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Balancing the oxidation of endogenous organics and macronutrient recovery from human urine treated with fenton's reagent: A targeted metabolomics study

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ABSTRACT

Human urine is a promising resource for circular fertiliser production, but its high concentrations of organic and inorganic compounds present both challenges and opportunities for effective treatment. This study evaluates Fenton oxidation for selectively degrading endogenous organic metabolites in acidified, unhydrolysed urine while preserving critical nutrients such as urea. Using targeted metabolomics, over 200 organic metabolites were identified in urine, with creatinine, citric acid, hippuric acid, and methylhistidine comprising half of the total organic metabolite load (ΣOMs = 3.23 g L^{-1}). Under optimised conditions (pH 4.0, 1:1 Fe^{2+}: H_2O_2 molar ratio), 59 % of ΣOMs were degraded in unconcentrated urine treated with 1 g H_2O_2 L^{-1}. Increasing the H_2O_2 dose in unconcentrated urine, or treating concentrated urine obtained through evaporative water removal, resulted in higher ΣOMs degradation but also increased urea oxidation, highlighting a trade-off between efficient COD removal and nutrient recovery. COD removal was 38 % at pH 4.0 and 27 % at pH 6.0, suggesting that Fenton oxidation could be applied to H_2O_2 stabilised urine without strict pH adjustment. Real urine differed significantly from synthetic urine, requiring five times more Fe^{2+} catalyst for complete H_2O_2 activation, with peroxide consumption occurring within five minutes compared to two hours in synthetic urine. Organic compounds in urine scavenged Fe^{3+}, forming iron-organic complexes that disrupted Fe^{2+} regeneration and contributed to iron precipitation at higher pH values. These findings demonstrate that Fenton oxidation can be optimised to achieve selective degradation of undesirable organics while preserving plant-essential nutrients in urine collected within resource-oriented sanitation systems.

1. Introduction

Human urine is a widely available yet underutilised biological resource that holds large potential for circular fertiliser production [1, 2]. Globally, its recycling could replace 25 % of the nitrogen and phosphorus currently supplied by synthetic fertilisers [3]. Before urine can be safely used in agriculture, it must typically be treated to stabilise plant-essential nutrients and, in some cases, to reduce or remove undesirable organic compounds. Urine contains a diverse mixture of endogenous metabolites and exogenous substances [4], many of which can interfere with treatment technologies aimed at recovering nutrients. For instance, organic compounds can cause foul membranes in filtration processes [5], alter adsorption dynamics of pollutants [6], and lower the purity and yield of precipitated mineral products [7]. Whether an organic compound in urine is considered undesirable depends on its potential to interfere with treatment processes, cause operational complications, or reduce product quality, and is therefore inherently dependent on the treatment technology and the intended application of the recovered product. Any effective urine treatment must therefore balance the degradation of these compounds while preserving plant-essential nutrients, particularly urea, which comprises >80 % of urine's nitrogen content [8].

Advanced oxidation processes, such as Fenton oxidation, offer a promising approach for organic degradation in urine (Eq. (1)) [9]. Fenton oxidation generates highly reactive (E° = 2.73 V) hydroxyl radicals (•OH) in-situ from Fe^{2+} catalysed decomposition of H_2O_2.

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Fenton oxidation follows a sequence of reactions (Eq. (2–8) [10], initiated when ferrous ions (Fe^{2+}) catalyse the formation of hydroxyl radicals (•OH) in an acidic medium (Eq. (2)). The reaction chain is sustained by Fe^{2+} regeneration, as the ferric (Fe^{3+}) ions produced in Eq. (2) further react with H_2O_2 (Eq. (3)).

$$\bullet OH + \text{organics} \rightarrow \text{Oxidation products} \quad (1)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^- \; k_1 = 63.5\,M^{-1}s^{-1} \quad (2)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + \bullet HO_2 + H^+ \; k_2 = 0.001 - 0.01\,M^{-1}s^{-1} \quad (3)$$

$$\bullet OH + H_2O_2 \rightarrow \bullet OOH + H_2O \quad (4)$$

$$\bullet OH + Fe^{2+} \rightarrow Fe^{3+} + OH^- \quad (5)$$

$$Fe^{3+} + \bullet OOH \rightarrow Fe^{2+} + O_2 + H^+ \quad (6)$$

$$Fe^{2+} + \bullet OOH + H^+ \rightarrow Fe^{3+} + H_2O_2 \quad (7)$$

$$\bullet OOH + \bullet OOH \rightarrow H_2O_2 + O_2 \quad (8)$$

The mechanism by which hydroxyl radicals interact with organic compounds is well understood, occurring primarily by abstraction of hydrogen from C–H, N–H, or O–H bonds, or by addition to C = C double bonds and aromatic rings [11,12]. While Fenton oxidation has been widely studied for wastewater treatment [13], its application to human urine remains largely unexplored, especially at the molecular level. Human urine presents several unique challenges for Fenton treatment. First, unlike typical wastewater matrices, urine is a high-strength organic and inorganic matrix, with a chemical oxygen demand of 5–10 g O_2 L^{-1} [14], containing salts, endogenous metabolites, and exogenous substances like pharmaceutical drug residues [15]. Second, urine has an exceptionally diverse metabolome, comprising amino acids, phospholipids, amines, organic acids, acylcarnitines, sphingomyelins and nucleotides, which can influence oxidation pathways [4]. Third, Fenton chemistry in urine is further complicated by radical scavenging, as urine contains high concentrations of chloride (3–6 g L^{-1}) and urea (8–14 g L^{-1}) [16], both of which are highly reactive with •OH [17].

