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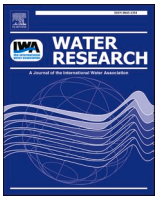

Selective degradation of endogenous organic metabolites in acidified fresh human urine using sulphate radical-based advanced oxidation

Ali Peter Mehaidli [a], Rupasri Mandal [b], Prithvi Simha [a,*]

[a] Swedish University of Agricultural Sciences, Department of Energy and Technology, SE 75007 Uppsala, Sweden
[b] The Metabolomics Innovation Centre, Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada

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ABSTRACT

The human urine metabolome is complex, containing a wide range of organic metabolites that affect treatment of urine collected in resource-oriented sanitation systems. In this study, an advanced oxidation process involving heat-activated peroxydisulphate was used to selectively oxidise organic metabolites in urine over urea and chloride. Initial experiments evaluated optimal conditions (peroxydisulphate dose, temperature, time, pH) for activation of peroxydisulphate in unconcentrated, non-hydrolysed synthetic urine and real urine acidified to pH 3.0. Subsequent experiments determined the fate of 268 endogenous organic metabolites (OMs) and removal of COD from unconcentrated and concentrated real urine (80–90% mass reduced by evaporation). The results revealed >90% activation of 60 mM peroxydisulphate in real unconcentrated urine heated to 90 °C for 1 h, resulting in 43% ΣOMs degradation, 22% COD removal and 56% total organic carbon removal, while >94% of total nitrogen and >97% of urea in real unconcentrated urine were recovered. The mechanism of urea degradation was identified to be chemical hydrolysis to ammonia, with the rate constant for this reaction determined to be 1.9×10^{-6} s^{-1} at pH 3.0 and 90 °C. Treating concentrated real urine resulted in similar removal of COD, ΣOMs degradation and total nitrogen loss as observed for unconcentrated urine, but with significantly higher chloride oxidation and chemical hydrolysis of urea. Targeted metabolomic analysis revealed that peroxydisulphate treatment degraded 157 organic metabolites in urine, of which 67 metabolites were degraded by >80%. The rate constant for the reaction of sulphate radicals with oxidisable endogenous organic metabolites in urine was estimated to exceed 10^8 M^{-1} s^{-1}. These metabolites were preferentially oxidised over chloride and urea in acidified, non-hydrolysed urine treated with peroxydisulphate. Overall, the findings support the development of emerging urine recycling technologies, including alkaline/acid dehydration and reverse osmosis, where the presence of endogenous organic urine metabolites significantly influences treatment parameters such as energy demand and product purity.

1. Introduction

Source separation and recycling of domestic wastewater fractions such as human urine is a popular area of research in the water sector (Aliahmad et al., 2022). Significant progress has been made in developing technologies for treating human urine, particularly over the past 20 years (Harder et al., 2019; Larsen et al., 2021). Many of these technologies have been piloted at different scales and are approaching industrialisation (Larsen et al., 2021; Simha and Ganesapillai, 2017). Source separation involves segregating human urine from other domestic wastewater and treating it separately to recover nutrients, chemicals, energy and/or water. Human urine contributes 70–90% of the nitrogen, 50–70% of the potassium and 45–65% of the phosphorus in domestic wastewater, but only 1% of the total volume (Vinnerås et al., 2006). Recycling human urine, a biological resource, offers an approach to accelerate the transition of the water sector to circularity, e.g. through returning plant-essential nutrients in urine to farmland as crop fertiliser (Martin et al., 2020). The added benefit with such recycling is that it reduces the nutrient load to existing centralised wastewater treatment plants, which in turn lowers the demand for energy, space and chemicals for treating domestic wastewater (Wilsenach and van Loosdrecht, 2006). It also reduces the flux of reactive nitrogen and phosphorus to surface water and groundwater, lowering the impact of poor wastewater treatment on aquatic environments, e.g. through reduced eutrophication

* Corresponding author at: Department of Energy and Technology, Swedish University of Agricultural Sciences, P.O. Box 7032, SE 75007, Uppsala, Sweden.
E-mail addresses: Prithvi.Simha@slu.se, Prithvi.Simha@mespom.eu (P. Simha).

