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Factors influencing the recovery of organic nitrogen from fresh human urine dosed with organic/inorganic acids and concentrated by evaporation in ambient conditions

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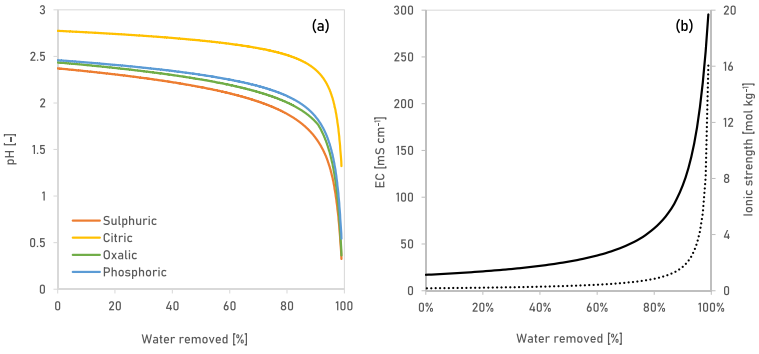
HIGHLIGHTS

- Oxalic/citric acid have high capacity to buffer pH changes in urine contaminated by urease.
- Nitrogen loss during acid dehydration likely due to urea degradation to ammonium cyanate
- Bio-based solid fertiliser produced by concentrating human urine by acid dehydration
- Acid dehydration yields fertiliser products with high nutrient content (>21 % N) and no calcite.

GRAPHICAL ABSTRACT

Effect of removal of water on (a) pH and (b) electric conductivity (EC, mS cm − 1; solid line) and ionic strength (mol kg − 1; dashed line plotted on the secondary y-axis) in fresh human urine dosed with four different organic and inorganic acids.



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ABSTRACT

To feed the world without transgressing regional and planetary boundaries for nitrogen and phosphorus, one promising strategy is to return nutrients present in domestic wastewater to farmland. This study tested a novel approach for producing bio-based solid fertilisers by concentrating source-separated human urine through acidification and dehydration. Thermodynamic simulations and laboratory experiments were conducted to evaluate changes in chemistry of real fresh urine dosed and dehydrated using two different organic and inorganic acids. The results showed that an acid dose of 1.36 g H_2SO_4 L^{-1}, 2.86 g H_3PO_4 L^{-1}, 2.53 g $C_2H_2O_4{\cdot}2H_2O$ L^{-1} and 5.9 g $C_6H_8O_7$ L^{-1} was sufficient to maintain pH ≤ 3.0 and prevent enzymatic ureolysis in urine during dehydration. Unlike alkaline dehydration using $Ca(OH)_2$ where calcite formation limits the nutrient content of fertiliser products (*e.g.* <15 % nitrogen), there is greater value proposition in acid dehydration of urine, as the products contain 17.9–21.2 % nitrogen, 1.1–3.6 % phosphorus, 4.2–5.6 % potassium and 15.4–19.4 % carbon. While the treatment recovered all phosphorus, recovery of nitrogen in the solid products was 74 % (± 4 %). Follow-up experiments revealed that hydrolytic breakdown of urea to ammonia, chemically or enzymatically, was not the reason for the nitrogen losses. Instead, we posit that urea breaks down to ammonium cyanate, which then reacts with amino and sulfhydryl groups of amino acids excreted in urine. Overall, the organic acids evaluated in this study are promising for decentralised urine treatment, as they are naturally present in food and therefore already excreted in human urine.

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1. Introduction

Recycling of human urine is currently attracting much attention within the research community (Harder et al., 2019; Larsen et al., 2021) and in popular media (Christofaro, 2022; Wald, 2022), mostly because of soaring fertiliser prices. Human urine is a year-round source of urea, phosphorus, potassium and micronutrients (Vinnerås et al., 2006). If all global urine-derived nutrients were to be returned to farmland, up to 25 % of the nitrogen and phosphorus demand in agriculture could be met (Simha, 2021b). Such recycling could make local food systems more resilient, as they would be less vulnerable to changes in the supply of synthetic and mineral fertilisers (Perez-Mercado et al., 2022). It would also reduce the negative impacts of agriculture and poor wastewater management on the environment, *e.g.* by reducing the flux of reactive nitrogen and eutrophication of water bodies (Tidåker et al., 2007; van Puijenbroek et al., 2018).

Human urine can be segregated from other household wastewater (Gundlach et al., 2021) and applied as liquid fertiliser at small scale (Kvarnström et al., 2006). However, to achieve mainstream urine recycling, especially at city scale, urine must be treated as close to the source as possible and converted into a concentrated liquid or solid fertiliser. Otherwise, transporting, storing and applying urine to farmland would be impractical and uneconomical (Trimmer and Guest, 2018), given that urine consists of 95 % water (Friedler et al., 2013). One of the best methods to remove water without losing nutrients is to treat fresh urine to capture ammonia (5–10 % of total nitrogen) and to inhibit urease-catalysed degradation of urea (about 85 % of total nitrogen) (Simha et al., 2021; Vasiljev et al., 2022). In fresh urine, urease can be reversibly inactivated by increasing the pH to >10 (Simha et al., 2022) or decreasing the pH to <4 (Ray et al., 2018).

