Metabolomic insights into balancing the trade-off between oxidation of endogenous organics and macronutrient recovery from human urine treated with Fenton's reagent

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Abstract

The high concentrations of organic and inorganic compounds in human urine present both challenges and opportunities for its treatment using advanced oxidation processes. This study evaluates Fenton oxidation for selectively degrading endogenoous organic metabolites in urine while preserving critical nutrients such as urea. Using targeted metabolomics, over 200 organic metabolites were identified in acidified 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 and reaching equilibrium 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.

Keywords: Advanced oxidation; Wastewater treatment; Resource recovery; Source separation; Peroxide; Sustainable sanitation

Synopsis: Metabolomics-guided optimisation of Fenton oxidation enables selective degradation of unwanted organics in urine while preserving urea and preventing chlorinated by-products, advancing source-segregated sanitation systems.

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1. Introduction

Advanced oxidation processes use highly reactive radical species to facilitate the non-selective oxidation and breakdown of organic and inorganic compounds (Eq. 1.). One of the most reactive free radicals, the hydroxyl radical ($E°$ = 2.73 V), can be generated by activating hydrogen peroxide (H_2O_2) with ozone, UV light, ultrasound, and transition metals (Neyens and Baeyens, 2003). H_2O_2 activation with iron salts, colloquially known as Fenton oxidation, consists of a sequence of reactions (Eq. 2 -8) (Barb et al., 1949). The chain reaction begins when ferrous ions (Fe^{2+}) catalyse the formation of hydroxyl radicals (•OH) in an acidic medium (Eq. 2). The reaction chain regenerates Fe^{2+} as the ferric (Fe^{3+}) ions produced in Eq. 2 further react with H_2O_2 (Eq. 3).

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

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

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

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

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

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

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

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

Fenton oxidation has been widely studied as a treatment method for degrading organic pollutants in water and wastewater (Nidheesh and Gandhimathi, 2012). 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 (Buxton et al., 1988; von Sonntag et al., 1997). Comprehensive reviews on Fenton chemistry in various matrices can be found in the works of Pignatello et al. (2006), (Deng and Englehardt, 2006) and (Bautista et al., 2008). Compared to other advanced oxidation processes, Fenton oxidation offers practical advantages, as it does not require complicated equipment or additional energy inputs, such as those needed for ozone-, UV-, or ultrasound-based activation of H_2O_2. However, the classical Fenton chain reaction has limitations, including a narrow optimal pH range (2-4), safety concerns regarding the handling, transport and storage of corrosive reagents, such as H_2O_2, concentrated acids, and iron catalyst, and the formation of iron sludge, which requires proper disposal (Zhang et al., 2019).

Human urine presents a unique matrix for studying Fenton chemistry due to several key factors. First, compared to domestic wastewater, human urine is highly concentrated in both organic and inorganic compounds, with a chemical oxygen demand typically ranging from 5 to 10 g O_2 L^{-1} (Putnam, 1971). These compounds include salts like sodium chloride, endogenous metabolites such as urea, uric acid and creatinine, as well as exogenous substances like pharmaceutical drug residues (Simha et al., 2024). Second, the human urine metabolome encompasses a diverse range of organic molecules, including amino acids, phospholipids, amines, organic acids, acylcarnitines, sphingomyelins and nucleotides, that contribute to its complex chemical profile (Bouatra et al., 2013). Third, studies investigating Fenton chemistry in real urine are scarce, as most existing research has been conducted in synthetic urine (Gonzaga et al., 2021; Miao et al., 2023). Fourth, urine naturally contains trace amounts of transition metals, which can activate H_2O_2 (Bouatra et al., 2013). Lastly, in the emerging field of resource-oriented sanitation, there is growing recognition that the presence of endogenous organic metabolites in human urine can significantly impact the performance and efficiency of unit operations used to recover resources. For instance, organic compounds in urine can cause fouling in filtration processes (Guizani et al., 2022), alter