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(Tidåker et al., 2007).

Water makes up >95% of urine by composition (Friedler et al., 2013). Urination is the main route by which the human body eliminates water-soluble wastes, including metabolic breakdown products of food, drugs and environmental contaminants, endogenous wastes and bacterial byproducts (Bouatra et al., 2013). Urea is the most abundant metabolite in human urine, contributing 36% of the dissolved solids content and 75% of the total nitrogen content (Putnam, 1971). Inorganic ions such as sodium, potassium, chloride, sulphate, phosphate and ammonium account for 38% of the dissolved solids content in urine (Putnam, 1971), while hundreds of organic metabolites, typically endogenous compounds, including a wide range of amino acids, peptides, hydroxy acids, amines, fatty acid esters, carbohydrates and lipids, make up the remaining 26%. The concentration of organic substances is about 10 g COD L^{-1} (Udert et al., 2006).

The organic matrix present in urine can affect its treatment in several ways. Organic metabolites form complexes with divalent ions such as iron (Fe^{2+}), which lowers the purity of vivianite ($Fe(II)_3(PO_4)_3{\cdot}8H_2O$) precipitated from human urine (Simbeye et al., 2023). Proteins can complex with uncharged molecules such as urea (Pronk et al., 2006), while organic acids (*e.g.* uric acid dihydrate) can foul membranes used for treating urine by nanofiltration and reverse osmosis (Courtney and Randall, 2022). Organic metabolites also determine the pH buffering capacity of urine and the solubility of different chemicals, e.g. citric acid (Ray et al., 2018) and magnesium hydroxide (Simha et al., 2022). Urobilin, the compound that gives urine its characteristic amber colour, competes with pharmaceuticals for biochar adsorption sites and reduces adsorptive removal of pharmaceuticals from urine (Solanki and Boyer, 2019). Studies focusing on degradation of micropollutants using dichromatic ultraviolet irradiation (185 and 254 nm) have shown that organic metabolites in urine competitively absorb light radiation and scavenge free radicals (Demissie et al., 2023, 2024). Volatile organic compounds emitted from urine, including dimethyl disulphide and 4-heptanone, also cause malodour (Liu et al., 2017).

The majority of previous research on urine recycling has focused on developing technologies for treating human urine to recover energy (Asiain-Mira et al., 2022; Santoro et al., 2020), water (Kamranvand et al., 2018; Volpin et al., 2019) and/or macronutrients (Christiaens et al., 2019; Courtney and Randall, 2022; Fumasoli et al., 2016; Kavvada et al., 2017; Ray et al., 2020; Vasiljev et al., 2021) or to remove micropollutants (Decrey et al., 2011; Ronteltap et al., 2007). Organic metabolites other than urea have been largely neglected, despite their significant contribution to the composition (metabolome) and properties of urine. In studies that have included organic metabolites, only a few metabolites that are relatively abundant in urine have been analysed, e.g. creatinine (Kamranvand et al., 2018), or different fractions of organic carbon such as low-molecular-weight organic acids have been analysed together, *e.g.* by size exclusion chromatography (Heusser et al., 2023). It is common practice to determine the concentration of organic metabolites in urine by analysing total organic carbon content or chemical oxygen demand (COD) of urine. However, some emerging urine treatment technologies could benefit from considering the effect of specific organic urine metabolites on treatment inputs (e.g. energy demand) and outcomes (e.g. product purity). For technologies with treatment objectives that are significantly influenced by organic metabolites in urine, such as those highlighted in the previous section, it may also be of interest to develop techniques to selectively recover, remove or degrade organic metabolites.