Much work has been conducted with alkalised urine, presumably because alkaline earth chemicals are inexpensive and can be dosed passively to fresh urine (Randall et al., 2016; Simha et al., 2022). Technologies to concentrate alkalised urine and produce solid urine-based fertilisers are advancing rapidly, and many have been demonstrated at pilot scale (Riechmann et al., 2021; Simha et al., 2020a). However, this has not been the case for fresh urine treatment by acidification. Hellström et al. (1999), who conducted one of the earliest studies on acidification of urine, found that a one-time dose of 60 meq acid L^{-1} inhibited urea degradation in fresh urine for several months. More recently, Andreev et al. (2017), Ray et al. (2018) and Saetta and Boyer (2017) evaluated the inhibition of hydrolysis of urea in acidified urine and developed practical ways to perform acidification at source. Those studies showed that acidification can also prevent the precipitation of minerals in waterless sanitation systems that collect urine separately. However, little further analysis has been performed on the acidified urine produced in previous studies.

There is little value in acidifying urine if it is only intended to be stored and applied as liquid fertiliser. In that case, it would be simpler to allow urine to hydrolyse naturally and apply hydrolysed urine as a liquid fertiliser after storage. In this study, research on urine acidification was extended by evaluating whether fresh urine dosed with different acids can be dehydrated in ambient conditions to recover nutrients as dry solids. The one-time dose of two organic and two inorganic acids required to keep the pH of urine to <3 during acidification and dehydration was determined in laboratory experiments and thermodynamic simulations of chemical speciation for the system. Changes in physicochemical properties of the urine, the fate of nutrients and the composition of dehydrated urine products were examined. Advantages and disadvantages of dehydrating fresh urine after acid treatment, in comparison with dehydrating urine after alkaline treatment, were then considered. Overall, this study adds to the growing body of research on sanitation systems with the emphasis on recycling rather than removing nutrients from domestic wastewater.

2. Methods

2.1. Materials

Fresh urine was collected from 20 volunteers (male and female, aged 20–65 years) every day using sterile 500 mL polypropylene flasks. Donations were made at different times of the day and used in experiments on the same day. In total, about 75 L of fresh urine were collected through >240 donations. As fresh urine contains <5 % nitrogen in the form of ammonia nitrogen (Simha et al., 2021), the donations were pooled, mixed and fortified with 1.45 g ammonium chloride (NH_4Cl) L^{-1} before use in the experiments. Addition of NH_4Cl increased the concentration of ammonia nitrogen to about 10 % of total nitrogen in urine and allowed quantification of the different forms of nitrogen recovered during urine treatment (Vasiljev et al., 2022). The analytical method used for measuring nitrogen in this study had a standard deviation of at least ±5 %.

Two organic acids and two inorganic acids of reagent grade were evaluated. These were: citric acid ($C_6H_8O_7$), oxalic acid dihydrate ($C_2H_2O_4{\cdot}2H_2O$), sulphuric acid (H_2SO_4) and phosphoric acid (H_3PO_4). These acids were selected because they are already produced industrially and available globally. All chemicals used were of analytical grade.

2.2. Thermodynamic simulations

To inform the study design, thermodynamic simulations were first performed to estimate the acid dose required to reduce the pH of four different previously reported fresh urine compositions (FU_1-FU_4) to a value of ≤3 (Tables S1 and S2 in Supporting Information (SI)). Since the simulations did not consider organic substances excreted in human urine, the average simulated acid dose was approximately doubled to give the experimental acid dose. Additional simulations were later conducted to estimate the acid dose required to treat the fresh urine composition used in this study (FU_{12}) and other urine compositions reported in the literature (FU_5-FU_{11}) (Table S3 in SI). The effects of removing water on physicochemical properties (pH, electrical conductivity and ionic strength), chemical speciation and major solids formed in acidified urine at thermodynamic equilibrium were also simulated. All these simulations were carried out using the 'Mixed Solvent Electrolyte' model option in the software OLI Stream Analyser (OLI Systems Inc, 2020). The urine compositions used in the modelling are shown in Table S1.

2.3. Experimental procedure

The experiment involved dehydrating acidified fresh urine in polypropylene boxes (17 × 21 × 11 cm; Jysk, Sweden) placed inside a modified incubator (Heratherm IGS400, Thermo Scientific, USA). The incubator had four stainless steel trays, each of which could hold up to six boxes. A computer fan (DP201A, SUNON, 2550 rpm) was placed above each box for ventilation.

At the start of the experiment, 250 mL of fresh urine were added to each box and the boxes were then dosed with the predetermined amount of acid required to treat a total of 4 L of urine per box. The boxes were placed inside the incubator and urine was dehydrated in ambient conditions (20 ± 2 °C and 20 % relative humidity). These drying conditions were chosen so as to avoid chemical hydrolysis of urea (Simha et al., 2020b). To start a new drying cycle, 100–750 mL fresh urine were added and the boxes were returned to the incubator. The experiment concluded when a total of 4 L of fresh urine had been dehydrated per box. The average evaporation rate of urine in the incubator was 16 ± 5 kg d^{-1} m^{-2}.