Studies on Fenton oxidation in real urine remain scarce, with most research conducted using synthetic urine [18,19], which lacks the chemical complexity of real urine and may not fully capture Fenton's performance in practice. Furthermore, many studies rely on bulk parameters, such as COD and total organic carbon, which do not provide molecular level insights into oxidation processes. As a result, it remains unclear which specific compounds in urine are oxidised, which persist, and how nutrient recovery is influenced.

In this study, we hypothesise that selective oxidation can be achieved in real urine using Fenton's reagent to target complex organic metabolites while preserving urea, the primary endogenous metabolite and dominant nitrogen compound. This hypothesis is grounded in the known kinetics of hydroxyl radical reactions: many urine constituents, including pharmaceuticals (10^9–10^{10} M^{-1} s^{-1}), amino acids (10^7–10^8 M^{-1} s^{-1}), and carbohydrates (10^8–10^{10} M^{-1} s^{-1}), react significantly faster with •OH than urea, which has a lower second-order rate constant of 7.9×10^5 M^{-1} s^{-1} [17]. To evaluate this, we applied targeted metabolomics to track the fate of over 200 endogenous metabolites in real human urine before and after Fenton treatment.

The overall aim was to develop Fenton oxidation as a targeted treatment process for the selective degradation of organic compounds in urine, while minimising the oxidation of chloride and urea. Specifically, we investigated how: i) key operating parameters (H_2O_2:Fe^{2+} ratio, pH, urine concentration factor, and peroxide dose) influence the trade-off between organics removal and nutrient recovery; (ii) urine concentration factor (CF) affects peroxide activation and organic degradation; (iii) differences between real and synthetic urine matrices affect Fenton chemistry. Overall, this study provides critical insights into both the feasibility and challenges of integrating a Fenton-based advanced oxidation process in resource-oriented sanitation systems aimed at recycling urine.

2. Materials and methods

2.1. Urine collection and concentration

Fresh urine was collected throughout the day in sterile bottles from male and female donors, aged 25–60 years old. At the end of each day, the donations were pooled into a 25-litre plastic container and mixed with 1.5 g L^{-1} of a 95 % sulfuric acid solution to lower the pH to ≤ 3.0 and prevent urea hydrolysis [20]. Portions of the acidified urine were concentrated by evaporation at 40 °C, reducing its mass by 80 % or 90 % to achieve mass concentration factors (CF) of 5 and 10, respectively. Unconcentrated acidified urine was designated as CF1. Detailed urine compositions are provided in Table S1 of the Supplementary Information (SI). Before use in experiments, urine was filtered through 0.45 μm syringe filters (Filtropur S, Sarstedt) to remove any solids precipitated during storage or evaporative concentration.

2.2. Experimental procedure

Four sets of experiments were conducted to evaluate the effects of key operating parameters on Fenton oxidation of urine (Table 1). These were structured as follows: Experiments 1 and 2 focused on identifying optimal operating conditions by varying the H_2O_2:Fe^{2+} molar ratio and initial urine pH, respectively. In Experiment 4, a higher peroxide dose (2 and 4 g L^{-1}) was applied to CF1 urine matrix; this treatment condition is referred to as CF1X throughout the manuscript. Experiment 3 investigated the influence of urine matrix strength by comparing concentrated and unconcentrated urine (CF1, CF4, CF6).

For each experiment, 75 mL of acidified urine was placed in 100 mL Erlenmeyer flasks kept on a magnetic stirrer. Hydrogen peroxide (50 % H_2O_2, w/w in water) and iron (II) sulphate heptahydrate ($FeSO_4{\cdot}7H_2O$) from a prepared 0.44 M stock solution were added according to the conditions specified in Table 1. The pH was adjusted once, after the addition of all reagents, using 1 M H_2SO_4 or 1 M NaOH, and the reactions were allowed to proceed for 2 h at 20 ± 1 °C in covered flasks.

Additional experiments were conducted using concentrated and

Table 1
Summary of experimental operating conditions.