Organic substances can be degraded by advanced oxidation processes involving hydroxyl radicals (•HO) or sulphate radicals ($SO_4^{\bullet-}$) as oxidants. The redox potential of sulphate radicals (E^0 = 2.5–3.1 V) is higher than that of hydroxyl radicals (E^0 = 2.7 V) (Mahdi Ahmed et al., 2012). Sulphate radicals are highly reactive with most organic and inorganic substances, with rate constants in the range 10^7–10^{10} M^{-1} s^{-1} (Neta et al., 1988). In aqueous solution, sulphate radicals react with organic substances in the following order: non-aromatic organic compounds with -C=C- double bonds > substances with π electrons on aromatic rings > substances containing α-H > substances not containing α-H bonds (Wang and Wang, 2022). Inorganic substances, such as halide ions, also react with sulphate radicals, scavenging the radical and affecting the efficiency of the advanced oxidation process to degrade pollutants (Xue et al., 2022). Additionally, the reaction of halide ions with sulphate radicals can produce reactive halogen species that can further react with organic substances, leading to the formation of potentially toxic halogenated byproducts (Lu et al., 2016).

Generation of sulphate radicals involves activating precursor ions, peroxydisulphate ($S_2O_8^{\bar{}}$) or peroxymonosulphate ($HSO_5^{\bar{}}$), using heat, UV-light, transition metals, alkaline conditions or electric current (Matzek and Carter, 2016). Cleavage of the peroxide bond (-O-O-) in peroxydisulphate during its activation generates sulphate radicals with half-life of 40 μs (Wang and Wang, 2022), which is much longer than the half-life of hydroxyl radicals (<1 μs) (Hoelderich and Kollmer, 2000). If peroxydisulphate is activated using heat or UV-light, two sulphate radicals are generated, whereas activation with transition metals and alkaline pH generates only a single radical (Deng and Zhao, 2015). Activating peroxydisulphate in real urine with UV-light is likely to be challenging, since organic compounds in urine also absorb UV radiation in the wavelength range 190–400 nm (Demissie et al., 2024). The activation energy needed to generate sulphate radicals from peroxydisulphate ions using heat is 100–116 kJ mol^{-1} in acidic conditions, 119–129 kJ mol^{-1} in neutral conditions, and 134–139 kJ mol^{-1} in alkaline conditions (House, 1962). This suggests that heat activation of peroxydisulphate is favourable in acidified urine, especially if the treatment technologies used for recovering resources from human urine already involve acidification and heating (*e.g.* evaporation and distillation). However, considering the significant amount of chloride excreted in urine (in the range 1.9–8.4 g L^{-1} according to Putnam (1971)), there is risk of chloride oxidation during the treatment of urine with heat-activated peroxydisulphate.

The overall aim of this study was to develop an advanced oxidation process for selective degradation of organic metabolites in human urine with minimal loss of urea and minimal oxidation of chloride. Specific objectives were to (i) evaluate the effect of different parameters (temperature, pH, time, peroxydisulphate concentration, type of urine) on activation of peroxydisulphate in urine and (ii) determine the fate of endogenous organic metabolites and that of different forms of nitrogen, chloride, and COD in urine treated with heat-activated peroxydisulphate.

2. Methods

2.1. Materials

Real fresh human urine was collected from around 20 male and female donors, aged 20–65 years old. All the donations (n = >80) were pooled, dosed with 1.5 g L^{-1} of 96% H_2SO_4 to reduce the pH to ≤3.0 in order to prevent enzymatic hydrolysis of urea to ammonia (Simha et al., 2023), and mixed using an overhead stirrer (OHS 40 Digital, Velp Scientifica, Italy). Acidification ensured that subsequent treatment of urine using peroxydisulphate was mediated with sulphate radicals, which are predominant at pH <7.0, rather than hydroxyl radicals, which are predominant at pH >7.0 (Oliveros et al., 2021). Portions of the acidified urine were concentrated by evaporation at 40 °C to reduce the mass by 80%, *i.e.* a concentration factor (CF) of 5 (CF5 urine), or 90% (CF10 urine) or left unconcentrated (CF1) at room temperature (20 ± 1 °C) for about 14 days before use in experiments. Synthetic fresh human urine was prepared according to a protocol described in Simha et al. (2022) (see Table S1 in Supplementary Information (SI)) and acidified using H_2SO_4. All chemicals used in the experiments were of reagent grade.

