Effect of storage on physicochemical characteristics of urine for phosphate and ammonium recovery as struvite

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ABSTRACT

Since human urine alone contributes to 90% of phosphorous (P) and 50–70% of nitrogen (N) load to municipal wastewater, retrieval of nutrients in the form of struvite (magnesium ammonium phosphate) from urine has gained much attention as it can act as an excellent slow-release fertilizer for plant growth. The present study aimed to comprehend the effect of urine storage on its physicochemical characteristics for the recovery of ammonium and phosphate in the form of struvite by adding magnesium chloride as an external source of magnesium (Mg). Various influencing factors such as pH, conductivity, salinity, total dissolved solids, and ion concentrations of phosphate, ammonium, calcium, nitrate, sodium and potassium were monitored over a period of 25 days to delineate its role on struvite recovery. The highest yield of 619.3 ± 54.41 mg L^{-1} of struvite was obtained on day 24. It was found that phosphate and ammonium recovery reached up to 90% with the storage of 25 days. Excessive addition of Mg increased both struvite yield and phosphate recovery (by 20%) but affects struvite purity undesirably due to the formation of struvite analogs. Statistical analysis with Spearman's co-efficients revealed that total dissolved solids exhibited a strong positive correlation for struvite yield.

1. Introduction

Every individual produces 1–1.5 L of urine per day in 4–5 times and as an average excretes 500 L urine per year (Karak and Bhattacharyya, 2011). Urine accounting for only 1% of the volume of wastewater, actually contains over 50–70% and 90% of the Nitrogen (N) and Phosphorous (P) excreted by humans, making it an ideal feedstock for struvite recovery (Bonvin et al., 2015; Tilley et al., 2008a). Struvite ($MgNH_4PO_4 \cdot 6H_2O$) is a bioavailable phosphate fertilizer recovered from wastewater having the potential to replace commercial fertilizers derived from the fast depleting, finite phosphate reserves. For instance, China's municipal domestic wastewater contains P approximately equivalent to 5.5% of its chemical fertilizer phosphorus consumption. Urine contains a meagre concentration of heavy metals (Simha and Ganesapillai, 2017), which is also lower than that of farmyard manure and has less cadmium than artificial P-fertilizers, making them cleaner fertilizers. Substantial separation of urine at source would thus allow nutrient recycling from a concentrated nutrient solution and, at the same time, obviate advanced nutrient removal including nitrification, de-nitrification and phosphorus elimination (Wilsenach and Van Loosdrecht, 2004).

The fertilization value of human urine and its use as a crop nutrient source has received greater attention among researchers in recent times (Karak and Bhattacharyya, 2011). It has been used in varying extents for a wide array of crop fertilization in different countries. For instance, in the Manipur state of India, harvests of potatoes and chilies were very good when urine was used as fertilizer as compared to crops fertilized with chemical fertilizer such as diammonium phosphate, urea and potash (Karak and Bhattacharyya, 2011). Yet, its direct use as fertilizer has not been advocated because human urine is a fast-acting liquid fertilizer that requires careful application and regulation, the absence of which, can cause volatilization of intrinsic ammonia (a greenhouse gas), increase soil conductivity, salinity and pH resulting in poor agro-productivity or in some instances, crop failures (Heinonen-Tanski and Van Wijk-Sijbesma, 2005). Stored urine has a pungent smell and is prone to N losses due to ammonia volatilization; the high water content renders transport to cropping regions costly; and undesired constituents (such as pathogens, emerging contaminants like synthetic hormones, pharmaceuticals and their metabolites) pose a health risk to farmers and consumers. Moreover, the life cycle assessment of cross-comparisons

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with conventional wastewater treatment plants (WWTPs) indicate that significantly large volumes of urine are required to provide a fertilising effect equivalent to synthetic fertilisers. Large volumes necessitate additional investment for urine collection, handling, storage and transportation to farmlands which tends to reduce systemic efficiency and cost savings versus conventional systems (Tidåker et al., 2007). However, many researchers globally advocated struvite precipitation as a favored approach for the removal and recovery of considerate amount of phosphorus and nitrogen as NH_4^+ from human urine.

To date, struvite precipitation takes place under alkaline conditions, which is being achieved by the addition of caustic soda to increase the pH and induce precipitation with magnesium. Still, researchers around the globe were trying to explore the cheaper alternatives for chemical additions to escalate the pH. During storage under non-sterile conditions, the urea present in urine is hydrolyzed to ammonia/ammonium and carbonate due to microbial activity, which in results raises the pH to around 9 and triggers the precipitation of calcium and magnesium in the form of carbonates and phosphates (Udert et al., 2003a). The enzyme urease (urea amidohydrolase) hydrolyzes urea to ammonia and carbamate, where the latter compound decomposes spontaneously to carbonic acid and a second molecule of ammonia.

(LIU et al., 2008) reported that urea could be hydrolyzed entirely in the collection tank in a day if urease was added with sufficient mixing and the hydrolysis temperature was maintained at 25 °C. The increase of pH and ammonia concentration by ureolysis promotes hydroxyapatite formation and enables the precipitation of struvite and calcite (Udert et al., 2003b). Although previous studies show low or no presence of citrate and hippurate in stored urine, there is limited published data on the extent of endogenous metabolite degradation during storage. Other impacts of storage and hydrolysis include the presence of a high concentration of ammonia, which can act as a biocide to reduce the number of pathogens (Jagtap and Boyer, 2018).

Previous research works on the storage of urine were carried out even up to a duration of 8 months to analyze the desired conditions for struvite precipitation (Gethke et al., 2015). However, continuous monitoring of the physicochemical parameters of urine will enable us to arrive at the required conditions at a comparatively earlier time. The novelty of this research lies in the day-wise monitoring of ten key parameters and elucidating the kinetics of phosphate and ammonium concentration during struvite formation. Another modernity of the work is studying the effect of excess addition of magnesium on struvite purity and formation of its analogs.

The present study aimed to comprehend the effect of storage on the changes of physicochemical characteristics of human urine that favored for the efficient recovery of N and P as struvite. The physicochemical characteristics of human urine, such as pH, conductivity, salinity, total dissolved solids (TDS), along with the ion concentrations of phosphate, ammonium, calcium, nitrate, sodium and potassium were analyzed daily to evaluate the effect of storage on struvite recovery. Further, the impact of excess addition of magnesium on struvite recovery was studied. The formation and the purity of the struvite were verified by the detailed characterization of obtained crystals through X-ray diffractometer (XRD) and Fourier transform infrared (FTIR) spectroscopic analysis. The kinetics of struvite formation, along with recovery percentage and yield, were used to evaluate the overall process efficiency. The effect of the addition of excess magnesium on the purity of struvite obtained has also been elucidated.