adsorption dynamics of pollutants (Heusser et al., 2024), and influence the purity and yield of precipitated minerals (Simbeye et al., 2023). Many treatment technologies also require collected urine to be acidified, as it prevents mineral precipitation, inhibits reactions that lead to nitrogen loss, and inactivates pathogens (Hellström et al., 1999). This pre-acidification is advantageous for Fenton processes, which operate most effectively within a pH range of 2–4 (Pignatello et al., 2006). Moreover, if source-separated urine is stabilised with H_2O_2 to inhibit urease activity (Arve and Popat, 2021), any residual H_2O_2 can subsequently be activated by dosing Fe^{2+}, providing dual functionality that strengthens the case for applying Fenton oxidation for treating urine.

Since the urine matrix is complex, it is important to establish key operating parameters for Fenton oxidation, including the optimum H_2O_2 dose, the H_2O_2 to Fe^{2+} ratio, and pH. In addition, source-separated urine is typically concentrated to remove water and reduce transport costs (Chipako and Randall, 2020). It is therefore important to understand how the urine mass concentration factor (CF) affects the Fenton process, as both the composition and concentration of organic compounds can significantly impact the optimal pH range for effective treatment (Zhang et al., 2019). Additionally, Fenton oxidation can be inhibited by certain inorganic ions, such as sulphate, phosphate, bromide, and chloride (Pignatello et al., 2006). This inhibition may result from hydroxyl radical scavenging or iron precipitation, particularly in the presence of phosphate, leading to the formation of iron(III) phosphate or vivianite (Simbeye et al., 2023), which in turn influences the optimal doses of H_2O_2 and Fe^{2+}. Another key consideration is the reactivity of •OH with chloride (k=4.3×10^9 M^{-1}s^{-1}) and urea (k = 7.9×10^5 M^{-1}s^{-1}) (Dorfman and Adams, 1973). The oxidation of chloride can lead to the formation of chlorinated by-products, such as chlorate, perchlorate, organic and inorganic chloramines (Clark et al., 2021), whereas the oxidation of urea can result in nitrogen loss, reducing the value of fertiliser products derived from treated urine.

The overall aim of this study was to investigate Fenton oxidation as a targeted treatment process for the selective oxidation of organic metabolites in human urine, with a focus on minimising the oxidation of chloride and urea. Specific objects were to (i) evaluate the effect of different operating parameters (ratio of H_2O_2:Fe^{2+}, pH, concentration factor, H_2O_2 dose) and (ii) determine the fate of urea, total ammoniacal nitrogen (TAN), chloride, COD, and >250 endogenous organic metabolites in urine treated using Fenton's reagent. Overall, this study provides valuable insights into both the feasibility and challenges of integrating advanced oxidation processes in resource-oriented sanitation systems aimed at recycling human 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 (Simha et al., 2023). 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 **Error! Reference source not found.** 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: the H_2O_2 to Fe^{2+} ratio, pH, H_2O_2 dose, urine concentration factor, and initial chloride concentration. Experimental conditions, including chemical dose, specific ratios, pH levels, and concentration factors, are detailed in **Table *1***. 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 specified conditions. 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 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.

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 4* 6**	1 5 10	1	4	Determine effect of CF on urea, ammonium, Cl, COD and organic metabolites
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.

2.3. *Analytical methods and calculations*

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 (114555, Merck KGaA, Germany) in the range 500-10000 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 (Arve and Popat, 2021). 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 (Vergara et al., 2023), 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 (1990).

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

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 (Bouatra et al., 2013). All statistical analyses were performed in RStudio (version 2023.12.0 + 369) using R (version 4.3.2) (RStudio Team, 2015).