2.2. Peroxydisulphate activation in synthetic urine

Initial experiments were conducted using acidified, non-hydrolysed synthetic urine (CF1 urine and CF10 urine) to determine optimal parameters for activating peroxydisulphate. A one-variable-at-a-time experimental design was used where each parameter was varied while the rest were fixed (Table S2 in SI). The parameters tested were urine temperature, pH, activation time and initial peroxydisulphate concentration. The procedure involved placing 200 mL synthetic urine with adjusted pH (3.0–12.6) in a 250 mL Pyrex® borosilicate glass bottle, sealing and heating the bottle on a hot plate with magnetic stirring (SBS-MR-1600/6, Steinberg Systems, Germany) to the desired temperature (50–70 °C), dosing peroxydisulphate from a stock solution of 2.4 M sodium peroxydisulphate (30–60 mM) and treating urine for the desired time (15 min-6 h). Following treatment, urine was cooled to room temperature and then sampled to determine activation of peroxydisulphate (see Section 2.5).

2.3. Peroxydisulphate activation in real urine

An experiment with real urine was conducted based on optimal parameters that maximised peroxydisulphate activation in synthetic urine, but the results showed significantly lower peroxydisulphate activation in real urine compared with synthetic urine. Therefore, a new set of experiments was conducted to optimise peroxydisulphate activation in real urine acidified to pH 3.0 using the one-variable-at-a-time approach, where urine temperature was varied between 70 and 90 °C, initial peroxydisulphate concentration between 60 and 90 mM, and activation time between 0.5 and 3 h (Table S3 in SI).

2.4. Treatment of real urine with heat-activated peroxydisulphate

In the next experiment, the fate of metabolites in real urine treated with heat-activated peroxydisulphate was evaluated. Parameters identified in the previous experiment—temperature, time, pH, and peroxydisulphate dose—as optimal for peroxydisulphate activation in real urine were applied and three types of real urine (CF1, CF5, CF10) were studied. The peroxydisulphate dose for CF5 urine and CF10 urine was set to be 5-fold and 10-fold higher, respectively, than the optimal peroxydisulphate dose for CF1 urine, to account for the higher COD content of concentrated urine (Table S3 in SI). Physicochemical properties of urine and the concentrations of different forms of nitrogen, chloride, COD and 268 endogenous metabolites were measured before and after treatment.

2.5. Controls

A follow-up experiment assessed potential interference by potassium dichromate (an oxidant used for measuring COD concentration in urine) in determination of residual peroxydisulphate concentration in urine. Real acidified fresh urine was dosed with known amounts of peroxydisulphate, varying from 0 mM to 30 mM, mixed and immediately analysed for concentration of COD.

A second experiment was conducted to determine degradation of urea in the absence of peroxydisulphate. Real acidified fresh urine was distributed into 10 mL glass vials, which were closed and placed in a digital dry block heater (QBD4, Grant Instruments, UK) at 90 °C. The vials were destructively sampled after 1, 2, 3, 4.5 and 24 h and the contents were analysed for concentration of urea and ammonia.

A third experiment was carried out to determine the concentration of free, total and combined chlorine (Cl_2) in real acidified CF1 urine after treatment with 60 mM peroxydisulphate at 90 °C for 1 h. This experiment was repeated without any gas headspace for comparison.

A fourth experiment evaluated the effect of chloride concentration on degradation of urea during peroxydisulphate treatment of urine. Synthetic CF1 urine without chloride or dosed with 16- and 32-fold higher chloride concentrations was treated using 60 mM peroxydisulphate at 90 °C for 1 h. The fate of urea and chloride in urine was determined.