2. Materials and methods

2.1. Urine collection and preservation

The undiluted human urine samples were collected from healthy male individuals on a regular diet without any medical preconditions and pooled together to store at −20 °C before use for struvite precipitation experiments. In order to comprehend the effect of storage under various temperatures, a portion of the sample was kept continuously on a deep freezer at −20 °C throughout the study period and earmarked as a control sample for the study. While initiating the struvite precipitation experiments, rest of the samples were trifurcated and the experiments were carried out at ambient room temperature (25 ± 3 °C) for a period of 25 days under three varying dosages of Magnesium salt such as without the addition of Mg, with equimolar addition of Mg and excessive addition of Mg. In this study, magnesium chloride hexahydrate ($MgCl_2.6H_2O$) was chosen as the external source of Mg and the duration was 25 days based on pH variation without influencing the purity and recovery of struvite. For analysis of physicochemical parameters and struvite precipitation, the required volume of urine samples was withdrawn from the corresponding container each day and examined. For analysis, it was maintained at 4 °C until it was completely thawed and later analyzed.

2.2. Characterisation and compositional analysis of urine

For the present study, undiluted human urine samples were collected from the healthy male individuals in the early morning for three consecutive days, on a regular diet without any medical preconditions and pooled together before use. Batch experiments were carried out to estimate the struvite yield with the real-time human urine solutions, thus neglected the consideration of heavy metals and pharmaceuticals existing at an insignificant level. The physical parameters of urine, such as pH, conductivity, total dissolved solids (TDS) and salinity was measured using a multiparameter water quality meter (LMMP-30 Labman Scientific Instruments Pvt. Ltd. India). The phosphate concentration was estimated by the spectrophotometric method of Murphy and Riley using stannous chloride at the absorbance of 650 nm. Ammonium estimation was done by the phenol-hypochlorite method using a double beam UV–Vis Spectrophotometer (Model 2230, Systronics, India) at 630 nm. The salicylic method was utilized for the estimation of nitrate concentration at 410 nm. Potassium, sodium and calcium concentrations in the urine sample were detected by using a microprocessor-based flame photometer (Model 1385, ESICO, India). All the analysis was carried out in triplicates and the values were recorded.
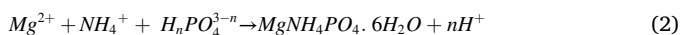
2.3. Struvite precipitation experiments

In this study, magnesium chloride hexahydrate ($MgCl_2.6H_2O$) of analytical grade procured from HiMedia (India) was used for struvite precipitation experiments. The magnesium salt was added to 100 ml of urine sample in a 1:1 M ratio (Mg: PO_4^{3-}). The stirring of the sample was carried out at a rate of 120 rpm for 30 min. The sample was then centrifuged at 7000 rpm for 10 min. The supernatant was discarded and the pellet was washed well with deionized water. The struvite pellet was air-dried at 50 °C and analyzed. All the precipitation experiments were carried out in triplicates. The experimental struvite yield obtained corresponds to the amount of struvite precipitated for 100 ml of urine. The theoretical yield was calculated based on the fact that 1 mol of phosphate yields 1 mol of struvite. The % struvite yield was calculated using Eq. (1):

$$Percent\ yield = \frac{Actual\ yield}{Theoretical\ yield} \times 100\% \tag{1}$$

2.4. Kinetics of struvite precipitation

Struvite is formed by the hydrogen bonding between phosphate, ammonium and magnesium in the ratio of 1:1:1. Therefore, the overall reaction of this process can be represented as Eq. (2).

$$Mg^{2+} + NH_4^+ + H_nPO_4^{3-n} \rightarrow MgNH_4PO_4 \cdot 6H_2O + nH^+ \qquad (2)$$

Therefore, the rate of struvite formation (Eq. (3)) can be obtained as follows:

$$\frac{dX}{dt} = K[Mg^{2+}][NH_4^+][H_nPO_4^{3-n}] \qquad (3)$$

where K is the rate constant for struvite precipitation (hr^{-1}), X is the mass of struvite ($mol\ L^{-1}$) and t represents the reaction time (Day). Since the struvite formation generally follows first-order rate kinetics (Bayuseno et al., 2020; Purnomo et al., 2019), the rate constant can be arrived by plotting the concentration of magnesium, phosphate and ammonium versus the rate of change of struvite yield. However, the rate of struvite formation has not been calculated in this work as it might also be influenced by other factors such as mixing intensity, supersaturation ratio and molar ratio (Mg: PO_4^{3-}) (Rahaman et al., 2008).

In the case of human urine, the concentration of magnesium is almost negligible compared to phosphate and ammonium ions. Nearly all of the magnesium that is naturally present in urine gets used up during spontaneous precipitation, making it unavailable for further struvite precipitation. Therefore, Eq. (3) has been deduced to Eq. (4) as follows:

$$\frac{dX}{dt} = K_2[NH_4^+][H_nPO_4^{3-n}] \qquad (4)$$

Here, K_2 is the reaction rate constant for variation in phosphate and ammonium concentrations corresponding to struvite formation. For efficient struvite recovery, the variation in these concentrations over time has to be observed. The phosphate and ammonium recovery can be calculated using Eqs. (5) and (6), respectively.

$$\eta_P\% = \frac{P_I - P_R}{P_I} \qquad (5)$$

where, η_P is the recovery efficiency of phosphate, P_I is the initial mass concentration of phosphate ($mg\ L^{-1}$) and P_R is the mass concentration of residual phosphate ($mg\ L^{-1}$) (Wang et al., 2019).

$$\eta_{N-NH4+}\% = \frac{N_I - N_R}{N_I} \qquad (6)$$

where, N_I is the initial mass concentration of ammonium ($mg\ L^{-1}$) and N_R is the mass concentration of residual ammonium ($mg\ L^{-1}$) (Bas, 2007).

2.5. Struvite precipitation with excess addition of magnesium

In this study, $MgCl_2.6H_2O$ was added to 100 ml of urine sample in a 1:1 M ratio with ammonium ions (Mg: NH_4^+) which is approximately a five-fold increase in magnesium-dose compared to Mg: PO_4^{3-}. The remaining procedure was carried out as similar to the previous struvite precipitation protocol (Sec 2.3).

As per Eq. (2), synthesis of struvite is occurring by the amalgamation of magnesium, ammonium and phosphate in an equimolar ratio. Taken into consideration that only a negligible amount of magnesium is present in the case of human urine, the phosphate concentration tends to be the limiting reactant for struvite precipitation. In the existing literatures, magnesium was added according to the moles of phosphate present in urine and the Mg: PO_4^{3-} ratio was varied between 1 and 2. However, the current experiments were conducted to elucidate the effects of adding excess magnesium on struvite yield.

