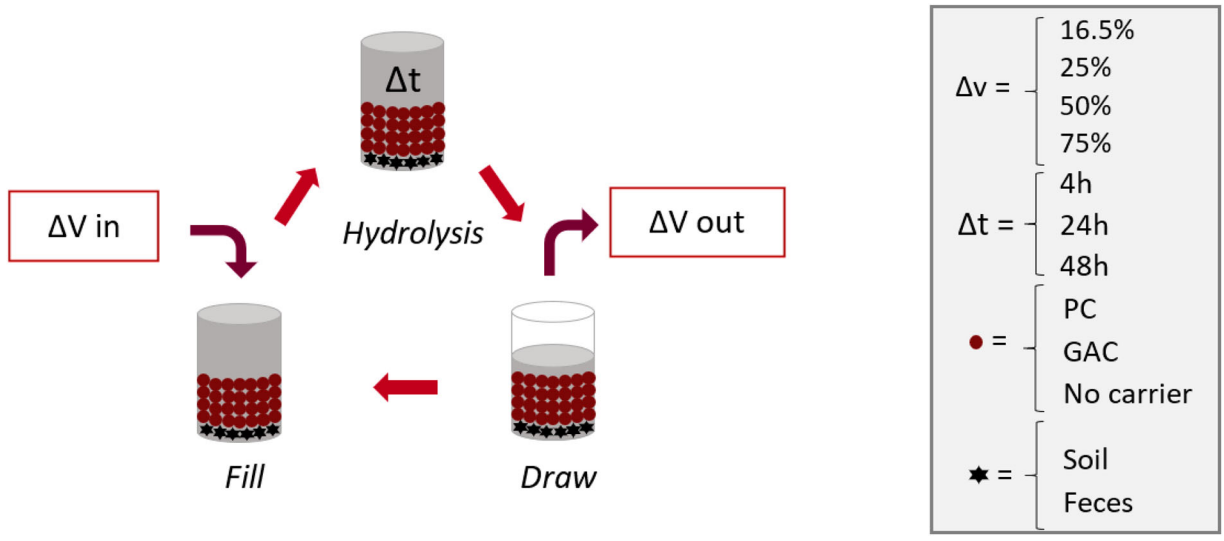

Figure 1. Urea hydrolysis was analyzed by adjusting the volume percentage and draw time in a sequencing batch reactor.

step). That percentage of drawn hydrolyzed urine was replaced with fresh urine (i.e. the 'fill' step). No stirring or aeration was provided other than what might have occurred passively during draw and fill steps. A syringe was used to draw and fill urine.

Inocula and carriers were added to the reactors to investigate their potential contribution to accelerating urea hydrolysis. Inocula included untreated feces and soil. Feces was collected from a volunteer at the University of California, Berkeley (Committee for Protection of Human Subjects, Protocol 2019-04-12054). Soil was collected from Codornices Park in Berkeley, CA. Approximately 41 g of human feces or 50 g of soil was added to selected urea hydrolysis reactors to obtain a urine to feces/soil ratio of 5.2:1 (volume/ volume) based on densities determined in prior research [23]. Carriers included granular activated carbon (GAC) (Fisher Scientific, Waltham, MA) and plastic carriers (PC) (K1 Filter Media, Wholesale Koi Farm, Norco, CA). Approximately 40 g of GAC (Activate Charcoal, Sigma Aldrich) and 100 cm^3 of PC were added to selected urea hydrolysis reactors. GAC typically settled to the bottom of the reactor (Figure S1). The plastic carriers were less dense than urine and remained at the top of the reactor.

Four phases of research were conducted to address the three specific objectives, with the main operating conditions summarized in Table 1 and the changes between phases displayed in Figure S2. Phase 1 served as a broad screening of inoculum type, carrier type, and HRT (where HRT (d) = Δt / ΔV, units of d / %) using

Table 1. Description of four research phases to investigate the impact of inoculum type, carrier type, and hydraulic retention time (HRT) on accelerating urea hydrolysis.