2.4. Follow-up experiments evaluating fate of nitrogen

Two follow-up experiments were performed to evaluate the fate of urea and ammonia nitrogen during the treatment. In the first of these experiments, a solution containing 120 g urea and 4 g sulphuric acid in 500 mL

Milli-Q water was prepared and distributed between 50-mL centrifuge tubes. This solution was intended to simulate fresh urine acidified with a one-time dose and concentrated by a factor of eight (assuming that fresh urine contains 15 g urea L^{-1}). The tubes were stored at 20 ± 2 °C and sampled destructively in triplicate every week for a period of four weeks. Changes in pH, electrical conductivity and concentration of total ammonia nitrogen were then monitored. In the second follow-up experiment, synthetic fresh urine concentrated by factor of 16, 32, 64 and 100 was prepared, using a similar procedure to that described by Simha et al. (2022). The synthetic urine was acidified using sulphuric acid to pH <1.0, distributed between 50 mL centrifuge tubes, and changes in pH and electrical conductivity were monitored using duplicate samples taken every week for a total of six weeks.

2.5. Sampling and analysis

For analysis of the initial nutrient composition, a representative sample of fresh urine used in the experiment was taken and acidified to pH <2 by adding 1 M H_2SO_4 and stored at 20 °C. The weight of the boxes at the start and end of every drying cycle was measured using a weighing balance with 0.01 g precision (Adventurer Pro AV2102, OHAUS Europe, Switzerland). Fresh urine and dehydrated urine collected at the end of the experiment were analysed for total solids (TS) and volatile solids (*VS*). Samples were dried for 7 days at 30 °C to determine TS, and further dried at 80 °C for 24 h and at 105 °C for 24 h to determine TS at those temperatures. They were then combusted for 6 h at 550 °C to determine *VS*. Mass concentration factor was calculated using the equation presented in Vasiljev et al. (2022).

The pH and electrical conductivity (EC) of fresh urine were measured before and after it was added to the boxes, and after the urine had been dehydrated. The pH was measured using an electrode (Accumet 12-620-AE6, Fisher Scientific, United States) attached to a pH meter (Accumet AE150, Fisher Scientific, United States). The EC was measured using a TetraCon 325 probe attached to a handheld EC meter (Cond 340i, WTW, Germany).

The concentration of total nitrogen, ammonia nitrogen, urea and chemical oxygen demand (COD) in fresh urine was determined colorimetrically using Spectroquant® test kits (Merck KGaA, Darmstadt, Germany) and a photometer (NOVA 60 A, Merck KGaA, Darmstadt, Germany). To determine concentration of total nitrogen, samples were diluted 1000-fold, digested using a Spectroquant® Crack-Set 20 test kit (114963) and analysed for concentration of nitrate nitrogen in the range 0.1–25 mg L^{-1} (kit 109713). To determine concentration of total ammonia nitrogen, samples were diluted 10-fold and analysed using a Spectroquant® ammonium test kit (100683) in the concentration range 2–150 mg L^{-1}. Concentration of urea nitrogen was determined as the difference in concentration of total ammonia nitrogen between fresh urine and fresh urine completely hydrolysed by addition of jack bean (*Canavalia ensiformis*) urease (EC 3.5.1.5). To determine COD, fresh urine was diluted 100-fold and analysed using a Spectroquant® COD Cell Test (109772) in the concentration range 10–150 mg L^{-1}.

The concentrations of phosphorus, potassium and other elements (Ca, Na, S, Mg) were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES) using an Optima Avio 200 (PerkinElmer, USA) optical emission spectrometer. Prior to ICP-OES, 1 mL fresh urine or 0.5 g of dehydrated urine was digested using a mixture of 9 mL 65 % HNO_3 and 1 mL 30 % H_2O_2 and diluted with Milli-Q water. The total nitrogen and total carbon content of dehydrated urine was measured by dry combustion using an elemental analyser (TruMac CN, LECO Corp., USA).

2.6. Statistical analysis

One-way analysis of variance (ANOVA) at 95 % confidence interval was performed to test whether the different acids added to fresh urine significantly influenced: a) recovery of nitrogen from the urine; b) physicochemical properties of the urine; and c) elemental composition of the dehydrated urine produced in the experiment. When significant differences were found, Tukey's honest significant difference (HSD) post-hoc test at 95 % confidence interval was applied. When variables of interest were found, linear regression analysis was also performed. RStudio version 1.2.5042 and R version 4.0.0 (RStudio Team, 2016) were used to perform all statistical analyses.

3. Results

3.1. Simulation of urine acidification

Thermodynamic simulations for four urine compositions (FU_1-FU_4) used in a previous study by our research group (Simha et al., 2022) suggested that dosing fresh urine with 0.35 g sulphuric acid L^{-1}, 2.5 g citric acid L^{-1}, 0.8 g oxalic acid L^{-1} or 0.76 g phosphoric acid L^{-1} would reduce the pH of urine from 6.2 (±0.2) to ≤3. Therefore, in laboratory experiments fresh urine was dosed with 0.8 g sulphuric acid L^{-1}, 4.5 g citric acid L^{-1}, 1.8 g oxalic acid L^{-1} or 1.8 g phosphoric acid L^{-1} (Table S2). Further simulations on the urine composition used in the experiments (FU_{12}) showed that 0.4 g sulphuric acid L^{-1}, 2.6 g citric acid L^{-1}, 0.64 g oxalic acid L^{-1} or 0.86 g phosphoric acid L^{-1} was required to decrease the pH value to ≤3 (Fig. 1). Overall, for the broad range of urine compositions considered (FU_1-FU_{12}), the average dose required to acidify fresh urine to pH ≤3 in simulations was 0.5 ± 0.1 g sulphuric acid L^{-1}, 3.2 g ± 0.7 citric acid L^{-1}, 0.8 ± 0.2 g oxalic acid L^{-1} or 1.1 ± 0.2 g phosphoric acid L^{-1}.