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 (Figure 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, chemical sludge formation, and residual Fe in solution. As shown in Figure 1A, a 1:1 molar ratio of H_2O_2 to Fe^{2+} was required to ensure complete H_2O_2 activation. Beyond this ratio, the COD removal decreased marginally. Urine required approximately five times more Fe catalyst than synthetic urine (Figure S2 in SI) to ensure complete H_2O_2 activation. Furthermore, the Fenton reaction reached equilibrium within five minutes in urine but took over two hours in synthetic urine (Figure S3 in SI). These observations suggest that the cycle of Fe^{2+} regeneration (Equation 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 (Ma et al., 2022) thus preventing the regeneration of Fe^{2+}. Supporting this hypothesis, Simbeye et al. (2023), 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 (Luo et al., 2016) and citric acid (Seol and Javandel, 2008) 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 **Error! Reference source not found.**B and **Error! Reference source not found.**C. 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 (Altmann et al., 2016), 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 (Cetinkaya et al., 2018). The reduced COD removal at low pHs is attributed to high H^+ concentration, which facilitates the formation of $H_3O_2^+$ through Equation 10 (Kwon et al., 1999), thus reducing the availability of H_2O_2 for •OH generation. In addition, H^+ ions can scavenge •OH radicals via Equation 11 (Sun et al., 2007), 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 (Gogate and Patil, 2015). In addition, at higher pHs, hydroxyl radicals tend to undergo secondary reactions with hydroxide ions (OH^-), forming less reactive species like the superoxide radical ($\cdot O_2$) (Buxton et al., 1988). This was observed visually, with higher formations of sludge occurring in the pH range of 3.5-6. However, the COD removal at pH 6 (27%) is still relatively high compared to the maximum removal at pH 4 (38%), suggesting that Fenton oxidation could be effectively applied to freshly excreted urine stabilised with peroxide, without requiring strict pH correction.

The residual Fe^{2+} concentration in urine was highest at pH 2.5 (28% of added Fe^{2+}) and decreased with increasing pH (Figure 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 (Fig S1 in SI). Minimal sludge formation was observed at pH 2.5–3, likely due to partial reduction of Fe^{3+} back to Fe^{2+} (Equation 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 (Simbeye et al., 2023). 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.