Exp No.	Urine CF (-)	H_2O_2 dose (g/L)	Molar ratio H_2O_2: $FeSO_4$ (1: x)	Initial pH (-)	Aim
1	1	1	0.125, 0.25, 0.5, 1, 1.5	3	Determine optimum ratio of H_2O_2 to Fe^{2+}
2	1	1	1	2, 2.5 3, 3.5, 4, 5, 6	Determine the effect of initial pH
3	1	1	1	4	Determine effect of CF on urea, ammonium, Cl, COD and organic metabolites
	4*	5			
	6**	10			
4	1	1,2,4	1	4	Determine the effect of H_2O_2 dose on urea, ammonium, Cl, COD and organic metabolites

* The addition of $FeSO_4{\cdot}7H_2O$ and 50 % H_2O_2 (w/w) in experiments diluted CF5 urine to CF4 urine.

** The addition of $FeSO_4{\cdot}7H_2O$ and 50 % H_2O_2 (w/w) in experiments diluted CF10 urine to CF6 urine.

unconcentrated synthetic urine to better understand Fenton chemistry in a simplified urine matrix. The experimental sets on synthetic urine mirrored those conducted on urine, with details provided in Table S2 in the Supplementary Information.

2.3. Analytical methods

The concentration of urea, cations and anions were determined colorimetrically on an automated Gallery™ Discrete Analyzer (ThermoFisher Scientific, USA) using standard methods of the equipment. Soluble chemical oxygen demand (COD) was measured using a Spectroquant COD Cell Test (114,555, Merck KGaA, Germany) in the range 500–10,000 mg L^{-1}. UV absorbance was measured using a LAMBDA 365 double-beam UV–Vis Spectrophotometer (PerkinElmer-Inc, USA). The concentration of hydrogen peroxide was measured by reacting urine with titanium(IV) oxysulfate in sulfuric acid solution (27–31 % H_2SO_4 basis) and measuring the absorbance at 405 nm [21]. All samples were diluted using Milli-Q water and the pH was adjusted where necessary to fall within the range of the analytical method.

A targeted quantitative metabolomics approach was employed to profile 268 endogenous metabolites in urine, using direct-injection mass spectrometry (MS) integrated with reverse-phase liquid chromatography-tandem mass spectrometry (LC-MS/MS). This custom assay (TMIC MEGA) described elsewhere [22], identified and quantified amino acids, phospholipids, biogenic amines, organic acids, acylcarnitines, sphingomyelins, and nucleotides/nucleosides via derivatisation, analyte extraction, and selective detection through multiple reaction monitoring pairs, with isotope-labelled internal standards and other internal references for quantification. The assay utilised a 96-well deep plate format with an attached filter plate for sample preparation. For metabolites other than organic acids, samples were thawed, vortexed and centrifuged at 13,000 × *g* and dried under nitrogen flow, before phenyl-isothiocyanate derivatisation. Post-incubation, the spots were re-dried using an evaporator, and metabolites were extracted with 300 μL of extraction solvent. Extracts were centrifuged into a lower 96-well plate and diluted with MS running solvent. Organic acids were prepared separately with 3-nitrophenylhydrazine derivatisation, followed by the addition of BHT stabiliser and water before LC-MS injection. Mass spectrometric analyses were performed on an ABSciex 5500 Qtrap® MS coupled with an Agilent 1290 UHPLC system (Agilent Technologies, Palo Alto, USA). Data processing was performed using Analyst 1.6.3 software (Sciex, USA). In this paper, the term "organic metabolites" (OMs) refers specifically to the organic compounds in urine quantified through the targeted metabolomics approach outlined above. Notably, this term excludes urea, which, despite being an organic metabolite, was quantified separately.

2.4. Calculations and statistical analyses

The degradation of metabolites organic metabolites (OMs) was calculated using Eq. (9), where C_0 and C_t represent the metabolite concentration in untreated and treated urine, respectively. When metabolite concentration in treated samples was below the limit of detection (LOD), C_t was estimated as the LOD divided by the square root of two, following Hornung and Reed [23]. Urea was excluded from the ΣOMs calculation despite being an organic metabolite, as it is present in much higher concentrations than other metabolites and has a significantly lower reactivity towards hydroxyl radicals ($k = 7.9 \times 10^5$ M^{-1} s^{-1} according to [17]).

$$Degradation = \left(1 - \frac{C_t}{C_0}\right) \times 100\ [\%] \quad (9)$$

Principal component analysis (PCA) was conducted to examine whether the variability in the degradation of organic metabolites in urine could be accounted for by computed or predicted property descriptors of these metabolites, as provided in the human urine metabolome database. The descriptors included water solubility, LogP, LogS, pKa, hydrogen bond acceptor and donor counts, polar surface area, rotatable bond count, ring count, refractivity, and polarisability [4]. All statistical analyses were performed in RStudio (version 2023.12.0 + 369) using R (version 4.3.2) [24].