Phase	Inoculum Type	# reactors	Carrier Type	Hydraulic Retention Time (HRT, days)	Draw Volume Percentage (ΔV, %)	Draw Time Interval (Δt, days)	Duration of Experiment (days)
1	None	1	None (1)	2 days (1)	50% (1)	1 d (1)	21 days
	Feces	5	None (2)	1.3 days (1)	75% (1)	1 d (5)	21 days
			PC (3)	2 days (2)	50% (2)		
				4 days (2)	25% (2)		
	Soil	12	None (4)	1.3 days (3)	75% (3)	1 d (12)	21 days
			GAC (4)	2 days (3)	50% (3)		
			PC (4)	4 days (6)	25% (6)		
2	Feces	2	PC (1)	2 days (2)	50% (2)	1 d (2)	4 days
			None (1)				
	Soil	3	GAC (1)	2 days (5)	50% (3)	1 d (3)	4 days
			PC (1)				
			None (1)				
3	Soil	3	GAC (1)	2 days (3)	50% (3)	1 d (3)	10 days
			PC (1)				
			None (1)				
	Liquid*	3	None (3)	2 days (3)	50% (3)	1 d (3)	10 days
4	Soil	2	GAC (2)	2 days (2)	50% (1)	1 d (1)	4 days
					17% (1)	0-4-8h (1)	
	None	1	None (1)	2 days (1)	50% (1)	1 d (1)	4 days

PC = plastic biofilm carrier, GAC = granular activated carbon. HRT (d) = $\Delta t/\Delta V$ (d/%). The numbers in parentheses indicate the number of reactors at each operating condition. *Liquid from the three reactors from Phase 2-soil (GAC, PC, none).

18 SBRs (Figure 1; Table S1). Therefore, for each inoculum type, a variety of carriers and HRTs were considered. Each reactor had 250 mL of urine and up to 60 mL of headspace. In Phase 2 (Table S2), we collected and analyzed samples on an hourly basis for a single HRT (50% draw volume and 24 h time interval) to provide more insight into hydrolysis rate immediately after draw/fill. One reactor from each carrier/inoculum combination from Phase 1 was used in Phase 2 (Figure S2), and urine from the reactors was drawn and refilled once a day for four days. Reactors were filled to the top (310 mL) to reduce nitrogen loss through volatilization. In Phase 3 (Table S3), we investigated if the urease-positive bacteria inside the reactors were suspended in the liquid phase or attached to carrier surfaces, reactor walls, or in sediment that accumulated in the bottom of the reactors. Half of the liquid volume from an 'original' running reactor was drawn and placed in an empty 'liquid only' reactor. Both sets of reactors ('original' and 'liquid only') started with 50% volume of liquid from the 'original' reactors and 50% fresh urine. Only soil inoculum was studied based on results from Phases 1 and 2. Reactors were filled to the top as in Phase 2. In Phase 4, we investigated draw volumes and timing (Table S4). A control of no inoculum or carrier was compared to two other reactors: one reactor with soil and GAC that had 50 mL of urine added three times a day (at 0, 4, and 8 h), and another reactor with soil and GAC that had 150 mL of urine added once at the beginning of the day.

2.2 Analytical techniques

Physical and chemical parameters such as temperature, pH, electrical conductivity, and nitrogen species were monitored using analytical techniques. Temperature and pH were measured with a handheld Oakton pH 150 meter, and electrical conductivity was measured with an Oakton PC 450 probe (Vernon Hills, IL). Ammonium concentration was measured in fresh and hydrolyzed urine using ion chromatography (IC) (Dionex, IonPac CS12 column, 30 mM methane-sulfonic acid eluent, 1.0 mL/min, 40 °C). To prepare samples for the IC, a 40-uL sample of urine was diluted and 2 drops of 2 M nitric acid were added. Next, 650 uL of the combined solution was pipetted into the IC vial. Initial urea concentrations in fresh urine were calculated by subtracting the total ammonia nitrogen (TAN) concentration in a fresh urine sample from the TAN concentration in a sample that had been hydrolyzed with Jack Bean urease ($urea_{IFU} = TAN_{JB} - TAN_{IFU}$) (Figure S3). Jack Bean urease, in doses of 25–49 mg/L, can provide complete conversion of urea into ammonium in 1.5 h [27]. Therefore, to back calculate the urea concentration, 50 mg/L of Jack Bean urease was added with a contact time of 4 h. To achieve this concentration, 15 mL urine was pipetted into a 15-mL centrifuge tube, and then 0.75 mg Jack Bean urease (powder urease from *Canavalia ensiformis*, type IX, Sigma Aldrich) was added. A 40-uL sample was taken after 4 h. The extent of urea hydrolysis was calculated by dividing the TAN concentration after hydrolysis (TAN_H) by the initial total nitrogen (TN) concentration, which equals the TAN concentration after Jack Bean urease addition (TAN_{JB}) (i.e. TN was composed of the initial TAN (TAN_{IFU}) and initial urea in fresh urine ($urea_{IFU}$); very little organic nitrogen exists apart from urea and thus was considered negligible) [28]. Also, the initial TAN concentration in fresh urine was low (around 5% of the TN), so its impact on TAN/TN was minimal.