3.2. Simulation of dehydration of acidified urine

The thermodynamic simulations showed that removing water from fresh urine dosed with any one of the four acids decreased the pH (Fig. 2a). To reduce the simulated pH to <2, 75 % of the water had to be removed from urine dosed with sulphuric, phosphoric or oxalic acid, whereas 95 % of the water had to be removed from urine dosed with citric acid. When 99 % of the water was removed, the pH was predicted to drop to <0.5, except for urine dosed with citric acid where the minimum pH was 1.4. Irrespective of the acid used, with removal of water the EC increased from 16.9 to 300 mS cm^{-1}. The ionic strength also increased, from 0.17 mol kg^{-1} at 0 % water removal to 16.1 mol kg^{-1} at 99 % water removal (Fig. 2b).

No solids were predicted to form in fresh acidified urine according to the simulations. However, urea ($CO(NH_2)_2$), halite (NaCl), salammoniac (NH_4Cl), biphosphammite (KH_2PO_4), sylvite (KCl) and aphthitalite ($K_2SO_4{\cdot}KNaSO_4$) were predicted to form only when >99 % of the water was removed from urine composition FU_{12}. Similar simulations performed for urine compositions FU_1 and FU_7 suggested that syngenite ($K_2SO_4{\cdot}CaSO_4{\cdot}H_2O$) and gorgeyite ($K_2SO_4{\cdot}5CaSO_4{\cdot}H_2O$) were also formed. In addition to halite, natroxalate ($Na_2(COO)_2$) was predicted to form in urine dosed with oxalic acid and sodium dihydrogen citrate ($NaH_2(C_6H_5O_7)$) was predicted to form in urine dosed with citric acid.

3.3. Experimentally determined changes in physicochemical properties

On the first day of the treatment, overdosing fresh urine with the different acids decreased the pH value to between 0.9 (sulphuric acid) and 1.7 (citric acid). Drying acidified urine further decreased the pH (Fig. 3), as predicted by the thermodynamic model (Fig. 2a). Adding fresh urine to concentrated acidified urine always increased the pH. For all treatments, there was a linear fit ($R^2 > 0.92$; $p < 0.01$) between the volume of urine treated per equivalent of acid dosed and the pH of urine measured after every urine addition. The volume of urine that could be treated before the pH increased to >3 was 735, 350, 395, and 170 mL per gram of sulphuric, phosphoric, oxalic and citric acid, respectively. When urine was treated beyond this pH threshold, drying urine increased the pH, unlike at the beginning of the treatment.