3. Results and discussion

3.1. Optimum operating parameters

In CF1 urine matrix, the optimal operating conditions for Fenton oxidation, to ensure complete H_2O_2 activation and maximum COD removal, were determined to be a molar H_2O_2 to Fe^{2+} ratio of 1:1 and pH of 4.0. Under these conditions, 38 % of the COD was removed in urine (Fig. 1B). Activation refers to the percentage of H_2O_2 that has reacted or been converted to other forms, indirectly indicating hydroxyl radical formation. The Fe^{2+} dose is important because it catalyses the decomposition of H_2O_2, but should be minimised to reduce operating costs, sludge formation, and residual Fe in solution. As shown in Fig. 1A, a logistic curve fitted to both the H_2O_2 activation and COD removal data suggests that a 1:1 molar ratio of H_2O_2 to Fe^{2+} was sufficient for complete peroxide activation. COD removal approached saturation at a ratio of 0.96, beyond which further increases in Fe^{2+} provided only marginal additional removal. When comparing the required Fe catalyst to ensure complete H_2O_2 activation, urine (Fig. 1A) needed approximately five times more Fe catalyst than synthetic urine (Figure S1 in SI). Furthermore, H_2O_2 activation was complete in less than five minutes in urine, compared to over two hours in synthetic urine (Figure S2 in SI). Due to this fast reaction, only the initial and final H_2O_2 concentrations (at 0 and 5 min) could be measured, precluding detailed kinetic analysis. As such, kinetic curves are only shown for synthetic urine in the Supplementary Information (Figure S3).

These observations suggest that the cycle of Fe^{2+} regeneration (Eq. (2)) in urine is being interrupted. It is hypothesised that the organic compounds present in urine scavenge Fe^{3+} ions, forming insoluble iron (III) organic complexes through a ligand exchange mechanism [25] thus preventing the regeneration of Fe^{2+}. Supporting this hypothesis, Simbeye et al. [7], observed co-precipitation of organics with iron during vivianite recovery from urine dosed with $FeSO_4$. The use of reducing agents such as L-cysteine [26] and citric acid [27] may enhance Fe^{2+} regeneration in the presence of organic inhibitors by aiding in electron transfer.

An analysis of the effect of operating pH on COD removal, residual Fe^{2+} and orthophosphate concentrations is shown in Fig. 1B and Fig. 1C. Complete H_2O_2 activation was achieved at all tested pH levels. The removal of COD increased from 22 % at a pH 2.5 to a maximum of 38 % at pH 4.0 and then decreased to 27 % at pH 6.0. UV_{254} removal, a surrogate parameter for predicting organic carbon content in wastewater [28], followed the same trend as COD removal (Fig. S4 in SI) and showed a moderate correlation with it ($R^2 = 0.6$, $p = 0.06$). A similar trend has been observed in studies investigating the treatment of landfill leachate using Fenton oxidation [29]. The reduced COD removal at low pHs is attributed to high H^+ concentration, which facilitates the formation of $H_3O_2^+$through Eq. (10) [30], thus reducing the availability of H_2O_2 for •OH generation. In addition, H^+ ions can scavenge •OH radicals via Eq. (11) [31], further limiting organics oxidation in urine.

$$H_2O_2 + H^+ \rightarrow H_3O_2^+ \quad (10)$$

$$\bullet OH + H^+ \rightarrow H_2O \quad (11)$$

The reduced COD removal at higher pH values is hypothesised to be due to the rapid conversion of $Fe^{2+}$to Fe^{3+} and the formation of ferric oxyhydroxide compounds which react more slowly with H_2O_2 [32]. In addition, at higher pHs, hydroxyl radicals tend to undergo secondary reactions with hydroxide ions (OH^-), forming less reactive species like

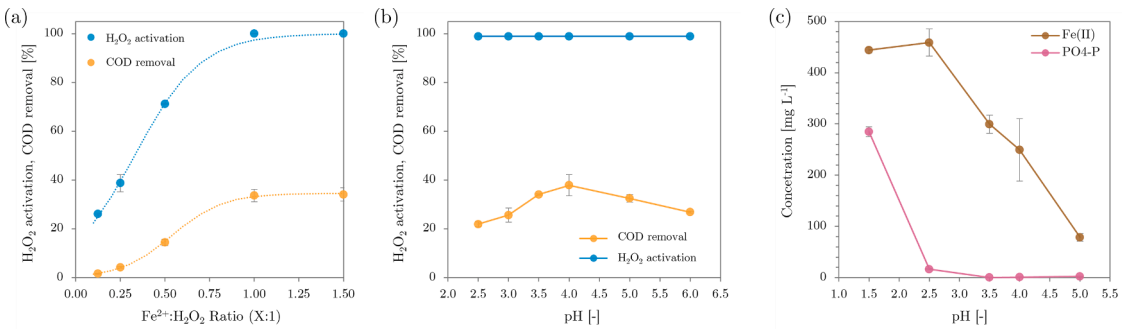

Fig. 1. (a) H_2O_2 activation and COD removal as a function of H_2O_2:Fe^{2+} molar ratio at a fixed pH of 3.0 and 1 g L^{-1} H_2O_2 dose in CF1 urine. A logistic function ($y = 100 / [1 + \exp(-5.4 \times (x - 0.33))]$) was fitted to the H_2O_2 activation data, and another logistic function ($y = 34.51 / [1 + \exp(-7.07 \times (x - 0.54))]$) was fitted to the COD removal data. (b) H_2O_2 activation and COD removal as a function of pH at a fixed 1:1 H_2O_2: Fe^{2+} molar ratio and 1 g L^{-1} H_2O_2 dose in CF1 urine; (c) residual $Fe^{2+}$and PO_4-P concentration in CF1 urine after treatment with 1 g L^{-1} H_2O_2 at different pH levels and a fixed 1:1 H_2O_2: Fe^{2+} molar ratio.