2.3 Statistical and kinetic analysis

Statistical analysis was conducted using ANOVA (single factor) with data from all four phases. If the null hypothesis was rejected in ANOVA, Tukey's post-hoc test was used to identify the specific pairs of means that were statistically different. A simple t-test was used to compare 'original' reactors to their 'liquid only' counterparts in Phase 3. Additionally, a kinetic analysis was conducted on the hourly data from Phase 2 to characterize rates and order of reaction when active urea hydrolysis was occurring. The kinetic analysis only considered data in the first eight hours due to low urease activity between 8 and 24 h. TAN values from each reactor were plotted using linear forms of zero-, first-, and second-order equations, and the slope and R^2 value of the linear regression trendline were tabulated for further analysis.

3 Results

3.1 Phase 1: screening of inoculum type, carrier type, draw volume, and time

The SBRs operated under different operational conditions (inoculum, carrier, draw volume, and time) in Phase 1 produced mean TAN/TN values above 0.9 (i.e. near complete urea hydrolysis after 24 h reaction time) except for the 'No Inoculum, No Carrier' reactor, which had a mean TAN/TN of 0.11 (Table 2). The inoculum produced immediate and consistent urea hydrolysis as the mean TAN/TN was above 0.9 for the duration of the experiment (21 days) for reactors containing feces or soil. In contrast, the hydrolysis activity in the reactor without inoculum and carrier (which was started on day 7) increased over time, with the TAN/TN increasing from 0.07 on day 8–0.26 on day 21 (Figure S4), likely

Table 2. Hydrolysis extent (TAN/TN) for reactors in Phase 1 with different hydraulic retention times (HRTs), carriers, and inoculum.

Inoculum	Carrier	HRT (d)	*n*	TAN/TN (AVG ± SD)
Soil	Plastic Carrier	4	20	0.94 ± 0.23
		2	10	0.96 ± 0.15
		1.33	10	0.96 ± 0.08
Soil	No Carrier	4	20	0.98 ± 0.13
		2	10	0.97 ± 0.06
		1.33	10	1.03 ± 0.23
Soil	Granular Activated Carbon	4	20	0.93 ± 0.26
		2	10	0.94 ± 0.14
		1.33	10	0.95 ± 0.19
Feces	Plastic Carrier	4	10	0.93 ± 0.13
		2	10	0.87 ± 0.33
		1.33	10	0.94 ± 0.09
Feces	No Carrier	4	10	0.90 ± 0.10
		2	10	0.93 ± 0.10
No Inoculum	No Carrier	2	6	0.11 ± 0.08

TAN = Total Ammonia Nitrogen and TN = Total Nitrogen. One reactor was studied for each condition, with samples (n) collected at the end of each 24-h hydrolysis period in the SBR (draw cycle; Figure 1).

due to the growth of bacteria with urease activity. There were no statistically significant differences among reactors with inoculum present (ANOVA, $p = 0.96$). Thus, no differences could be distinguished between the effects of inoculum type, carrier type, presence of a carrier, and the HRT on the extent of hydrolysis under the conditions studied. Given that urea was nearly fully hydrolyzed at the lowest HRT tested (1.33 days), in Phase 2 we analyzed samples on an hourly basis to determine if the HRT could be further reduced.

3.2 Phase 2: focused hourly study of different carriers and inocula

During each daily reaction period, the TAN/TN in reactors with an inoculum of soil rose an average of 0.46 compared to 0.24 in reactors with a feces inoculum (Figure 2). The differences in the average daily increase in TAN/TN values in the five reactors were statistically significant (ANOVA, $p < 0.02$). Specifically, the Feces No Carrier reactor and the Feces PC reactor were significantly lower than the Soil No Carrier reactor, according to Tukey's post-hoc test. As expected, in the five reactors that were fed once per day (50% volume replaced with fresh urine), the TAN/TN dropped by approximately 50% after each fill with fresh urine. The increase in TAN/TN was greatest during the first eight hours (Figure 1, Table S5).