2.6. Analysis of standard parameters

Activation of peroxydisulphate was estimated by determining concentration of peroxydisulphate anions in urine spectrophotometrically at 400 nm, using the modified iodometric titration method described in Liang et al. (2008). These measurements were performed on a Lambda 365 UV–Vis spectrophotometer (PerkinElmer, USA) with 1 cm optical path length and Milli-Q water for background absorbance. A calibration curve correlating absorbance with pre-determined concentrations of peroxydisulphate was developed (Fig. S1 in SI).

Electrical conductivity (EC) and pH were measured using a Metrohm iUnitrode with Pt1000 temperature sensor and a 4-wire conductivity measuring cell with integrated Pt1000 temperature sensor connected to a 914 pH/Conductometer (Metrohm, Switzerland). Concentration of COD was measured using a Spectroquant® COD Cell Test in the range 500–10000 mg L^{-1}. Interference in COD measurements caused by residual peroxydisulphate (up to 30 mM) was less than 5% and was ignored, as there was more than 80% activation of peroxydisulphate and the treated samples were diluted by a factor of 10. To measure soluble COD, urine samples were first passed through a 0.45 μm syringe filter (Filtropur S, Sarstedt, Germany). Free and total chlorine were analysed with a Spectroquant® Chlorine test, using the DPD spectrophotometric method (100599, Merck KGaA, Germany).

Treated and control urine samples were passed through a 0.45 μm syringe filter (Filtropur S, Sarstedt, Germany) and analysed spectrophotometrically for concentration of urea, total ammonia, nitrate and chloride using a Gallery™ Discrete analyzer (98610001, Thermo Fisher Scientific, USA). To determine total nitrogen concentration, urine samples were digested using a Spectroquant® Crack-Set 20 test kit (114963) and analysed for concentration of nitrate in the range 0.1–25 mg NO_3-N L^{-1} using a Spectroquant® test kit (109713, Merck KGaA, Germany) and a NOVA 60 A photometer (Merck KGaA, Germany).

2.7. Targeted urine metabolomics

A targeted quantitative metabolomics approach involving a combination of direct-injection mass spectrometry (MS) and reverse-phase LC-MS/MS custom assay (TMIC MEGA) was used to determine endogenous metabolites in urine (Vergara et al., 2023). The custom assay was used in combination with an ABSciex 5500 QTrap® mass spectrometer (Applied Biosystems/MDS Analytical Technologies, Foster City, USA) for targeted screening, identification and absolute quantification of 268 organic metabolites in urine, including amino acids, phospholipids, biogenic amines, organic acids, acylcarnitines, sphingomyelins and nucleotides/nucleosides. The method combined derivatisation and extraction of analytes with selective mass-spectrometric detection using multiple reaction monitoring pairs. Isotope-labelled internal standards and other internal standards were used for metabolite quantification. The custom assay comprised a 96 deep-well plate with a filter plate attached with sealing tape, and reagents and solvents used to prepare the plate assay. The first 14 wells were used for one blank, three zero samples, seven standards and three quality control samples. For all metabolites except organic acids, samples were thawed on ice, vortexed and centrifuged at 13,000 × *g*. Portions of sample were then loaded onto the centre of the filter on the upper 96-well plate and dried in a stream of nitrogen, after which phenyl-isothiocyanate was added for derivatisation. After incubation, the filter spots were dried again using an evaporator and the metabolites were extracted by adding 300 μL of extraction solvent. The extracts were transferred by centrifugation into the lower 96-deep well plate, followed by a dilution step with MS running solvent. For analysis of organic acids, 10 μL aliquots of sample were loaded into the centre of wells of a 96-deep well plate, followed by addition of 3-nitrophenylhydrazine derivatisation reagent and internal standard solution. After

incubation for 2 h, BHT stabiliser and water were added before LC-MS injection. Mass spectrometric analysis was performed on an ABSciex 5500 Qtrap® tandem mass spectrometer (Applied Biosystems/MDS Analytical Technologies, Foster City, USA) equipped with an Agilent 1290 series UHPLC system (Agilent Technologies, Palo Alto, USA). The samples were delivered to the mass spectrometer by an LC method followed by a direct injection method. Data analysis was performed using the software Analyst 1.6.3 (Sciex, USA). Throughout the remainder of this paper, the term "organic metabolites" (OMs) is used to refer to organic metabolites in urine quantified using the targeted metabolomics approach described above. It is important to note that this term does not encompass urea, also an organic metabolite, which was quantified separately.