2.6. Statistical analysis

All the struvite precipitation experiments were carried out in triplicates. Mean and standard deviations were calculated, and the data was reported. Statistical calculations were made using the OriginPro 8.5, GraphPad software and differences were evaluated. Correlations between struvite yield, phosphate and ammonium recovery and their respective external influencing parameters were made using the Spearman test. Spearman's coefficients were calculated using Scipy 1.1.0. The r values of correlation coefficients up to 0.39 were considered to represent a weak relationship, 0.40–0.59 moderate association and 0.6–1.0 strong correlation. (+)ve and (−)ve values indicate positive and negative monotonic correlations, respectively. Multiple regression analysis was performed using Sklearn 0.20.2 to determine the interaction between the experimental factors that significantly affected the struvite yield, phosphate and ammonium recovery. Data were fit with a first-degree linear polynomial equation consisting of pH, conductivity, salinity, TDS and concentration of phosphate, ammonium, nitrate, calcium, sodium and potassium. The positive and negative coefficients were elucidated.

2.7. Characterisation of struvite

The dried struvite crystals were further characterized by using Philips X-Ray Diffractometer with Ni filter of Cu-Kα ($\lambda = 1.5418$ Å) at a scan rate of $4°$ (2θ) min^{-1} in a scanning range of $5°$–$70°$. Further, the purity of the synthesized struvite crystals was verified by Fourier transform infrared spectroscopy (FTIR). The analysis of FTIR for the struvite sample was recorded in the wavenumber range of 500–4000 cm^{-1} on an FTIR spectroscope (Bruker, Alpha E).

3. Results

3.1. Effect of storage on urine characteristics

3.1.1. Variations in the physicochemical characteristics of urine

Prior to struvite precipitation experiments, urine samples were stored to record the state of physical and chemical parameters. Data regarding the variation in the physicochemical characteristics of urine, such as pH, conductivity, salinity and TDS is represented in Fig. 1a. The pH of the urine sample at the time of collection was 6.2. It gradually increased to 9.3 at the end of day 25. The pH of 9 was attained on day 14, which was highly conducive for struvite precipitation. The pH value of the stored urine samples rises as a result of spontaneous degradation of urea present in urine to ammonia/ammonium and carbonate due to microbial activity, further triggering the precipitation of calcium and magnesium in the form of carbonates and phosphates. It could be attained enzymatically with the help of enzyme urease (urea amidohydrolase) secreted from the existing bacteria in urine or chemically by adding caustic soda. Generally, an alkaline pH is required for attaining the optimal environmental conditions for effective struvite precipitations.

Similarly, a rise in conductivity from 14.5 mS/cm to 21 mS/cm was observed during the course of storage from day 1–25. These changes could have occurred due to the release of ammonium ions as a result of ureolysis in the urine. Meanwhile, there was an increase in salinity and TDS. Salinity and TDS increased from approximately 8.5–12 psμ and 7.3–12.5 ppt, respectively. Though salinity and TDS do not have a direct impact on struvite precipitation, the levels of these parameters might affect the nature of struvite.

3.1.2. Ammonium and phosphate levels in urine during storage

Ammonium and phosphate ions are directly involved in the formation of struvite. The changes in the ammonium and phosphate concentrations were observed on a daily basis before struvite precipitation, to reach an optimum concentration of either of them for an enhanced yield of struvite. The initial concentration of ammonium was very low, which reached 15 $mg\ L^{-1}$ on day 1. The concentration almost kept doubling in the first 5 days and since then, the concentration shot up to approximately 320 $mg\ L^{-1}$ at the end of day 25 (Fig. 1b). This might have occurred due to the increase in pH by the hydrolysis of urea through urease releasing microbes in the urine. On the contrary, the phosphate
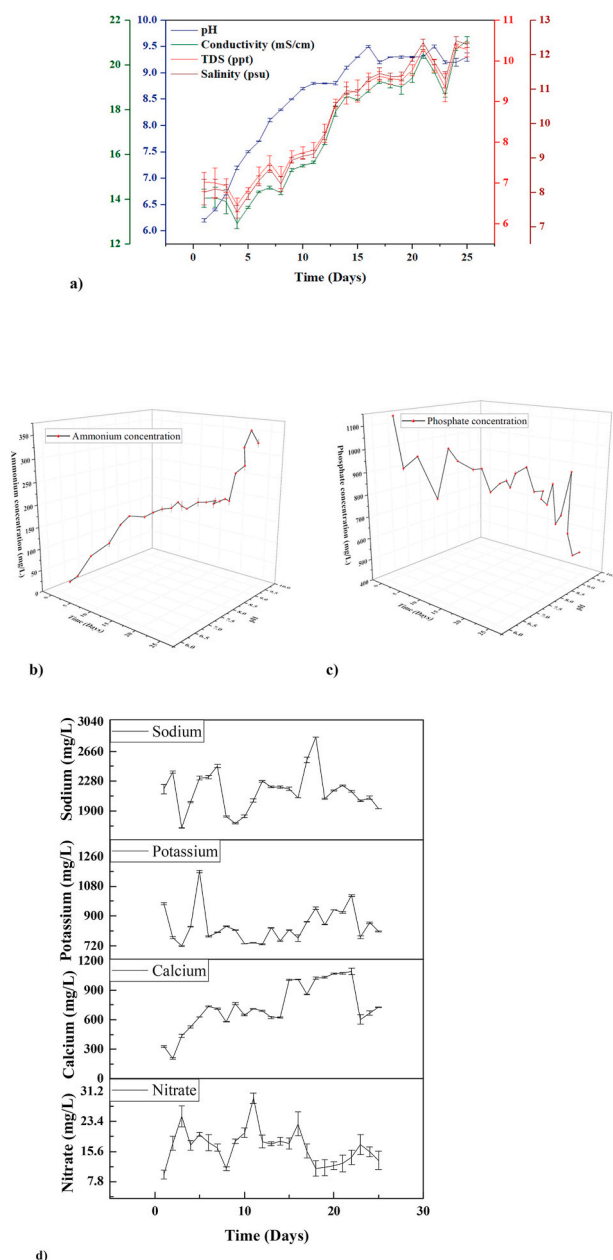
Fig. 1. Variation of a) physicochemical b) ammonium c) phosphate and d) co-existing ion concentrations in urine storage with time at a room temperature of 25 ± 3 °C.

concentration kept decreasing over time (Fig. 1c). The initial concentration of phosphate was found to be 1150 mg L^{-1}. It reached almost 510 mg L^{-1} by the end of the storage period of 25 days with a nearly 50% decline than that of its initial concentration. The reduction in phosphate minerals is due to its spontaneous precipitation occurring in stored urine; an average of 31% of phosphorus, 83% of calcium and 89% of magnesium were lost from the urine solution as precipitates in the first 14 days. Consequently, about 70% of phosphorus remained in urine solution after spontaneous precipitation and was available to be harvested in the form of struvite. Most of the earlier studies that focused on the non-spontaneous precipitation of phosphate suggested that the remained solution (after spontaneous precipitation) was ideal for struvite recovery (Tilley et al., 2008). Parallelly, the pH of the urine solution becomes much ideal for struvite recovery, while the non-existence of magnesium dictates the optimal environmental conditions for the non-spontaneous recovery of struvite.