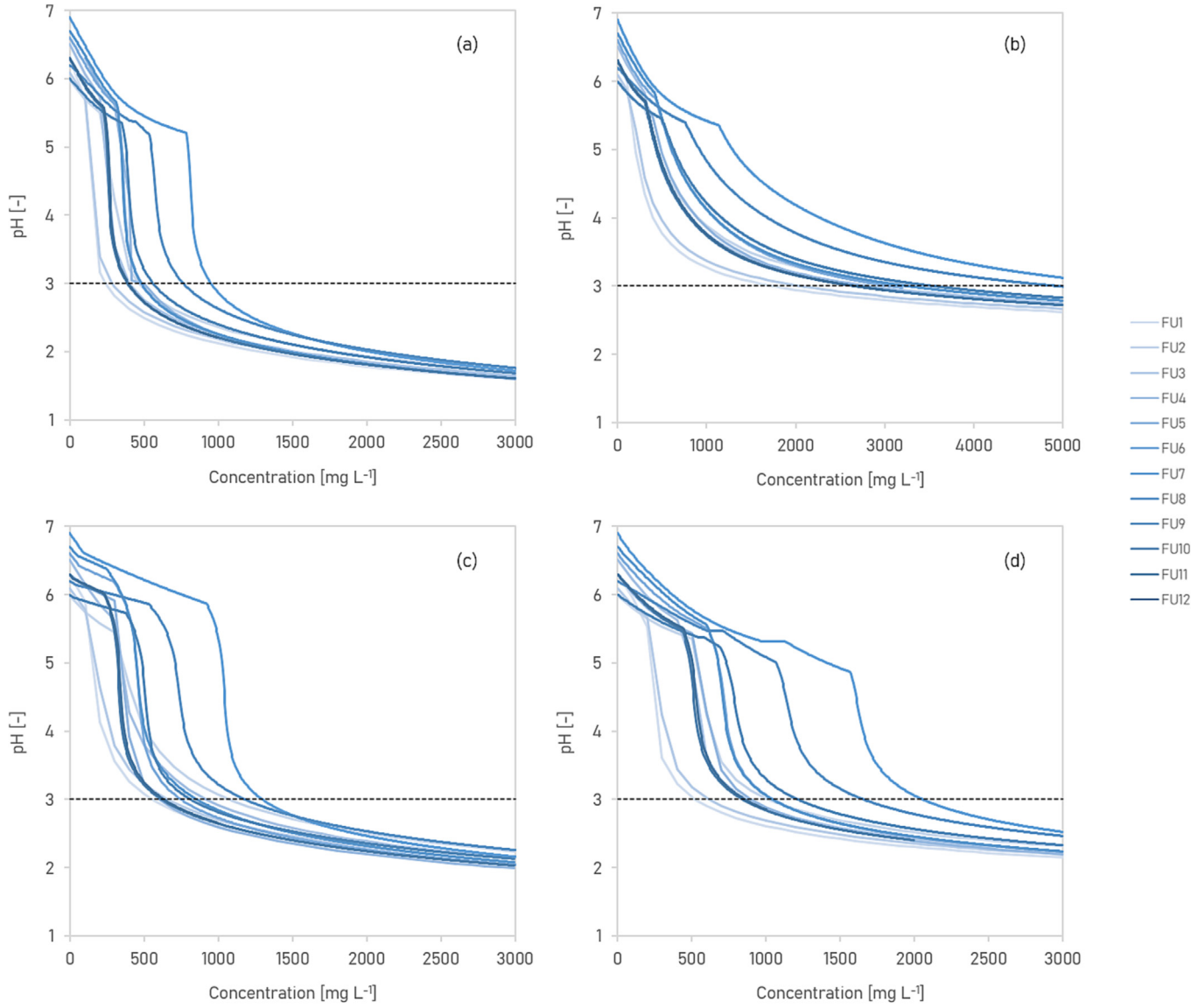

Fig. 1. Simulated change in the pH of fresh human urine when dosed with (a) sulphuric acid, (b) citric acid, (c) oxalic acid dihydrate and (d) phosphoric acid at 25 °C. The dashed line at pH 3.0 shows the acid dose required to inhibit urease-catalysed hydrolysis of urea in fresh urine. Urine composition (FU_1-FU_{12}) is described in Table S1 in Supporting Information.

3.4. Experimentally determined fate of elements

Fresh urine added in the treatments had a total nitrogen concentration of 6.1 g L^{-1}, of which 77 % was urea nitrogen, 9 % total ammonia nitrogen and 14 % organic nitrogen (excluding urea). Treatment by acidification and dehydration recovered 74 % ± 4 % of the total nitrogen present in fresh urine. There was 100 % recovery of phosphorus in all treatments. There was no significant difference ($p > 0.05$) in recovery of nitrogen and phosphorus from fresh urine between the different acid treatments (Table 1).

The TS content of the fresh urine added in the treatments was 25.9 ± 0.1 g L^{-1}. Dehydrating urine reduced its mass by >97 %, which is equivalent to a mass concentration factor (*CF*) of 36. The amount of dehydrated urine left at the end of the treatment varied between 24 g L^{-1} and 29 g L^{-1}. When dehydrated urine was further evaporated, either at 80 °C or 105 °C for 24 h, the mass of dried urine was reduced by an additional 8–17 %, as some water of crystallisation and volatile solids were lost (Fig. S1 in SI).

The elemental composition of the dehydrated urine on a wet-weight basis is summarised in Table 2. Since the same urine was added in all treatments, the variations in elemental content were due to type of acid used and differences in acid dose between treatments (from 1 g sulphuric acid L^{-1} to 5 g citric acid L^{-1}). Treatments that had a low acid dose yielded dehydrated urine with a significantly lower content of TS and significantly higher content of nitrogen. Treatments involving sulphuric and phosphoric acid produced dehydrated urine with significantly higher sulphur and phosphorus content, respectively. Treatments involving the two organic acids produced dehydrated urine with significantly higher carbon content. Concentrations of elements (Na, K, Ca, Mg) that were not supplied by adding acid to the urine did not vary in the dehydrated urine.

4. Discussion

4.1. Factors determining the fate of nitrogen

It has been shown that acidification of fresh human urine to pH values <4 can prevent enzymatic degradation of urea to ammonia (Ray et al., 2018; Saetta and Boyer, 2017). However, a pH of ≤3 is desirable because there is evidence of urease activity at pH 4 (Ray et al., 2018) and because inhibition of urease by acidification is reversible (Krajewska, 2009). In this study, a one-time dose of organic or inorganic acid added at the start