For kinetic analysis of urea hydrolysis from Phase 2, only the TAN results from the first 8 h were used because concentrations were monitored hourly. Also, it is likely that one or more factors were limiting hydrolysis after 8 h (and possibly sooner). Across all conditions and all days, the average R^2 value of the zero-order trendlines (0.66) was slightly higher than the first-order (0.63) and second-order (0.59) average R^2 values (Table S6). Looking only at the zero-order analysis, the mean R^2 values for reactors with feces were lower (0.39) than the reactors with soil (0.84). The mean zero-order rate constants (mM/hr) for reactors with a soil inoculum (15.1) were about three times higher than that of reactors with an inoculum of feces (4.9) (Table S6).

3.3 Phase 3: attached or suspended bacteria?

In Phase 3, we compared the performance of: (a) the 'original' reactors from Phase 2 with carriers (which provide additional surface area for attached growth); and (eb) 'liquid only' reactors with no carriers (and thus no additional surface area for attached growth). The 'original' and its 'liquid only' counterpart were compared using a t-test. We hypothesized that if the bacteria

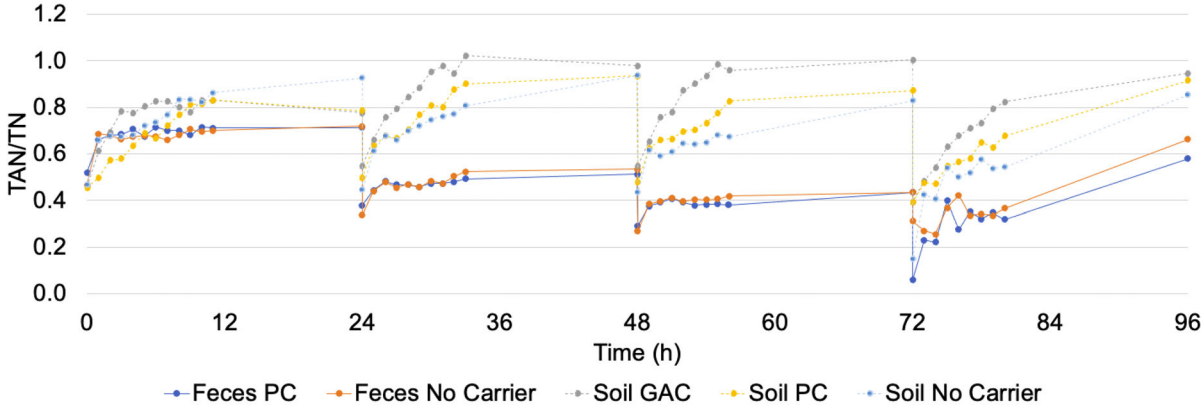

Figure 2. Time profile of urea hydrolysis (TAN/TN) for reactors in Phase 2 with different carriers and inocula drawn and filled every 24h. Carriers were granular activated carbon (GAC), plastic carrier (PC), and no carrier. Inoculum was soil or feces. TAN = Total Ammonia Nitrogen and TN = Total Nitrogen (n = 220; 44 for each reactor).

Table 3. Hydrolysis extent (TAN/TN) for two sets of reactors ('original' and 'liquid only') and three conditions of carriers (granular activated carbon (GAC), plastic carriers (PC), and no carrier) in Phase 3.

	GAC Original	GAC Liquid Only	PC Original*	PC Liquid Only*	No Carrier Original**	No Carrier Liquid Only**
Mean TAN/TN	0.99	0.95	0.66	0.79	0.82	0.56
Overall SD	0.19	0.20	0.20	0.27	0.27	0.23
Overall n	8	8	8	8	8	8

Reactors were fed once per day for ten days, and all 'original' reactors had an inoculum of soil. TAN = Total Ammonia Nitrogen and TN = Total Nitrogen. The hydraulic retention time was 2 days for all conditions. Asterisks indicate a statistically significant difference in average TAN/TN between pairs of reactors ('original' and 'liquid only') with the same carrier.

contributing to urea hydrolysis were suspended, the 'liquid only' reactors would perform as well as the original reactors.