To comprehend the behavior of ammonium and phosphate ions while storing the urine, the kinetics of the variation in the concentrations of these ions were studied. It was found that the rate of rise in ammonium concentration and the fall in the phosphate concentration was independent of reactant concentration. These variations were mainly dependant on the effect of pH. Since ureolysis is an enzyme-catalyzed reaction, the urea molecules attach to the active sites of urease enzyme to saturate and lead to the formation of an enzyme-substrate complex. This reaction progresses until the number of enzyme molecules becomes limited in comparison to the substrate and thus follows zero-order. It was analytically confirmed by fitting the ammonium concentration values in zero-order, first-order and second-order kinetic equations. Zero-order gave the best fit ($R^2 = 0.8506$) compared to first ($R^2 = 0.6881$) and second-order ($R^2 = 0.3772$). This reaction is time-bound and continues only until the reactant concentration diminishes. Also, the behavior suited well with the characteristics of zero-order, such as linearity of decrease in ammonium concentration over time. In the case of phosphate, the ions bind to calcium and magnesium in the urine to form salts of calcium and magnesium phosphate, leading to a decrease in the supernatant concentration of phosphate. The concentration of the reactant decreases linearly with time. Such reactions take place only for a narrow region of time until the limiting reactant is completely consumed or alters with a change in the physicochemical parameters. Thus the linearity is realistic over a limited duration, and hence zero-order kinetics is applicable for describing such reactions. On plotting the concentration values versus time, it was further confirmed that the zero-order had the highest correlation ($R^2 = 0.8338$) compared to first ($R^2 = 0.6452$) and second-order kinetics ($R^2 = 0.6186$).

3.1.3. Changes in the concentration of co-existing ions in urine

Apart from ammonium and phosphate ions, various other ions present in human urine are known to have a significant effect on struvite precipitation. Hence the concentrations of those ions such as calcium, nitrate, sodium and potassium were simultaneously observed daily in this study (Fig. 1d). The calcium levels highly fluctuated between ~200 and 1100 mg L^{-1}. The amount of calcium in urine was declining initially as it might have precipitated along with phosphates in the form of calcium phosphates. As time progresses, the change in pH favors struvite (Magnesium Ammonium Phosphate (MAP)) precipitation, which triggers the competition between calcium and magnesium to get precipitated. The steady rise in calcium concentration in the urine solution until day 14 might be primarily due to the replacement of magnesium ions in the precipitates by calcium. This is due to the fact that the spontaneously precipitated materials were not removed from the experimental study system until day 14 allows the ion exchange. However, beyond day 14, once the non-spontaneous precipitation of phosphate started on a daily basis based on invasive sampling, there is not much rise in calcium ion concentration. These observations were much corroborated with the earlier studies of Tilley et al. (2008), which postulated the replacement of calcium by magnesium ions during spontaneous precipitation.

The nitrate concentration showed only a slight variation in the range of ~10–20 mg L^{-1}. It was also found that nitrate ions being though a nitrogen species did not participate in the struvite precipitation. The percentage recovery of nitrate remained almost nil after the formation of struvite (Data not shown). The concentration of potassium throughout the storage period was ~840 mg L^{-1}, with a standard deviation of ~100 mg L^{-1}. While on the contrary, the sodium concentration was as high as ~1150 $\pm$ 250 mg L^{-1}.

3.2. Struvite precipitation experiments

The formation of struvite depends on the variations in ionic interactions, pH and temperature effects. Struvite was precipitated and processed under desired conditions of pH around 9 and drying temperature up to 50 °C (Tansel et al., 2018). Also, the FTIR and XRD analyses would play a major role in confirming the formation of struvite

crystals. When the optimum pH for struvite formation was obtained on Day 14, the precipitation experiments were conducted on a daily basis with varying physicochemical conditions. The overall kinetics of phosphate depletion and ammonium accumulation are given in Fig. 2. The actual yield of precipitates attained with the addition of magnesium salt in the case of equimolar and excessive addition is presented in Fig. 3. Since the phosphate concentration is rate-limiting and dictates the overall struvite yield, the theoretical yield was estimated based on stoichiometric calculations, and percent yield was evaluated.

3.2.1. Struvite precipitation without the addition of magnesium

As the pH increased with time, spontaneous precipitation of struvite along with other phosphate salts increased. This can be due to the presence of calcium and minuscule of magnesium present in urine, which caused the fall in phosphate concentration with time. In addition to struvite formed by the presence of the negligible amount of magnesium in urine, calcium or other phosphate salts gets precipitated. Hence, these salts deposited as a result of spontaneous precipitation is negated in this study.

3.2.2. Struvite precipitation with equimolar addition of magnesium

In this study, struvite was precipitated with a 1:1 M ratio of Mg: PO_4^{3-}. The phosphate and ammonium recovered was $60.87 \pm 1.48\%$ and $87.12 \pm 0.71\%$, respectively, at day 14 with the struvite yield of 238.4 ± 20.90 mg L^{-1}. As time progressed, the % PO_4^{3-} recovery was observed to be increased and reached a maximum of $85.81 \pm 0.62\%$ at day 24 (Table 1). However, only a slight variation could be seen with percentage ammonium recovery with time. On the contrary, the actual struvite yield was increased on every day with storage, and the highest yield of 619.3 ± 54.41 mg L^{-1} was obtained at day 24. Further, detailed characterization with FTIR and XRD was carried out to confirm the presence of struvite in the precipitates obtained.

3.2.3. Struvite precipitation with excess addition of magnesium

To investigate the effect of adding excess magnesium for precipitating struvite, magnesium chloride was added in a 1:1 M ratio with

ammonium. In this case, the concentration of magnesium was five times higher than Mg: PO_4^{3-}. The percentage of phosphate and ammonium recovery and struvite yield was calculated. It was noted that there was an increase in the amount of phosphate recovered and yield every day (Table 1). The phosphate recovered was ~10–20% higher than PO_4^{3-} recovered from the equimolar addition of Mg. Similarly, the yield of precipitates was almost doubled on every day with the excessive addition of Mg. However, only a modest change in the amount of ammonium recovered was observed. These data suggest that compounds other than struvite have also precipitated in this case. The FTIR data of the salt precipitated with excess magnesium support the above-made conclusion (Fig. 4a).