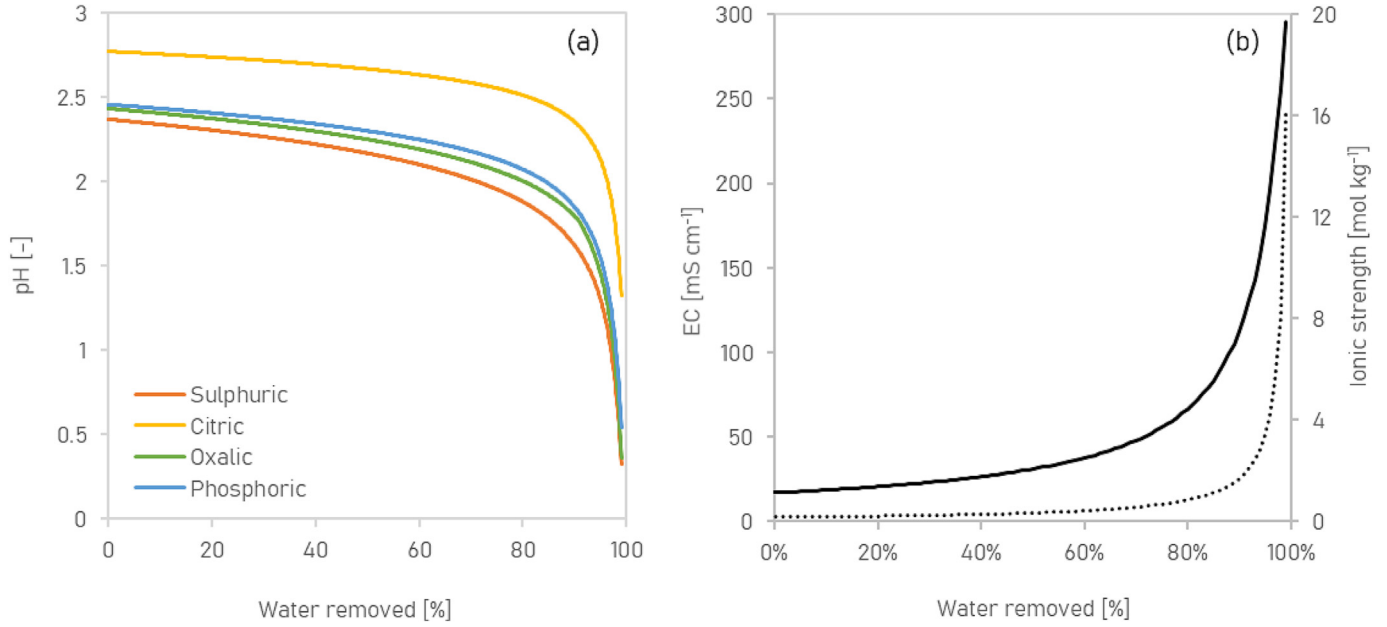

Fig. 2. Simulated effect of removal of water on (a) pH and (b) electric conductivity (EC, mS cm^{-1}; solid line) and ionic strength (mol kg^{-1}; dashed line plotted on the secondary y-axis) in fresh human urine dosed with four different organic and inorganic acids.

of the treatment was able to maintain pH ≤3 during consecutive cycles of urine addition and dehydration. The one-time dose needed to keep the pH to <3.0 was 1.36 g sulphuric acid L^{-1}, 2.86 g phosphoric acid L^{-1}, 2.53 g oxalic acid L^{-1} or 5.9 g citric acid L^{-1} of urine. The required acid dose was about three-fold higher than simulated by a thermodynamic model, which did not account for the influence of organic compounds present in real urine. The concentration of organic substances in fresh urine used in the study was 5.3 g COD L^{-1}. Our results are consistent with those of Ray et al. (2018) who showed that 2 g citric acid L^{-1} is needed to acidify synthetic fresh urine (containing no organic compounds except urea) to pH 3, but 6.2 g citric acid L^{-1} is needed to acidify real fresh urine.

Table 1
Recovery of nitrogen (N_{rec}) and phosphorus (P_{rec}) from fresh human urine dosed with different acids and dehydrated in ambient conditions. Values are mean ± standard deviation (n = 3). At 95 % confidence interval, there was no significant difference between the treatments in recovery of the two elements.

	Sulphuric acid	Phosphoric acid	Citric acid	Oxalic acid dihydrate
N_{rec}	72 ± 2	75 ± 5	73 ± 1	75 ± 4
P_{rec}	101 ± 3	96 ± 7	97 ± 9	101 ± 12

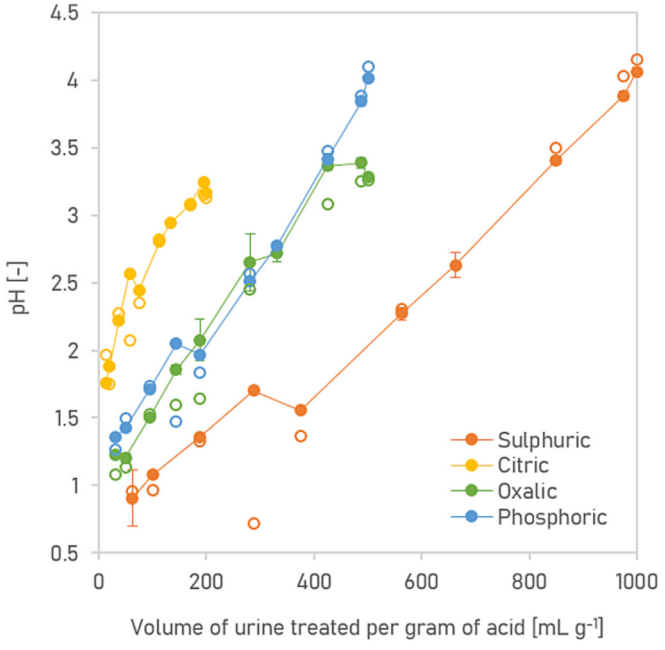

Fig. 3. pH of urine during treatment with different organic and inorganic acids. The acids were overdosed at the start of treatment and fresh urine was added and dehydrated cumulatively. Overall, 4 L urine was treated with 4 g sulphuric acid, 3.2 g phosphoric acid, 3.2 g oxalic acid dihydrate or 20 g citric acid. Filled circles show pH after urine addition and empty circles show pH after urine dehydration.

There was complete recovery of phosphorus in all treatments. However, irrespective of the acid used, the average recovery of nitrogen was 74 % (± 4 %). One explanation for the loss of nitrogen could be hydrolysis of urea, either chemically because of low pH and high ionic strength or enzymatically because of acid-resistant urease. As the half-life of urea at pH <4 and 20 °C is >500 days (Chin and Kroontje, 1963), the effect of chemical hydrolysis can be ignored. Human urine can contain urease-positive bacteria (Schönning et al., 2002). During the experiments, we observed fungal growth on the surface of urine, which could also be a source of urease (Kappaun et al., 2018). In unbuffered solutions such as human urine, there is evidence that *Helicobacter*

Table 2
Elemental composition (%) of the products obtained by dehydrating fresh human urine dosed with different organic and inorganic acids. The amount of dry solids (TS; g L^{-1}) collected at the end of the treatment is also shown.