The means of the two GAC reactors ('original' and 'liquid only') were not significantly different over a period of ten days (average TAN/TN of 0.97). The two other pairs (PC and No Carrier) had significantly different means and had an average TAN/TN ranging from 0.56–0.82 (Table 3). Two pairs of reactors (GAC and No Carrier) had higher average TAN/TN values in the 'original' reactor than in the 'liquid only' reactor, while the opposite was true for the PC reactor pair. Although all three 'liquid only' reactors clearly had urease activity, we cannot rule out the beneficial role of the GAC and PC in promoting urea hydrolysis because of the large difference in average TAN/TN between the No Carrier 'original' (0.82) and the No Carrier 'liquid only' (0.56) reactors.

3.4 Phase 4: draw volumes and frequency

In Phase 4 we investigated the effect of draw volumes and frequency by analyzing three reactors with the same 2-d HRT: a 'no carrier' reactor that had 50 mL drawn and filled three times a day (after 4, 8, and 24 h), a reactor with GAC that had 50 mL drawn and filled three times a day (GAC 3x/d), and a reactor with GAC that had 150 mL drawn and filled once a day (GAC 1x/d) (Figure 3, Figure S5). The three reactors' mean daily increase in TAN/TN were significantly different (ANOVA, $p = 0.004$). We determined utilizing Tukey's post-hoc test that the 'no carrier' reactor (0.13) had a significantly lower mean daily increase in TAN/TN value compared to the GAC 1x/d (0.47) and GAC 3x/d (0.43) reactors (Table S7). Thus, the GAC carrier provided conditions for more complete hydrolysis, but there was no benefit from more frequent draw/fill periods.

3.5 Physical and chemical indications of urea hydrolysis

Physical and chemical characteristics of urine such as pH and conductivity have been previously used to track the hydrolysis of urea [29]. In this study, fresh urine had a mean pH of 6.6 and hydrolyzed urine had a mean pH