3.3. Statistical analysis

Spearman's coefficients were used to analyze the effect of various factors affecting struvite yield, phosphate recovery and ammonium recovery (Fig. S1). pH and potassium concentration shows a weak positive correlation for struvite yield, whereas nitrate and calcium concentrations exhibit a weak negative correlation (Fig. S1a). Conductivity, salinity and sodium concentration have shown a greater relationship on struvite precipitation. TDS appears to have a positive and significant impact on struvite yield. In the case of P recovery (Fig. S2b), pH and concentrations of nitrate, calcium and potassium exhibit weak correlations. Conductivity, salinity and sodium concentrations show a moderate effect, while TDS appears to have a substantial impact on P recovery. Considering ammonium recovery (Fig. S2c), all the physical parameters except pH present a moderate negative correlation. Potassium and calcium ion concentrations show a weak negative effect, whereas a weak positive relationship is demonstrated by nitrate concentration. Sodium concentration exhibits a strong positive effect on ammonium recovery. Though a definite reason for this predicted effect has not yet been known, there are supportive literatures that the presence of higher sodium concentration decreased the magnitude of induction time of struvite. As the sodium concentration increased, the crystals grew wider (Kabdaslı et al., 2017).

Multiple regression analysis was performed to analyze the interaction between the significant parameters that affect struvite yield, phosphate recovery and ammonium recovery. The linear multiple regression equations obtained for struvite yield, phosphate recovery and ammonium recovery were shown in Eqs. (S1), (S2) & (S3) respectively, whose correlation coefficient (R^2) values obtained were 0.98, 0.97 and 0.84 respectively.

3.4. Characterisation of struvite and compositional analysis

The results of FTIR for the salts precipitated from equimolar and excess addition of magnesium are represented in Fig. 4a. The obtained struvite crystals showed characteristic absorption peaks at 1432 cm^{-1} attributes to NH_4^+ antisymmetric bending. The main peak at 991 cm^{-1} was due to the absorption of PO_4^{3-} asymmetric stretch. The peak at absorbance 753 attributes to the Mg–O bonding. Other peaks at 2314, 2389 and 2928 cm^{-1} correspond to the H–O–H stretching vibrations of the cluster of water molecules of crystallization. The absorption peaks at 1546 and 1648 cm^{-1} could be assigned to the H–O–H bending modes of vibrations. The broad asymmetric band observed between the range of 3800 to 2200 cm^{-1} ascribed to the O–H and NH stretching vibrations (Liao et al., 2019; Manzoor et al., 2019; Ameen and Mohamed, 2014). However, it can be seen from the figure that the intensity of P–O has increased with excess addition of Mg. Similar observations can be seen with Mg–O and NH_4^+. The decrease in % transmittance for the sample with excess magnesium indicates a high population of bonds. These results further suggest that excess Mg addition could lead to the formation of phosphate salts other than struvite.

The results of the XRD of the struvite sample obtained by the equimolar addition of Mg are presented in Fig. 4b. The diffraction pattern
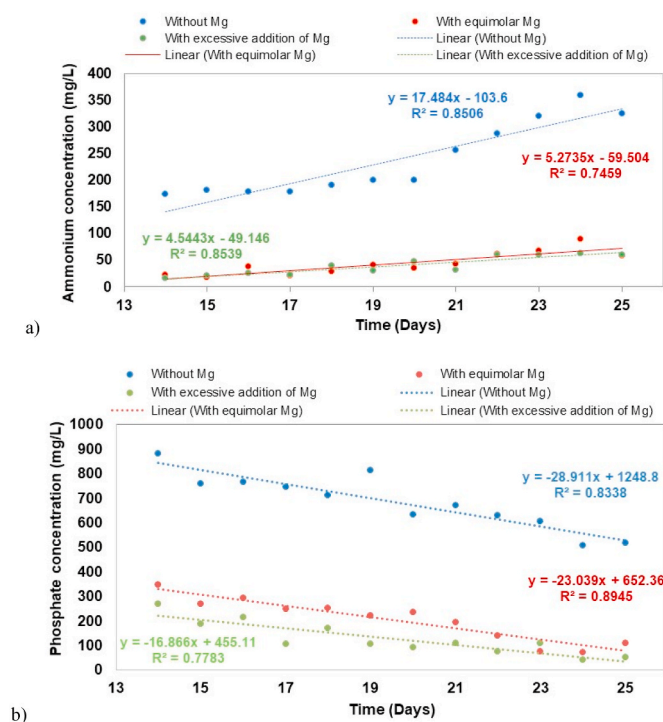

Fig. 2. Kinetics of a) ammonium accumulation and b) phosphate depletion from urine under various dosage of magnesium salt.
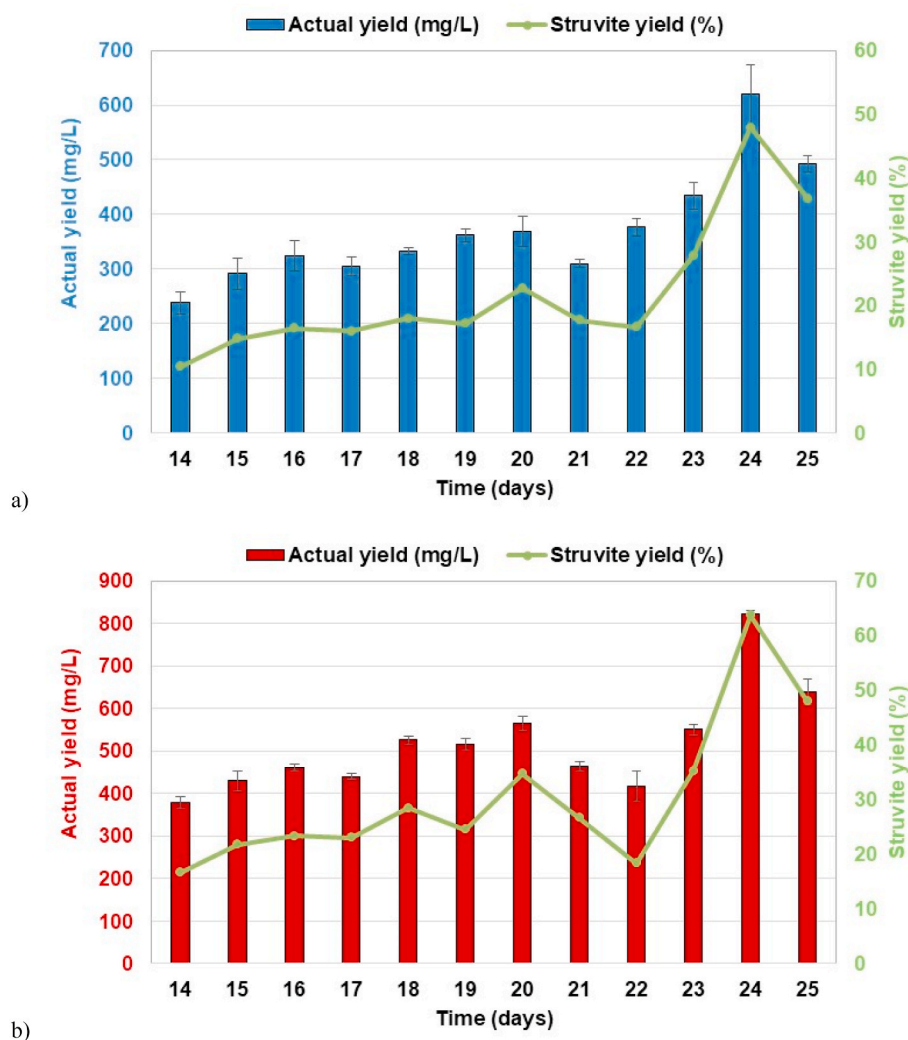
Fig. 3. Yield of actual precipitates and struvite yield in case of a) equimolar addition of Mg and b) excessive addition of Mg.