	Sulphuric acid	Citric acid	Phosphoric acid	Oxalic acid dihydrate
N	20.9 ^{a}	17.8 ^{b}	20.1 ^{a}	21.2 ^{a}
P	1.3 ^{b}	1.1 ^{b}	3.6 ^{a}	1.3 ^{b}
K	5.8 ^{a}	4.2 ^{b}	5.5 ^{a}	5.6 ^{a}
C	15.4 ^{b}	19.4 ^{a}	15.0 ^{b}	16.7 ^{a}
Na	5.2 ^{a}	3.6 ^{c}	4.8 ^{ab}	4.7 ^{ab}
S	2.6 ^{a}	0.94 ^{c}	1.17 ^{bc}	1.24 ^{b}
Ca	0.25 ^{a}	0.23 ^{b}	0.25 ^{a}	0.23 ^{b}
Mg	0.17 ^{a}	0.15 ^{ab}	0.16 ^{a}	0.16 ^{b}
TS	24.3 ^{c}	28.9 ^{a}	25.1 ^{b}	24.7 ^{b}

Values are average of three replicates. Within rows, values marked with different letters (a,b,c) are significantly different (α = 0.05).

pylori urease can hydrolyse urea at pH 3.0 (Ha et al., 2001). If there was urease contamination of the urine in our experiment and if this urease was active in the system, then urea hydrolysis should have elevated the pH of urine. The maximum pH of urine measured in the experiment was 4.2 (Fig. 3). According to our thermodynamic simulations, if only 5 % of the urea present in FU_{12} urine had hydrolysed, then the pH would have increased to >8 and dehydration would not have reduced the pH of urine to <4 (Fig. 4). We therefore believe that hydrolytic breakdown of urea to ammonia, chemically or enzymatically, was not the reason for the nitrogen losses.

According to Lilov and Kirilov (2019), urea can degrade in the presence of sulphuric acid. Those authors found that the pH of a solution containing 20 % nitrogen in the form of urea and 0.18 % sulphuric acid increased from 2.9 to 5.5 in 30 days, but offered no insights into the degradation pathway. To evaluate whether this was also the case in our study, in a follow-up experiment we monitored a solution of urea and sulphuric acid for 28 days. We found no increase in pH, while the increase in concentration of total ammonia nitrogen in relation to amount of urea added initially was negligible (Fig. S2 in SI). In a second follow-up experiment involving concentrated synthetic urine with initial pH of 1.0, we found that the pH increased slightly to 1.22 over 28 days (Fig. S3 in SI). This could be because urea was decomposed to ammonium cyanate. Dirnhuber and Schütz (1948) found that 0.8 % of urea was converted to cyanate when 0.25 M urea solution in acetate buffer (pH 5.0) was kept at 38 °C for seven days. Those authors also observed rapid decomposition of cyanate at pH <3. According to Vogels et al. (1970), carbonate, phosphate and citrate can also catalyse the decomposition of cyanate, and all are present in fresh urine (Putnam, 1971). In addition, it is known that cyanate reacts readily with amino and sulfhydryl groups of amino acids in physiologically relevant conditions (Stark et al., 1960). Amino acids are excreted in urine (Putnam, 1971), but their reaction kinetics with cyanate in the conditions prevailing in the present study are unknown. The EC of urine before the final drying cycle was 73 ± 5 mS cm^{-1} and the thermodynamic model simulated that the ionic strength of dehydrated urine was 16.1 mol kg^{-1}. To our knowledge, no previous study has evaluated the fate of nitrogen-containing organic compounds excreted in urine (*e.g.* urea, creatinine, hippuric acid, uric acid and amino acids) in such physicochemical conditions. Determining the fate and degradation pathway of major organic compounds in urine during acidification and dehydration could help develop treatment steps that limit the loss of nitrogen.

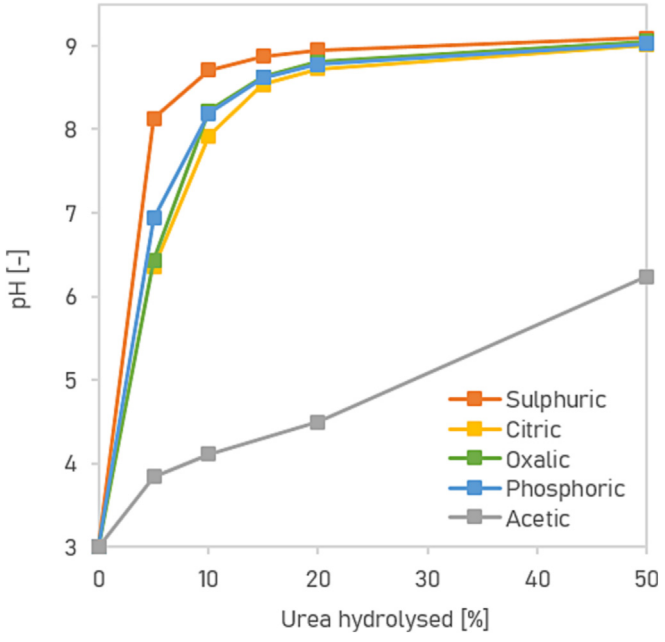

Fig. 4. Effect of urea hydrolysis on the pH of urine dosed with different acids. The simulation is for urine composition FU_{12} and acid doses are the same as those used in the experiments. For acetic acid, a dosage of 22 g L^{-1} was simulated.