the superoxide radical ($\cdot O_2$) [11]. These effects were consistent with our visual observations of increased sludge formation in the pH range of 3.5–6. While the COD removal at pH 6 (27 %) is lower than the maximum removal at pH 4 (38 %), it is still relatively high and suggests that Fenton oxidation may offer partial treatment of freshly excreted urine stabilised with peroxide, even without strict pH correction. It should be noted that both COD and UV_{254} measurements were conducted on filtered urine samples (0.45 μm), and thus represent only the soluble fraction. As such, some COD removal observed at pH 6 may also be attributed to the co-precipitation or sorption of organics onto ferric solids, in addition to limited oxidative degradation.

The residual Fe^{2+} concentration in urine was highest at pH 2.5 (28 % of added Fe^{2+}) and decreased with increasing pH (Fig. 1C). Phosphorus precipitation exceeded 94 % across all pH levels. According to our thermodynamic modelling, ferric phosphate ($Fe(PO_4)$) forms between pH 1.2 and 4.2, while iron hydroxide ($Fe(OH)_3$) and meta vivianite ($Fe_3(PO_4)_2{\cdot}8H_2O$) precipitate at pHs above 3 and 3.8, respectively (Figure S3 in SI). Minimal sludge formation was observed at pH 2.5–3, likely due to partial reduction of Fe^{3+} back to Fe^{2+} (Eq. (2)), limiting iron precipitation as $Fe(PO_4)$. At pH 3.5–6, significant sludge formation was observed visually, attributed to $Fe(OH)_3$ and $Fe_3(PO_4)_2{\cdot}8H_2O$ precipitation [7]. The initial phosphorus concentration was 8.6 mmol L^{-1}, indicating that depending on the type of iron precipitate formed, between 30 % and 44 % of the added Fe^{2+} may have been consumed in this reaction.

3.2. Fate of COD, urea, ammonium and chloride

When evaluating the effect of urine CF, the H_2O_2 dose was scaled proportionally to the CF whilst the H_2O_2:Fe molar ratio was fixed at 1:1 and pH 4. Complete H_2O_2 activation was observed in all cases. Increasing the CF had no significant effect on COD removal and residual Fe^{2+} concentration but did result in increased urea, chloride (Fig. 2A), and ammonium (Table S3 in SI) oxidation. Urea oxidation was approximately three-fold higher at CF4 and four-fold higher at CF6 compared to CF1. However, there was no clear correlation between the extent of urea oxidation and chloride oxidation across different CFs. Treating urine at CF1 with Fenton oxidation appears optimal, as it effectively targets the degradation of organic compounds while minimising nutrient losses. The oxidation of urea and ammonium is hypothesised to occur due to the presence of chloride ions, which scavenge hydroxyl radicals to form reactive chloride species that, in turn, oxidise nitrogen-containing compounds [33], with N_2 gas and nitrate as the final reaction products [34]. This aligns with the observed increase in nitrate concentration across all CFs (Table S3 in SI).

As expected, increasing the H_2O_2 dose and the iron catalyst increases hydroxyl radical formation, leading to higher oxidation of both organic and inorganic compounds in urine. Doubling the H_2O_2 dose significantly increased COD removal from 34.8 to 67.6 %. However, a subsequent doubling of the dose resulted in only a marginal additional removal of 7 %, indicating diminishing returns at higher dosages (Fig. 2B). A similar trend was observed by Kallel et al. [35] during Fenton oxidation of landfill leachate. Diminishing COD removal could be due to excess H_2O_2 or Fe^{2+} reacting with hydroxyl radicals [36]. It is also possible that easily oxidisable compounds are degraded first, leaving behind refractory, difficult-to-oxidise compounds. Hydroxyl radicals preferentially target bonds that are easier to break, and once these are exhausted, the remaining compounds are more resistant to further oxidation, leading to reduced oxidative efficiency at higher reagent doses [37]. The increase in COD removal with increasing H_2O_2 dose also suggests that organics are preferentially oxidised over urea and chloride, which exhibit slower reactivity with hydroxyl radicals. Urea oxidation increased from 3.3 to 10 % when the H_2O_2 dose was increased from 1 to 4 g L^{-1}. Thus, determining the optimal H_2O_2 dose requires balancing this trade-off on a case-by-case basis.