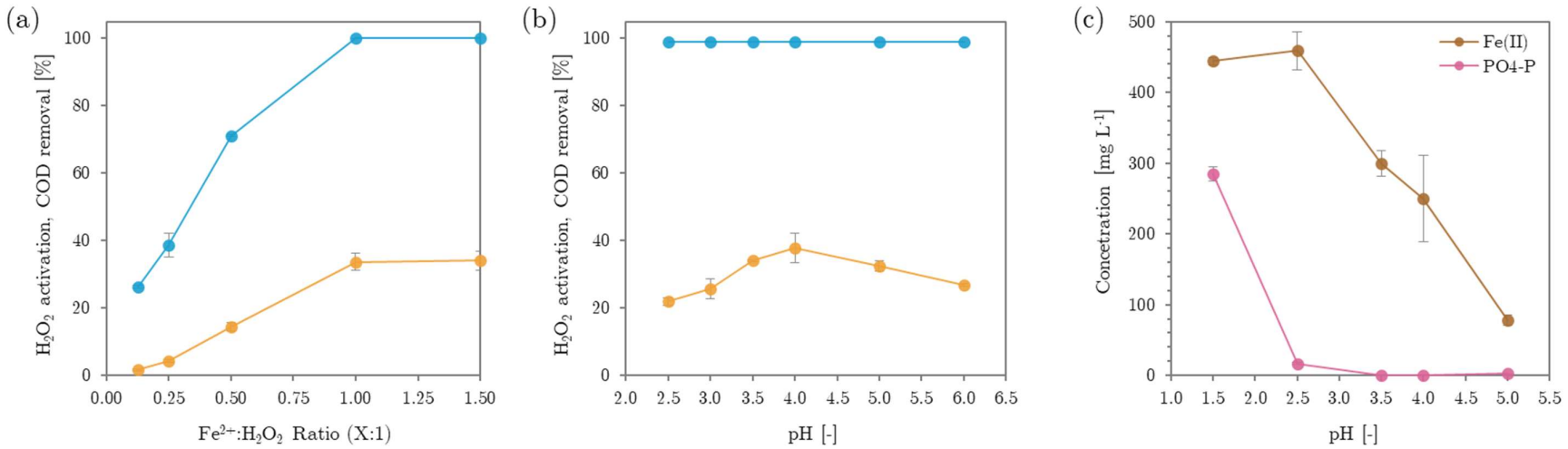

Figure 1: (a) H_2O_2 activation and COD removal as a function of H_2O_2:Fe^{2+} molar ratio at a fixed pH of 3.0 and 1 g L^{-1} H_2O_2 dose in CF1 urine; (b) H_2O_2 activation and COD removal as a function of pH at a fixed 1:1 H_2O_2: Fe^{2+} molar ratio and 1 g L^{-1} H_2O_2 dose in CF1 urine; (c) residual 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.

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 (Figure 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 (Clark et al., 2021), with N_2 gas and nitrate as the final reaction products (Cho and Hoffmann, 2014). This aligns with the observed increase in nitrate concentration across all CFs (Table S3 in SI).

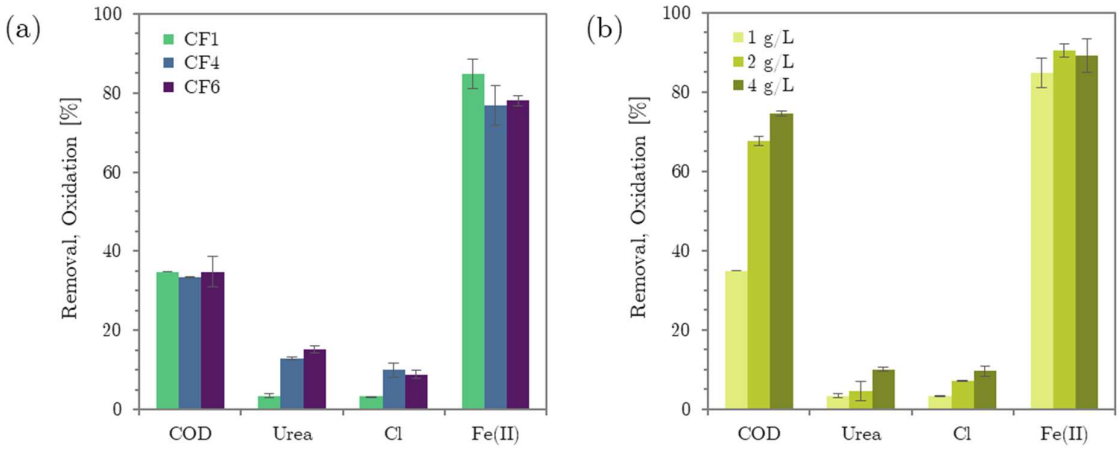

Figure 2: Fate of organic and inorganic compounds after Fenton oxidation of (a) different types of urine (CF1, CF4, and CF6) treated with 1, 5, and 10 g H_2O_2 L^{-1} , and (b) CF1 urine treated with different peroxide dose (1, 2, and 4 g H_2O_2 L^{-1}) at a H_2O_2: Fe^{2+} ratio of 1:1 and pH 4.

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 (Figure 2B). A similar trend was observed by Kallel et al. (2009) during Fenton oxidation of landfill leachate. Diminishing COD removal could be due to excess H_2O_2 or Fe^{2+} reacting with hydroxyl radicals (Tang and Huang, 1997). 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 (Pignatello et al., 2006). 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.

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 the total concentration of organic metabolites (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 (Figure 2). 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+} (Santana-Casiano et al., 2000) and the degradation of organic compounds (Lipczynska-Kochany et al., 1995), 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 (Figure 3). The resistance of lipids to oxidation is consistent with earlier work, where median degradation was less than 50% in urine acidified to pH 3.0 and treated with 60 mM peroxydisulphate at 90 °C for 1 h (Mehaidli et al., 2024). 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 (Pignatello et al., 2006).