Table 1
Variation of phosphate and ammonium ion removal at equimolar and excessive magnesium addition.

Day	Equimolar addition of Mg salt		Excessive addition of Mg salt	
	Phosphate removal (%)	Ammonium removal (%)	Phosphate removal (%)	Ammonium removal (%)
14	60.87 ± 1.48	87.12 ± 0.71	69.52 ± 3.00	89.79 ± 3.16
15	64.58 ± 3.82	90.39 ± 1.43	75.37 ± 5.16	88.95 ± 1.55
16	61.95 ± 0.73	79.24 ± 1.79	72.04 ± 1.38	76.12 ± 4.24
17	66.63 ± 2.43	88.66 ± 2.34	85.86 ± 1.57	87.17 ± 1.53
18	65.02 ± 1.60	85.15 ± 2.66	76.26 ± 2.79	88.54 ± 2.59
19	73.01 ± 4.56	79.83 ± 3.67	87.10 ± 4.72	82.81 ± 1.01
20	75.90 ± 3.38	83.03 ± 3.55	85.85 ± 2.49	86.66 ± 3.30
21	70.98 ± 0.95	83.33 ± 0.71	83.72 ± 0.84	84.73 ± 1.90
22	84.34 ± 1.88	78.45 ± 4.44	91.65 ± 1.73	79.16 ± 5.52
23	78.09 ± 3.30	78.85 ± 0.63	82.25 ± 3.92	81.16 ± 2.17
24	85.81 ± 0.62	79.05 ± 0.11	92.08 ± 1.45	82.29 ± 4.39
25	81.90 ± 3.40	81.93 ± 6.24	90.45 ± 1.67	81.65 ± 5.62

generated by the precipitate was registered within the 2θ angle range of 10–55° and showed characteristic peaks similar to those of MgNH$_4$PO$_4$.6H$_2$O (ICDC Standard #15–0762), which suggests that most of the crystals obtained were comparable with the pure struvite. The overall comparison of the struvite characteristics between equimolar and excessive addition of magnesium is given in Table 2.

4. Discussion

4.1. Variations in the physicochemical characteristics of urine

Physicochemical characteristics play an essential role in determining the chemical state and processes of any solution, thus demands the continual monitoring of these changes during the experiments. pH is a very crucial factor for struvite precipitation as it exerts a significant effect on the chemical and enzymatic urea hydrolysis. With an increase in pH, ureolysis is found to increase and vice versa. Likewise, pH in this study was found to increase prominently with the increase in time (Fig. 1a), which might be due to the release of ammonium ions by hydrolysis of urea. As well, pH could determine the type of compound that gets precipitated as the charge on the ion varies with acidity and alkalinity conditions as per the Pourbaix diagram. A pH interval of 8–10 is generally considered to be an operational pH range for struvite precipitation (Liao et al., 2019). The urine samples reached a pH of around 9 within 15 days, which is in accordance with the literature. Few studies have reported that it might take up to 40 days or even months to reach the desired pH as urine is highly variable with respect to age, gender and diet (Gethke et al., 2006; Lahr et al., 2016).

Conductivity was another indication of urea hydrolysis and it increased exponentially with time to reach a maximum of 21 mS/cm at the end of the study (Fig. 1a). It can be positively correlated with an increase in ammonium concentration over time and negatively
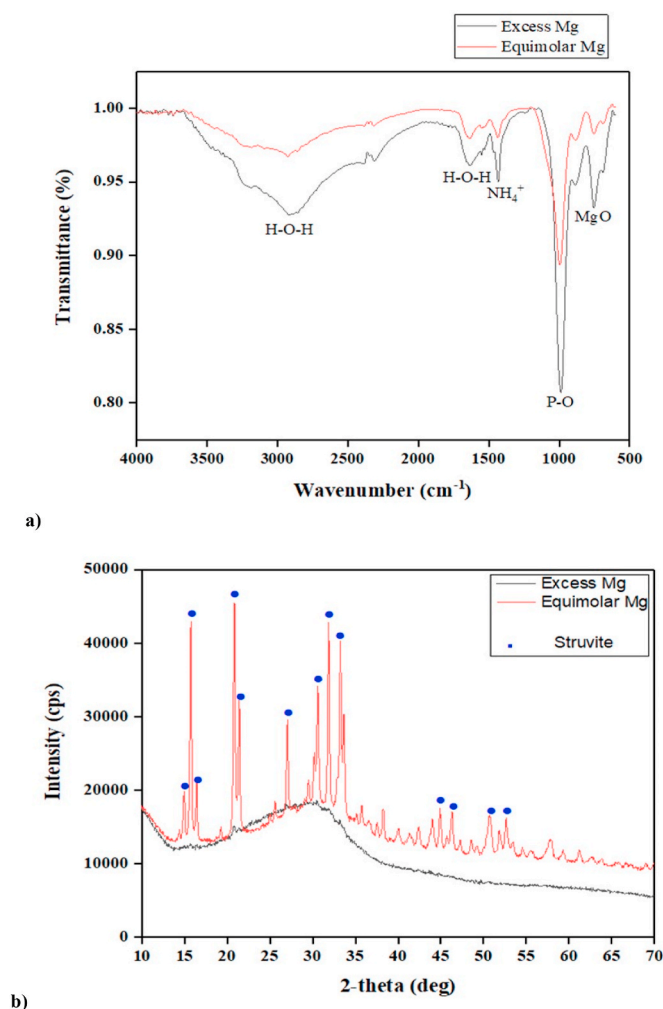
Fig. 4. Comparison of a) FTIR spectrum and b) XRD pattern for the struvite obtained on Day 14 from urine with equimolar and excessive addition of Mg.