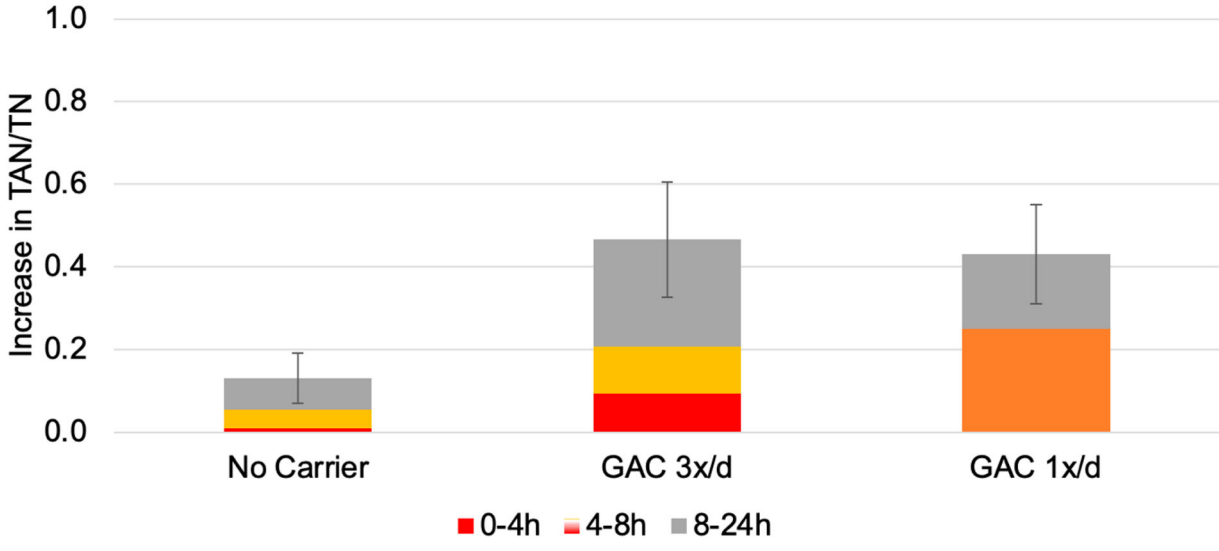

Figure 3. Daily increase in TAN/TN for three sets of reactors (No Carrier, GAC 3x/d, and GAC 1x/d) over four days in Phase 4. The 'No Carrier' scenario contained only stale urine and had 50 mL drawn and filled 3 times a day (after 4, 8, and 24 h). The 'GAC 3x/d' reactor was inoculated with soil, contained granular activated carbon (GAC) as a carrier, and had 50 mL drawn and filled 3 times a day. The 'GAC 1x/d' reactor was inoculated with soil, contained GAC as a carrier, and had 150 mL drawn and filled once a day. TAN = Total Ammonia Nitrogen and TN = Total Nitrogen. The hydraulic retention time was 2 days for all conditions. Given that half of the volume (150 mL) was drawn each day, the maximum possible increase in TAN/TN was 0.50.

of 9.1, consistent with previous studies (Figure S7) [20,30,31]. Conductivity can also be used to monitor the urea hydrolysis because as neutral urea is hydrolyzed to positively charged ammonium (depending on pH) and negatively charged bicarbonate, the conductivity increases. We found a linear relationship between conductivity and TAN concentration ($R^2 = 0.95$, Figure S8), consistent with previous research based on hydrolysis with Jack Bean urease [31].

4. Discussion

This research aimed to inform the design of a simple, low-energy (no mixing), passive reactor to accelerate urea hydrolysis. An empirical approach was used to test potential reactor configurations. Based on results, an SBR containing GAC and inoculated with soil appears to be a promising design. Below, we discuss additional insights from the results, including directions that could be pursued to develop a more fundamental understanding of the mechanisms and inform future design improvements.

4.1 Impact of inoculum on urea hydrolysis

Reactors inoculated with either feces or soil achieved much higher mean TAN/TN values (0.87 and above) compared to the reactor with no inoculum (0.11) in Phase 1 (Table 2). In Phase 2, reactors with a soil inoculum had mean increases in TAN/TN (0.46) that were over twice that of reactors with a feces inoculum (0.24) (Figure 1). One reason that the soil inoculum enabled greater hydrolysis could be that it contained more bacteria and/or urease activity than the feces inoculum, including free extracellular urease [24]. However, the experiments (Phase 1 – Phase 4) were conducted over several months and the reactors were only inoculated at the beginning of Phase 1. Thus, it appears that urease activity was maintained because urease-containing microorganisms persisted and grew in the reactors. Because the soil environment is generally more variable than the human intestine (e.g. temperature, moisture, dissolved oxygen), we hypothesize that the bacteria found in soil were better able to adapt to the variable conditions in the urea hydrolysis reactors. Another possible explanation is that we observed suspended fecal particles in the fecal inoculum reactors, which could subsequently have been removed from the reactor when urine was drawn, causing a reduction in the mass of urease-containing bacteria.

Based on the overall results, we recommend an inoculum of soil, which is both inexpensive and widely available, to promote urea hydrolysis. Many outstanding research questions remain that additional research could address. For example, soil from different environments and larger inocula could be tested. For inocula that perform well, characterizing the microbial biomass and the dominant bacterial species, their growth rates, the mass of urease enzyme, and the enzymatic activity could provide valuable insights.

In a kinetic analysis of TAN values in Phase 2, we determined that the hydrolysis rates most closely aligned with zero-order kinetics ($R^2 = 0.66$) (Table S6), although the linear fits were only slightly better than for first-order ($R^2 = 0.63$) and second-order ($R^2 = 0.59$) kinetics. Zero-order kinetics would be consistent with urease operating at the maximum rate with respect to urea, based on Michaelis-Menten kinetics. This interpretation seems reasonable for fresh urine. Reported values for the half-saturation constant (k_m) for plant and bacterial ureases range from 0.1–100 mM, with typical k_m values between 1 and 4 mM [22,24] (Figure S4). In the Phase 2 experiments, the initial molar concentrations of urea were between 30 and 60 mM, and the only time the urea molar concentration dropped below 4 mM was for Soil GAC on days 2 and 3. Because the urea molar concentration typically was well above 4 mM, it seems likely that urease was in the zero-order region with respect to urea throughout most of the experiment [22]. However, the rate clearly decreased over time with the higher rate being restored each time fresh urine was added, which suggests that another factor became rate-limiting. Further research is needed to determine which constituent in urine (e.g. carbon) other than urea could be rate-limiting, and under more controlled conditions to better enumerate the urease kinetics.