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 (Courtney and Randall, 2022) and sugars (Guizani et al., 2022), 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 (Figure 4). Glucose, ethanolamine, phenylacetylglutamine and threonic acid exhibited moderate resistance to oxidation, with both H_2O_2 dose and urine CF influencing their degradation efficiency. 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, 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 (Loo et al., 2022). Allantoin is a known oxidative product of uric acid (Tsahar et al., 2006). Guanidinopropionic acid and guanidoacetic acid were likely derived from the oxidative cleavage of guanidino-containing compounds such as arginine and creatinine (Marescau et al., 1992). The increase in N2-acetyl-ornithine can be attributed to the breakdown of ornithine (Majumdar et al., 2016), 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 (Liu et al., 2017).

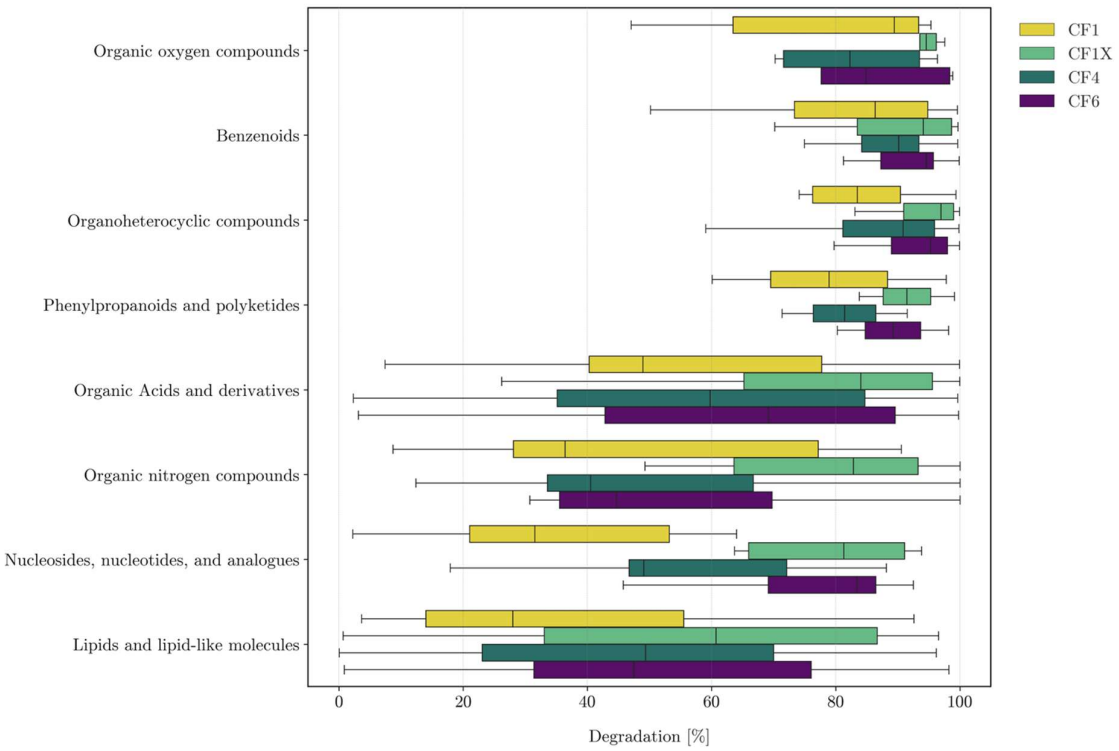

Figure 3: Box plot showing degradation (%) of metabolites in four types of urine treated with Fenton's reagent: unconcentrated urine treated with 1 g H_2O_2 L^{-1} (CF1), unconcentrated urine treated with 4 g H_2O_2 L^{-1} (CF1X), urine concentrated 4× by evaporation and treated with 5 g H_2O_2 L^{-1} (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.