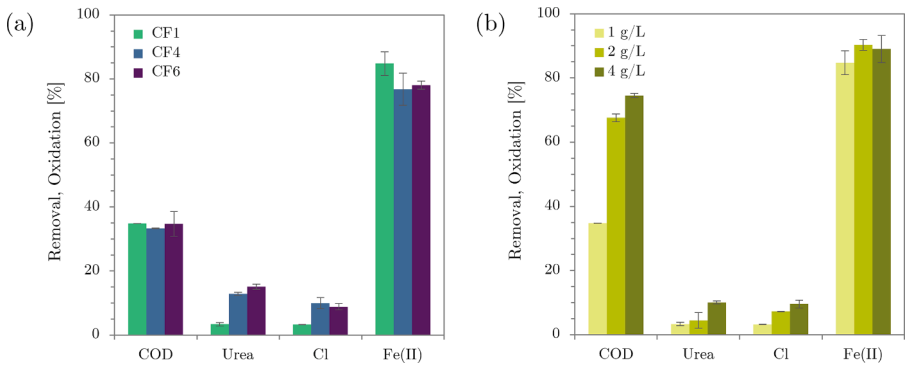

Fig. 2. Fate of organic and inorganic compounds after Fenton oxidation of (a) different types of urine (CF1, CF4, and CF6) treated with 1, 5, and 10 g H_2O_2 L^{-1}, and (b) CF1 urine treated with different peroxide dose (1, 2, and 4 g H_2O_2 L^{-1}) at a H_2O_2: Fe^{2+} ratio of 1:1 and pH 4.

3.3. Fate of organic metabolites

The targeted metabolomics assay identified 200 metabolites in acidified unconcentrated urine (Table S4 in SI). Creatinine, citric acid, hippuric acid, methylhistidine, histidine, glucose, and uric acid accounted for over 70 % of ΣOMs (sum of all quantified organic metabolites excluding urea) (ΣOMs = 3.23 g L^{-1}). Nearly 150 metabolites were oxidisable by Fenton's reagent, resulting in a ΣOMs degradation of 59 % in CF1 urine treated at pH 4.0 with 1 g H_2O_2 L^{-1} and 1:1 H_2O_2: Fe^{2+} molar ratio. Increasing the oxidant dose to 4 g H_2O_2 L^{-1} under the same conditions increased the number of oxidisable metabolites to 177 in CF1X urine, with ΣOMs degradation improving to 77 %, consistent with observed COD removal trends (Fig. 3). The ΣOMs degradation in concentrated urine matrices was higher than in unconcentrated urine when treated under identical conditions, with H_2O_2 doses scaled proportionally to the CF of the urine. While increased ionic strength at higher CFs can affect the oxidation kinetics of Fe^{2+} [38] and the degradation of organic compounds [39], the disproportionate increase in initial ΣOMs concentration with CF during evaporation likely also contributed to the higher observed degradation in concentrated urine. The mass concentration of ΣOMs in untreated CF4 urine and CF6 urine was 2.4 × and 5.7 × that of untreated CF1 urine, respectively, suggesting that some metabolites were not fully retained in solution during evaporation. For instance, metabolites such as glutamic acid, N2-acetylornithine, isoleucine, and glutamine were below the LOD in CF6 urine, despite being detectable in CF1 urine (Table S4 in SI). Overall, treating unconcentrated urine with a higher H_2O_2 dose resulted in the highest median degradation of metabolites across superclasses (Fig. 3), suggesting that the oxidant dose has a greater impact on organic degradation than the urine matrix.

Organic oxygen compounds, benzenoids, organoheterocyclic compounds, and phenylpropanoids were generally more oxidisable, with median degradation values exceeding 80 % across all treatments. In contrast, lipids, lipid-like molecules, and nucleosides were more resistant to oxidation, particularly in unconcentrated urine at the lower H_2O_2 dose (Fig. 3). The resistance of lipids to oxidation is consistent with earlier work, where median degradation was <50 % in urine acidified to pH 3.0 and treated with 60 mM peroxydisulphate at 90 °C for 1 h [40]. PCA revealed that molecular property descriptors poorly explained the variability in degradation, whether evaluated for individual metabolites or grouped into superclasses (Fig. S5 in SI). A weak positive correlation with polar surface area (PSA, r = 0.32) and a moderate negative correlation with molecular weight (MolWt, r=−0.36) suggested that smaller, more polar, and hydrophilic compounds are more susceptible to oxidation by Fenton's reagent. These findings align with studies in other wastewater matrices showing that molecular size and polarity are key determinants of oxidant reactivity [37].