4.2 Impact of carrier on urea hydrolysis

Carriers did not have a strong impact on urea hydrolysis in Phases 1 and 2. We found no significant difference in mean TAN/TN between reactors with carriers (PC or GAC) and reactors without carriers in Phase 1 (Table 2). In Phase 2, reactors without carriers had statistically similar increases in TAN/TN as reactors with PC or GAC carriers (Figure 1). Nonetheless, carriers did have a significant effect in Phases 3 and 4. In Phase 3, the No Carrier liquid only (0.56) reactor had a statistically significant difference in mean TAN/TN value compared to the GAC original (0.99) and GAC liquid only (0.95) reactors, highlighting the value of GAC in hydrolyzing urea to ammonia. Likewise in Phase 4, the mean daily increase in TAN/TN was significantly less in the no carrier reactor compared to reactors with GAC fed once or three times a day. A related conundrum is that in

Phase 3, the PC 'liquid only' reactor with no carriers achieved better hydrolysis than the PC 'original' reactor.

The decreasing performance of the reactor without carriers over time (relative to the reactors with carrier) suggests that the carriers contributed to sustaining the growth of the ureolytic microorganisms. Whether the carriers directly contributed by providing surfaces for attached growth is unclear. If biofilms were important, to explain the hydrolysis in the liquid phase in Phase 3, there must have been either significant migration of microorganisms into the liquid phase, or significant extracellular urease. The weak performance of the PC could be attributed to their small surface area and low contact with the inoculum as PC floated in the reactors due to being less dense than water. The mechanisms by which GAC improved urea hydrolysis is unclear. One hypothesis is that GAC may have indirectly affected the microbial community by adsorbing compounds that inhibited growth or enzyme activity.

4.3 Impact of hydraulic retention time on urea hydrolysis

We determined that HRT could be reduced while still hydrolyzing urea. In Phase 1, all reactors with feces and soil inocula had similar mean TAN/TN, regardless if the HRT was 4, 2, or 1.3 days. With an HRT of 2 days in Phase 2, hydrolysis was near complete in most reactors after 8 h. This suggests that a draw frequency of 8 h (0.33 days) could be paired with a draw volume percentage of 50% to produce an HRT of 16 h (0.67 days). In Phase 4, we determined that there was no significant benefit to more frequent feeding. One explanation is that urease was hydrolyzing urea at its maximum rate based on Michaelis-Menten kinetics [22]. Reducing the HRT to 16 h would be a large improvement over the days to weeks needed to hydrolyze urea without inoculum or carriers. Reducing HRT while still achieving near full hydrolysis can allow for reduced reaction times and/or reduced reactor volume. This will increase the feasibility of nitrogen recovery where space and treatment time are constrained.

5. Implications for urine treatment

Urea hydrolysis was accelerated by using soil as an inoculum and GAC as a carrier, achieving greater than 90% urea hydrolysis with an HRT of 2 days, which is lower than HRTs previously reported in the literature. Furthermore, the results suggest the HRT could be reduced to 16 h without reducing performance. The SBR configuration tested in the laboratory has the potential to be an adaptable design for scales ranging from individual toilets to larger building or neighbourhood systems. For example, in informal settlements with separating dry toilets [32], an SBR could passively hydrolyze the urine and discharge the ammonia-rich urine by gravity to a nitrogen recovery technology like ion exchange. Alternatively, where electricity is reliable and more mechanization is possible, such as the basement of an apartment building with separating flush toilets, an SBR for urea hydrolysis could be integrated with a number of nitrogen recovery technologies that require ammonia such as ammonia stripping, ion exchange, struvite precipitation, partial nitrification/distillation, and (bio)electrochemical methods. Additional research is warranted to inform optimal reactor design for these different contexts, such as the potential benefits of mixing.

Conductivity has the potential to provide real-time information on the extent of urea hydrolysis, in contrast to TAN measurements, which require time-consuming laboratory analysis. Conductivity can be measured either manually or with an online sensor for larger-scale designs, which is attractive for remote operation of decentralized systems. Overall, the findings from this research are promising for accelerating urea hydrolysis in fresh urine for subsequent nitrogen recovery processes that require ammonia.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author, KDO, upon reasonable request.

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