2.8. Calculations

Cumulative degradation of organic metabolites (OMs) in urine was calculated as:

$$\sum OMs\ degradation, \% = \left(\frac{\sum C_0 - \sum C_t}{\sum C_0}\right) \times 100$$

where C_0 and C_t is metabolite concentration in untreated and treated urine, respectively. If metabolite concentration in treated urine samples was below the limit of detection (LOD), then C_t was assumed to be a value equal to the LOD divided by the square root of two (Hornung and Reed, 1990).

2.9. Statistical analyses

The experiments were conducted in duplicate. One-way analysis of variance (ANOVA) at 95% confidence interval was used to evaluate (i) whether temperature, pH, time and peroxydisulphate dose significantly influenced activation of peroxydisulphate in urine and (ii) whether type of real urine (CF1, CF5 or CF10) significantly influenced degradation of urea and loss of total nitrogen during peroxydisulphate treatment. The experimental data were tested for normality and homogeneity of variance before ANOVA. Principal component analysis (PCA) was performed to explore whether the variance in degradation of organic urine metabolites could be explained by computed/predicted property descriptors of the metabolites available in the human urine metabolome database (water solubility, LogP, LogS, pKa, hydrogen acceptor count, hydrogen donor count, polar surface area, rotatable bond count, number of rings, refractivity and polarisability) (Bouatra et al., 2013). All statistical analyses were carried out in RStudio version 2023.12.0 + 369 and R version 4.3.2 (RStudio Team, 2016).