Table 2
Comparison of precipitation attributes at equimolar and excessive magnesium addition on urine.

Attributes	Equimolar Mg salt addition	Excessive Mg salt addition
Molar ratios of salt addition (Mg:P)	1:1	Approx. 3–5 times excess to 1:1
Phosphorus removal (%)	61.87–81.90	69.52–90.45
Ammonium removal (%)	78.45–90.39	76.12–89.79
Actual yield (mg/L)	238.4–491.8	378.5–639.7
Theoretical yield (mg/L)	1292.4–2282.6	1292.4–2282.6
Percent yield (%)	10.44–47.91	16.58–63.59
Purity of struvite	High	Low
Intensity of P–O bonds in FTIR spectrum	Low	High
Form of struvite crystals by XRD analysis	Crystalline	Amorphous

correlated with urea concentration in urine. Indeed, it helps to track the extent of ureolysis stage while precipitating the desired nutrients. Since urea is a non-electrolytic and neutral component, the further transformation of urea to ammonium ions results in the increment of conductivity during the storage of urine at normal room temperature. The result obtained were corroborated well with the information found in the literature (Fazil Marickar, 2010; Ray et al., 2018; Tao et al., 2019).

It is evident that all physical parameters of total dissolved solids

(TDS), salinity and conductivity are interrelated. In the case of stored urine samples, the salinity and TDS tend to increase with time might be due to the increase in bacterial activity during storage. It was well known that bacteria secrete out exopolysaccharides under various conditions of pH and temperature might raise the salinity. Though the salinity level did not affect the P removal, it might affect the crystal induction time. Altered morphogenesis of struvite crystals like elongation has been reported at high saline conditions (Liu and Wang, 2019; Zhao et al., 2019). A high TDS level has shown to have an effect of struvite formation, the actual role of TDS has not been well discussed in the literature (Fazil Marickar, 2010; Kabdasli et al., 2006).

All these changes in the physical characteristics of stored urine occurred as a result of bacterial activity. Due to the virtue of bacterial metabolism on stored urine, pH has been raised in response to enzyme secretion and subsequent reactions. This has been further confirmed by comparing with the urine samples stored at −20 °C, where the metabolism of bacteria present in urine was halted. It was observed that none of the samples stored at −20 °C shown any considerable changes in the physical characteristics of the urine even after a storage period of 25 days. It is noteworthy to mention that ceasing of bacterial metabolism while storage preserves the natural degradation as evident from the reported non-enzymatic decomposition of urea with a half-life time of 3.6 years at 38 °C (Udert et al., 2003a).

Similarly, the difference in the concentration of PO_4^{3-} and NH_4^+ was monitored on a daily basis. It can be noted from Fig. 1b that NH_4^+ concentration increases as pH increases with time as both the factors are majorly dependant on ureolysis. Though there is a sharp increase in NH_4^+ concentration until day 5 (pH 7.5), the rise becomes gradual in the following days. The stabilization of urea hydrolysis can be due to the utilization of ammonium in the spontaneous precipitation of struvite. Loss of NH_4^+ through ammonia volatilization during withdrawal from the storage container can also be considered a reason. Conventional hydrolysis of urea requires a long period of time and it is known from the literature that significant hydrolysis takes place from the 14th day of storage (Kabdaşli et al., 2006). According to the Eh-pH (pourbaix) diagram for nitrogen species, it exists as ammonium ion until a pH of 9.25 (Russell and Hall, 2009). Therefore, this study was terminated on day 25, above which the pH increased over 9.5. However, it can be found from previous works that the hydrolysis was not complete even until day 41, in which only 80% of conversion was observed (Lellis, 2003). Another study reported that only 25% of urea was found to be hydrolyzed in 30 days between pH 2 to 7.5, as well as, no hydrolysis would occur above pH 10 (Kabdasli et al., 2006). Further, it is noteworthy to mention that other negative impacts of raise in pH, such as that the mean struvite crystal size decreased with an increase in pH (Ahmad and Gupta, 2019). These variations in the time taken to attain a certain pH might be due to wider variations of initial urine characteristics that depending upon one's age, diet, lifestyle and other surrounding environmental conditions.

With respect to Eq. (4) it can be noted that the ammonium concentration increases with time due to enzymatic hydrolysis of urea. The change in ammonium concentration with respect to time is denoted as in Eq. (7).

$$\frac{d\left(NH_4^+\right)}{dt} = + K_2 \left[NH_4^+\right] \tag{7}$$

where K_2 is the rate constant for ammonium concentration, the positive sign indicates an increase in ammonium concentration with time. Fig. 2a shows the kinetics of the increase in ammonium concentration with time. It can be seen that the rise in ammonium concentration fitted well with zero-order kinetics. The slope, which is the rate constant K_2, was found to be 6.27 and 4.54 mg L^{-1}day^{-1} for equimolar and excessive Mg addition of struvite precipitation experiments. Though studies have reported that ureolysis followed zero-order kinetics (Kabdasli et al., 2006), no literature has reported the rate kinetics of the behavior of ammonium

concentration in urine with time.

Due to spontaneous reactions that occur during storage, phosphate concentration decreases gradually. An increase in pH aids spontaneous precipitation, leading to a decrease in phosphate concentration (Fig. 1c). Following Eq. (4) the change in phosphate concentration with time is denoted as Eq. (8).

$$\frac{d\left(H_nPO_4^{3-n}\right)}{dt} = -K_3\left[H_nPO_4^{3-n}\right] \tag{8}$$

where K_3 is the rate constant for phosphate ion concentration, the negative sign indicates the depletion of phosphate concentration with time. Fig. 2b shows the kinetics of phosphate concentration decrease with time. It can be noted that the fall in PO_4^{3-} concentration follows zero-order rate kinetics with the rate constant of -23.04 and -16.86 mg $L^{-1}day^{-1}$ for equimolar and excessive Mg addition of struvite precipitation experiments. Similar studies on the kinetics of change in phosphate concentration have not yet been reported. It is reported that the PO_4^{3-} concentration decreases by 25–35% during storage due to spontaneous precipitation (Etter et al., 2011; Tilley et al., 2008a). But the storage of urine becomes necessary as phosphorus exists as HPO_4^{2-} at pH ≥ 9 in which it will be directly available for intake by the plants (Ganrot, 2005; Tao et al., 2016). Though pH can be altered manually by chemical addition, storage is necessary to enhance the microbial activity and endogenous metabolite degradation required for the decomposition of urea. Although there is a reduction in phosphate levels, the recovery has increased by 20% with storage and reached a maximum of 86.44%.