There was efficient degradation of benzenoid hormones, including dopamine, norepinephrine, and epinephrine, as well as low-molecular-weight organics, such as citric acid, glutamic acid, and caffeic acid, with metabolite concentrations below the LOD in treated urine samples. Notably, organics that are known to foul membranes during urine treatment, such as uric acid [41] and sugars [5], were nearly completely degraded in all matrices. The degradation of creatinine, the major metabolite in urine, was 40 % in CF1 but improved to 65 % in CF1X with the higher H_2O_2 dose (Fig. 3). Glucose, ethanolamine, phenylacetylglutamine and threonic acid exhibited moderate resistance to oxidation, with both H_2O_2 dose and urine CF influencing their degradation (Fig. 4). In contrast, taurine and alanine were poorly oxidised across all treatments, suggesting low reactivity with hydroxyl radicals.

The concentration of several metabolites in urine increased post-Fenton oxidation (Fig. 4), with the most significant increases observed for allantoin, creatine, guanidinopropionic acid, methylamine, trimethylamine, glycine, N2-acetyl-ornithine, and guanidoacetic acid (Table S4 in SI). These increases likely result from the partial oxidation or breakdown of parent compounds present in urine. Complete degradation of trimethylamine N-oxide across all matrices likely contributed to the release of trimethylamine and methylamine [42]. Allantoin is a known oxidative product of uric acid [43]. Guanidinopropionic acid and guanidoacetic acid were likely derived from the oxidative cleavage of guanidino-containing compounds such as arginine and creatinine [44]. The increase in N2-acetyl-ornithine can be attributed to the breakdown of ornithine [45], which was degraded by 78 % in CF1 urine. Glycine may have been produced through the hydrolysis or oxidative cleavage of proteins, peptides, or amino acid derivatives [46]. Although these breakdown products are smaller than their parent metabolites, they

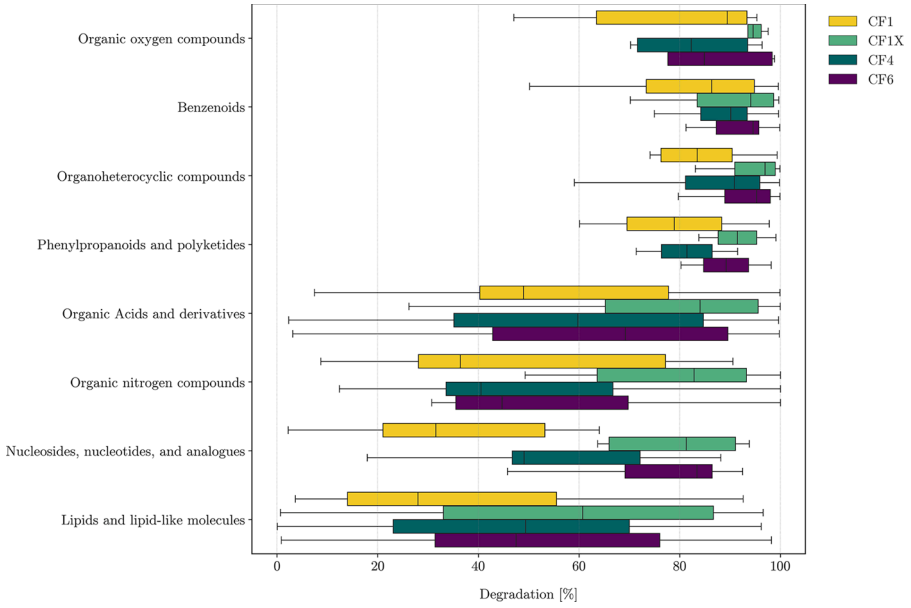

Fig. 3. Box plot showing degradation (%) of metabolites in four types of urine treated with Fenton's reagent: unconcentrated urine treated with 1 g H_2O_2 L^{-1} (CF1), unconcentrated urine treated with 4 g H_2O_2 L^{-1} (CF1X), urine concentrated 4 × by evaporation and treated with 5 g H_2O_2 L^{-1} (CF4), and urine concentrated 6 × by evaporation and treated with 10 g H_2O_2 L^{-1} (CF6). Metabolites are grouped into eight chemical superclasses based on the ClassyFire chemical taxonomy. Each box represents the median degradation and interquartile range, with whiskers extending to 1.5 times the interquartile range, excluding outliers.