4.2. Implications of this study for treatment of urine by dehydration

In previous studies by our research group, we reported on the technology *alkaline dehydration*, in which fresh urine is alkalised using $Mg(OH)_2$ (Vasiljev et al., 2022) and $Ca(OH)_2$ (Randall et al., 2016) and dehydrated at temperatures of 20–40 °C. All nutrients including nitrogen can be recovered if urine is dosed with 0.6 g $Mg(OH)_2$ L^{-1} to capture ammonia (Simha et al., 2022) and dosed with 6–12.5 g $Ca(OH)_2$ L^{-1} before dehydration to capture organic nitrogen (Riechmann et al., 2021; Simha, 2021a). One drawback with treating alkalised urine is dissolution of carbon dioxide present in air during dehydration, which converts hydroxides to carbonates, increases the TS content of urine and lowers the pH (Simha et al., 2018). Since urease inhibition is reversible below pH 10 (Geinzer, 2017), the treatment must either be stopped or more hydroxide must be added when the threshold pH is reached. In comparison, absorption of CO_2 in acidified urine has no effect on the pH of urine, since the pK_{a1} of H_2CO_3 is 6.8 at 25 °C (Nigretto, 2001). Therefore, the acid dose required to inhibit urease during urine dehydration is lower than the alkali dose required. As a result, dehydrated urine produced by acid treatment has lower TS content and higher nitrogen content (18–21 % depending on the acid used). In contrast, the highest nitrogen content measured for urine treated by alkaline dehydration is 11.6 % (Vasiljev et al., 2022).

Unlike previous studies on urine acidification (Saetta and Boyer, 2017), we do not recommend dosing fresh urine with acetic acid if the objective of the treatment is to concentrate urine by evaporation. According to our simulations, 22 ± 9 g acetic acid L^{-1} would be required to acidify fresh urine to an initial pH of 3.0 (Table S3). This acetic acid dose is 4-fold higher than that of other acids used in this study, and in fact almost the same as the TS content of fresh urine (26 g L^{-1}). Therefore, when acetic acid dosed fresh urine is concentrated (by drying or using other techniques), its nitrogen content cannot exceed 15 %. Concentrated acetic acid also has a lower flashpoint (39 °C) than citric acid (100 °C) and oxalic acid (166 °C) (NCBI, 2022).

The organic acids evaluated in this study exist as solids at room temperature. This makes them easier to store and apply in household-scale urine treatment systems. They are naturally present in food and therefore already excreted in human urine. The acid strength of oxalic acid (pK_a = 1.23) is higher than that of citric acid (pK_a = 3.1, 4.74 and 5.4) and acetic acid (pK_a = 4.75), but lower than that of sulphuric acid (pK_a = −3, 1.92) (Zumdahl and Zumdahl, 2007). In combination with their salts, organic acids also have higher capacity to buffer the pH of urine than inorganic acids (Fig. 4), which is important to prevent the loss of ammonia nitrogen when urine is contaminated with urease.

5. Conclusions

This study demonstrated that acid dehydration of fresh human urine is an effective treatment method for producing solid bio-based fertilisers with a high nutrient content. Organic acids were as effective as inorganic acids in stabilizing urine, maintaining the urine pH below 3.0 and recovering nutrients during consecutive cycles of fresh urine addition and dehydration. Organic acids showed higher capacity than inorganic acids in buffering changes in pH and preventing the loss of ammonia nitrogen from urine contaminated with urease. The volume of urine that could be treated without crossing the threshold pH necessary to inhibit urease-catalysed hydrolysis of urea (pH 3.0) was 735, 350, 395, and 170 mL per gram of sulphuric, phosphoric, oxalic and citric acid, respectively.

Unlike in alkaline dehydration, where higher alkali dose (*e.g.* 6–12.5 g $Ca(OH)_2$ L^{-1}) and calcite formation limit the nutrient content of the fertiliser products, acidification-dehydration required a lower acid dose (*e.g.* 1 g H_2SO_4 L^{-1}) and produced fertilisers with lower total solids content and higher nutrient content (18–21 % nitrogen).

Thermodynamic simulations and follow-up experiments involving two controls (urea solution and concentrated synthetic urine) provided further insights into pathways of nitrogen transformation and loss during acidification treatment. We concluded that hydrolytic breakdown of urea to ammonia, chemically or enzymatically, was not the reason for observed nitrogen losses. Instead, we posit that urea breaks down to ammonium cyanate, which then reacts with amino and sulfhydryl groups of amino acids excreted in urine. To limit the loss of nitrogen during acid dehydration, further research is necessary to evaluate the degradation pathways of nitrogen-containing organic compounds in concentrated urine.

Code availability

Code available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Prithvi Simha: Conceptualization, Formal analysis, Validation, Visualization, Writing – original draft. **Anastasija Vasiljev:** Conceptualization, Investigation, Data curation, Formal analysis, Writing – original draft. **Dyllon G. Randall:** Conceptualization, Software, Writing – review & editing. **Björn Vinnerås:** Conceptualization, Funding acquisition, Resources, Writing – review & editing.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Declaration of competing interest

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Appendix A. Supplementary data

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