Struvite precipitation can be hindered by the presence of co-existing ions in urine due to their ability to compete for ions to form complexes. In this study, the variation in the concentration of four significant ions (Ca^{2+}, Na^+, K^+ and NO_3^-), which are known to affect the precipitation process, are investigated.

The initial concentration of calcium in urine was found to be 325.65 $\pm$ 9.15 mg L^{-1} (Fig. 1d). The immediate reduction in calcium concentration on day 2 might be due to spontaneous precipitation. It is noteworthy to mention that the ion concentration fluctuates as the release of calcium by bacterial species present in urine. However, calcium has an inhibitory effect on struvite precipitation at high concentrations, as it competes with Mg to form calcium phosphates ($Ca_3(PO_4)_2$), di-calcium phosphate ($CaHPO_4\cdot2H_2O$) and hydroxylapatite ($Ca_5(PO_4)_3(OH)$) and thus leads to a rise in calcium concentration in the supernatant. Calcium is said to precipitate during ureolysis due to spontaneous precipitation. In general, these reactions occur when Ca^{2+}: Mg^{2+} ratio exceeds 0.25. Beyond this ratio, it could inhibit the struvite crystallization due to the variation in microbial communities and their activities with the change in pH. High calcium concentration in urine might influence PO_4^{3-} recovery, pH of the solution and struvite crystal characteristics such as size, shape and purity (Hu et al., 2020; Liu and Wang, 2019; Tilley et al., 2008b).

Nitrate ions are another form of nitrogen species present in human urine at almost all pH ranges. The fluctuations in the nitrate concentration (Fig. 1d) are due to the shift of electrons and reduction to nitrite with altering pH. It was evident from the experimental observations that nitrate concentration remained the same after struvite precipitation, which indicates that nitrate ions do not influence struvite formation. It is found from the previous research works that the presence of nitrate ions does not significantly affect the phosphate recovery. However, a decrease in the crystallization ratio was observed though NO_3^- favored crystallization of struvite (Shih et al., 2017; Tao et al., 2020).

K^+ and Na^+ are the two other cations which compete with ammonium ion to bind with Mg^{2+} and PO_4^{3-} in the urine to form K-struvite ($MgKPO_4.6H_2O$) and Na-struvite ($MgNaPO_4\cdot7H_2O$) respectively. The concentration of potassium during the course of storage was 842.68 $\pm$ 104.89 mg L^{-1}, which is quite high compared with the reported values (Xu et al., 2017). Such high concentrations can affect the purity of struvite crystals as they tend to co-precipitate with them. This process

takes place, especially at low ammonium concentrations. The sodium ion concentration during the storage is calculated to be 2151.12 $\pm$ 256.35 mg L^{-1}, which is similar to the data recorded previously (Gethke et al., 2006). The formation of both K-struvite and Na-struvite was unaffected by the variation in pH. But the possibility of K-struvite to be formed compared to struvite increased with an increase in pH. However, it was known that the optimal pH for Na-struvite crystallization was 12, and K-struvite is very soluble and precipitates with the rise in pH. At a molar ratio of Na: K greater than10, the precipitation of Na-struvite was more favourable than K-struvite. However, an increase in the reaction time reduced the effect of Na^+ on the formation of K-struvite (Hu et al., 2020; Huang et al., 2019a; Huang et al., 2019b).

4.2. Struvite precipitation experiments

Since the initial amount of magnesium present in urine is very meagre and tend to get used up in the event of spontaneous precipitation, there is always a need for external addition of Mg for attaining higher efficiency of struvite recovery. Yet, this study was carried out in two cases to elucidate the drawback of excess addition of magnesium on the yield and purity of struvite. Mg was added to maintain 1:1 of Mg: PO_4^{3-} ratio in a case and the other with Mg addition to maintaining equimolar concentration with ammonium (approx. 3–5 times excess to 1:1 of Mg: PO_4^{3-} ratio). The results of the struvite precipitation experiments revealed that the excessive addition of Mg enhances the PO_4^{3-} recovery and struvite yield. Although the storage doesn't have any considerate effect on the recovery percentage of NH_4^+, PO_4^{3-} recovery has increased by approximately 20% each day, which could correlate well with the results of an increase in struvite yield. However, it has been shown in the previous studies that struvite formation (and phosphorus removal) is optimized when Mg is added in excess even up to a molar ratio of 2 instead of the theoretical requirements of 1:1 of Mg: PO_4^{3-} ratio (Gethke et al., 2006; Lahr et al., 2016; Tilley, 2006).

The precipitation experiments were conducted from day 14 as the desirable pH for struvite formation was reached. Meanwhile, the ammonium recovery remained almost constant, be it with or without excess addition of Mg salt (Table 1). This can be explained by the fact that due to excessive addition of salt, analogs of struvite (K-struvite and Na-struvite) (Hu et al., 2020) and other phosphate salts ($MgHPO_4$, $Mg_3(PO_4)_2$, $Mg(H_2PO_4)_2$, hazenite, apexite) along with struvite got precipitated (Kampf et al., 2015; Yang et al., 2011), which could hinder the purity of the precipitated struvite (Tao et al., 2019). Though accurate and timely addition of Mg during large scale processes is merely impossible, the utmost care has to be taken in maintaining the molar ratios to ensure the quality of struvite. However, Mg addition can also vary depending on the application of struvite as fertilizer. In the case of phosphate deficient soil, higher addition of Mg can help improve PO_4^{3-} recovery and yield but will lack the presence of ammonium in the fertilizer. Therefore, careful attention has to be given while choosing the concentration of Mg dosage for struvite precipitation based on the desired applications.

5. Conclusion

The study observed the effect of storage time on human urine for the recovery of nutrients such as ammonium and phosphate in the form of struvite. The major outcomes of the study were as follows:

- It was found that the optimum pH required for struvite precipitation can be acquired by storage of 14 days.
- The enhanced struvite yield with PO43- and NH4+ recovery of 90% could be achieved with the storage of 25 days at a temperature of 25 °C.
- Effect of excessive addition of Mg salt revealed that PO43- recovery and precipitate yield increases due to struvite analogs with the decline in struvite purity.

- Statistical analysis revealed that total dissolved solids and potassium had a positive effect on struvite yield and PO43- recovery, whereas sodium ion exhibited a positive effect on NH_4^+ recovery.

It can be concluded that the storage of urine has a significant impact on struvite yield through PO_4^{3-} and NH_4^+ recovery by means of pH and the availability of ions.

CRediT authorship contribution statement

Nageshwari Krishnamoorthy: Data curation, Formal analysis, Writing - review & editing. **Baishali Dey:** Data curation, Formal analysis, Writing - review & editing. **Thirugnanam Arunachalam:** Supervision, Writing - review & editing. **Balasubramanian Paramasivan:** Supervision, Funding acquisition, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.ibiod.2020.105053.

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