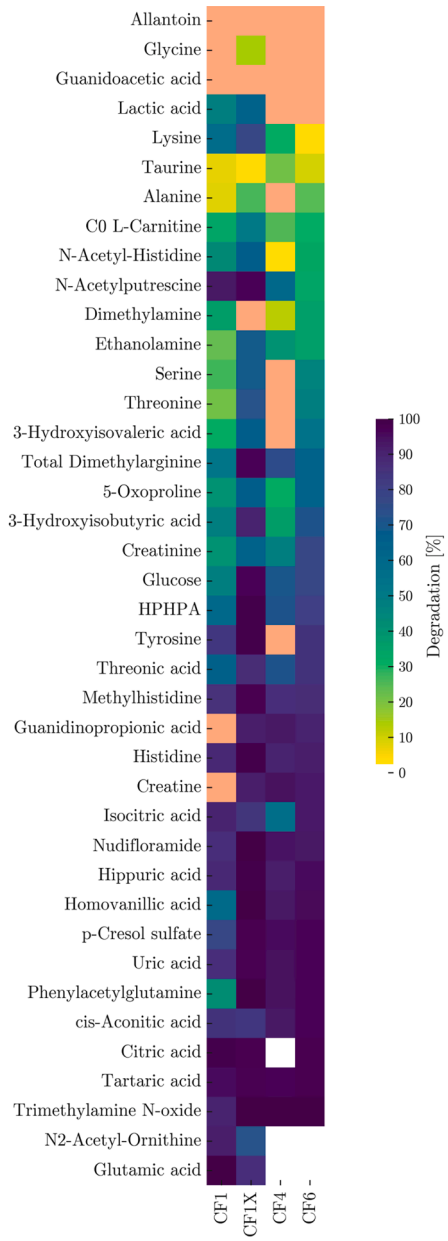

Fig. 4. Heatmap showing the degradation (%) of 40 major metabolites in urine treated with Fenton's reagent across four treatments: unconcentrated urine treated with 1 g H_2O_2 L^{-1} (CF1), unconcentrated urine treated with 4 g H_2O_2 L^{-1} (CF1X), urine concentrated 4 × by evaporation and treated with 5 g H_2O_2 L^{-1} (CF4), and urine concentrated 6 × by evaporation and treated with 10 g H_2O_2 L^{-1} (CF6). These metabolites collectively account for 95 % of the initial ΣOMs in unconcentrated urine. Orange cells represent metabolites that were produced during the treatment, while white cells indicate metabolites that could not be detected in either untreated or treated urine samples.

remain oxidisable and contribute to COD, which explains the limited overall COD removal despite substantial degradation of ΣOMs.

4. Conclusions and recommendations

This study demonstrates the potential and limitations of Fenton oxidation for selectively treating real human urine, balancing COD removal with the preservation of urea, a key fertiliser nutrient. Fenton oxidation in urine is optimised at pH 4.0 and a 1:1 Fe^{2+}: H_2O_2 molar ratio, achieving a ΣOMs degradation of 59 %, and retaining 97 % of the urea, in unconcentrated urine treated with 1 g H_2O_2 L^{-1}. The process remained effective at pH 6.0, suggesting that it could be applied to treat fresh urine stabilised with peroxide, without pH adjustment.

The formation of iron-organic complexes and iron phosphate precipitates in urine, resulting from Fe^{3+} scavenging, likely contributed to the observed fivefold increase in Fe^{2+} demand for complete H_2O_2 activation compared to synthetic urine. Future studies could explore alternative urine stabilisation strategies, such as citric acid instead of sulphuric acid, to improve Fe^{2+} regeneration and reduce sludge formation.

Fenton oxidation is best suited for treating unconcentrated urine, as higher mass concentration factors resulted in increased oxidation of urea, ammonium, and chloride, reducing nutrient recovery and overall treatment effectiveness. Notably, there was no clear correlation between the extent of urea and chloride oxidation across different CFs, suggesting that urea removal may not be directly linked to chloride scavenging alone. Although chloride oxidation was minimal (3 % in CF1 urine), further work is needed to determine the potential formation of chlorination by-products in urine.

The oxidant dose was found to be a stronger determinant of organic degradation than the urine matrix itself. Increasing the H_2O_2 dose to 4 g L^{-1} improved the degradation of ΣOMs to 77 %, however, this also increased urea (10 %) and chloride (9.5 %) oxidation. Organic oxygen compounds, benzenoids, organoheterocyclic compounds, and phenylpropanoids exhibited the highest degradation efficiencies (>80 %), whereas lipids, lipid-like molecules, and nucleosides were more resistant to oxidation. These findings suggest that Fenton's reagent preferentially oxidises smaller, more polar, and hydrophilic compounds in urine.

In conclusion, Fenton oxidation is a promising approach for degrading organic compounds in urine, provided treatment conditions are carefully optimised to minimise by-product formation and maximise nutrient recovery.

CRediT authorship contribution statement

Caitlin Courtney: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Abdullah Al-Saadi:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Prithvi Simha:** Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Prithvi Simha reports financial support was provided by Stiftelsen Lantbruksforskning. Prithvi Simha reports financial support was provided by Horizon Europe Research and Innovation Programme. Caitlin Courtney reports administrative support and travel were provided by August T Larsson Guest Researcher Programme. Abdullah Al-Saadi reports financial support was provided by Erasmus Plus. Prithvi Simha is a co-owner of Sanitation360 AB, a spin-off from the Swedish University of Agricultural Sciences that commercialises technologies for recycling human urine. Caitlin Courtney is a co-owner of PeeCycling, a spin-off from the University of Cape Town focused on a reverse osmosis-based treatment process to produce liquid fertiliser from human urine. The authors declare that the work reported in this study was conducted independently and was not influenced by their involvement in these companies..

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ceja.2025.100772.

Data availability

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

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