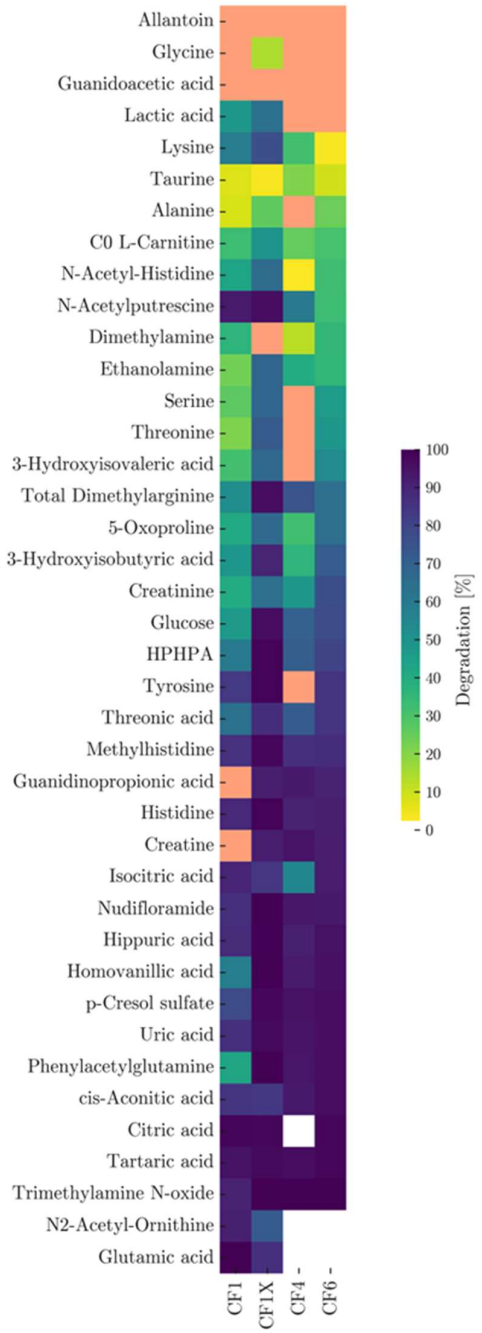

Figure 4: Heatmap showing the degradation (%) of 40 major metabolites in urine treated with Fenton's reagent across four treatments: unconcentrated urine treated with 1 g H_2O_2 L^{-1} (CF1), unconcentrated urine treated with 4 g H_2O_2 L^{-1} (CF1X), urine concentrated 4× by evaporation and treated 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.

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 efficiency 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 efficiency 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.

Author contributions

Caitlin Courtney: Conceptualisation, Methodology, Investigation, Formal analysis, Software, Writing – original draft. **Abdullah Al-Saadi**: Conceptualisation, Methodology, Investigation, Formal analysis, Data curation, Writing - Review & Editing. **Prithvi Simha:** Conceptualisation, Methodology, Investigation, Formal analysis, Visualisation, Writing – original draft, Funding acquisition, Resources, Supervision, Project administration.

Declaration of Competing Interest

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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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.

Supporting Information

The supporting information provides detailed experimental, modelling, and metabolomics data to support the findings of this study. It includes the compositions of real and synthetic urine used in the experiments (Table S1), experimental design of the synthetic urine trials (Table S2), thermodynamic modelling results predicting the forms of iron and phosphorus precipitates at varying pH levels (Figure S1), the kinetics of H_2O_2 activation in synthetic urine under different conditions (Figure S3), alongside the fate of ammonium and nitrate concentrations in treated urine (Table S3) are presented. The correlation between COD and UV_{254} removal is also shown to highlight the role of UV_{254} as a surrogate parameter for dissolved organic carbon in urine (Figure S4). Comprehensive metabolomics data, including over 200 organic metabolites, their

molecular properties, concentrations, and degradation efficiencies, are provided (Table S4). A PCA biplot highlighting molecular property descriptors and degradation patterns across treatments is also included (Figure S5).

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