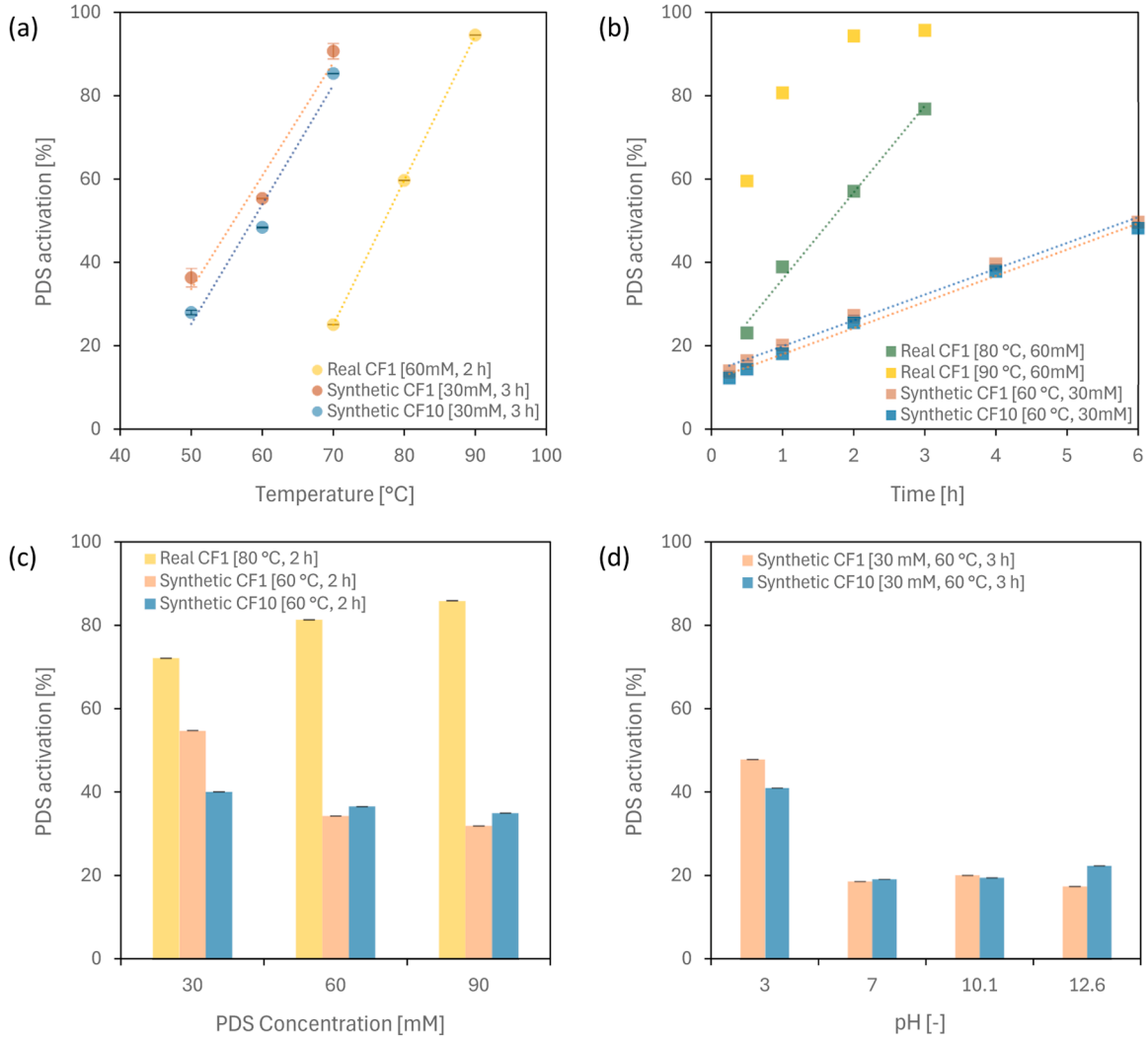

Fig. 1. Peroxydisulphate (PDS) activation (%) in real and synthetic urine at different (a) temperatures, (b) activation times, (c) PDS dose and (d) pH levels. Dotted lines show linear fit to experimental data (R^2>0.95), error bars show standard deviation of mean values (n = 2).

3. Results

3.1. Peroxydisulphate activation in synthetic urine and real urine

Temperature, peroxydisulphate dose and type of urine (real or synthetic) had significant effect on peroxydisulphate activation in urine (Fig. S2 in SI). Heating synthetic CF1 urine at 70 °C for 3 h resulted in >90% activation of 30 mM peroxydisulphate, whereas heating synthetic CF10 urine under the same conditions resulted in 85% activation of 30 mM peroxydisulphate (Fig. 1a). Lower doses of peroxydisulphate resulted in higher peroxydisulphate activation in synthetic urine regardless of urine CF. An initial pH of 3.0 resulted in the highest peroxydisulphate activation in synthetic urine (Fig. 1d).

Heating real CF1 urine at 70 °C for 3 h resulted in only 25% activation of 30 mM peroxydisulphate. For >90% activation of 60 mM peroxydisulphate in real urine, an activation temperature of 90 °C and reaction time of 1 h were needed. Kinetic analysis revealed a linear (R^2>0.99) increase in peroxydisulphate activation with time until 80% of peroxydisulphate was activated in real urine (Fig. 1b). For a peroxydisulphate dose of 60 mM, heating real urine at 90 °C for 1 h or at 80 °C for 4 h resulted in >90% activation. A higher peroxydisulphate dose resulted in higher peroxydisulphate activation in real urine (Fig. 1c). When real CF5 urine and real CF10 urine were treated using the same parameters and a CF-dependent peroxydisulphate dose, peroxydisulphate activation of 83% and 93%, respectively, was obtained.

Overall, optimal parameters for >90% peroxydisulphate activation in real CF1 urine were identified as: temperature 90 °C, peroxydisulphate dose 60 mM, urine pH 3.0 and reaction time 1 h.

3.2. Fate of COD, urea and chloride

Treatment at conditions that optimised activation of peroxydisulphate (90 °C for 1 h, pH 3.0, 60 mM peroxydisulphate) reduced the concentration of total COD in all types of real urine (Fig. 2a). Increasing the treatment time increased overall COD removal, but the difference was not significant beyond 2 h of treatment for CF1 urine (Fig. 2b). Increasing the peroxydisulphate concentration from 30 mM to 90 mM increased COD removal in CF1 urine from 17% to 32% (Fig. 2c). Removal of soluble COD was greater at higher CF. At lower CF, a much larger fraction of total COD was removed as insoluble organic precipitates (Fig. 3). In untreated urine, regardless of CF, >98% of the total COD was present as soluble COD.

Treating unconcentrated urine (CF1) with peroxydisulphate resulted in less than 3% degradation of urea and 6% loss of total nitrogen (Fig. 3). Treating CF10 urine with peroxydisulphate resulted in significantly higher degradation of urea ($p < 0.01$), but no significant difference in loss of total nitrogen ($p = 0.86$). Irrespective of urine CF, all treatments doubled the concentration of total ammonia in urine in comparison with the total ammonia initially present in urine. While no nitrate was detected in untreated urine, the average concentration in treated urine was 62 (±8.5) mg NO_3-N L^{-1}, corresponding to 1% of the total nitrogen content in untreated urine. There was visible precipitate formation during treatment, with higher precipitation observed when treating urine with higher CF. Based on a mass balance for nitrogen, between 1.3% (for CF10 urine) and 5.8% (for CF1 urine) of total nitrogen initially present in urine was bound in precipitates formed in the treated urine.

Treating real CF1 urine with initial pH 3.0 at 90 °C in the absence of peroxydisulphate resulted in urea being chemically hydrolysed to ammonia, which was retained in the urine and there was no loss of total nitrogen. Less than 2.5% of the urea present in urine was hydrolysed after 1 h, while 10.8% was hydrolysed after 24 h (Fig. 4). The rate of

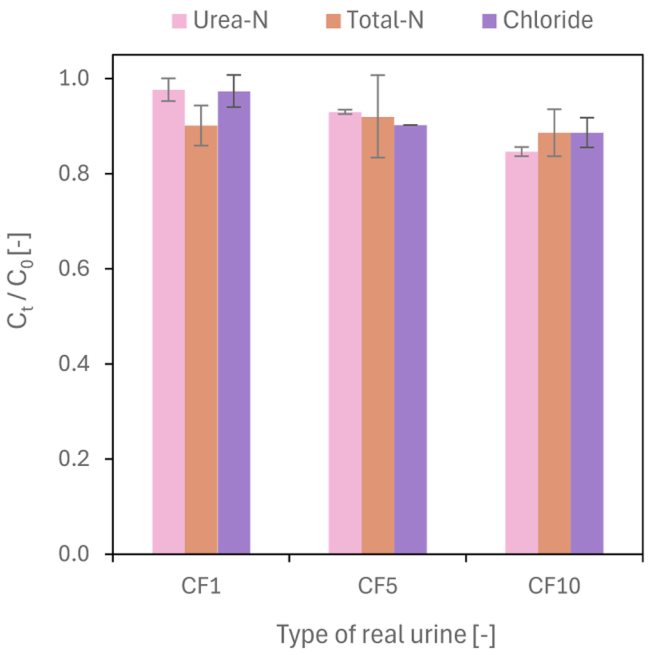

Fig. 3. Change in concentration of urea nitrogen, total nitrogen and chloride in different types of real human urine (CF, CF5, CF10) acidified to pH 3.0 and treated with 60 mM peroxydisulphate at 90 °C for 1 h. Mean of two replicates, error bars show standard deviation.

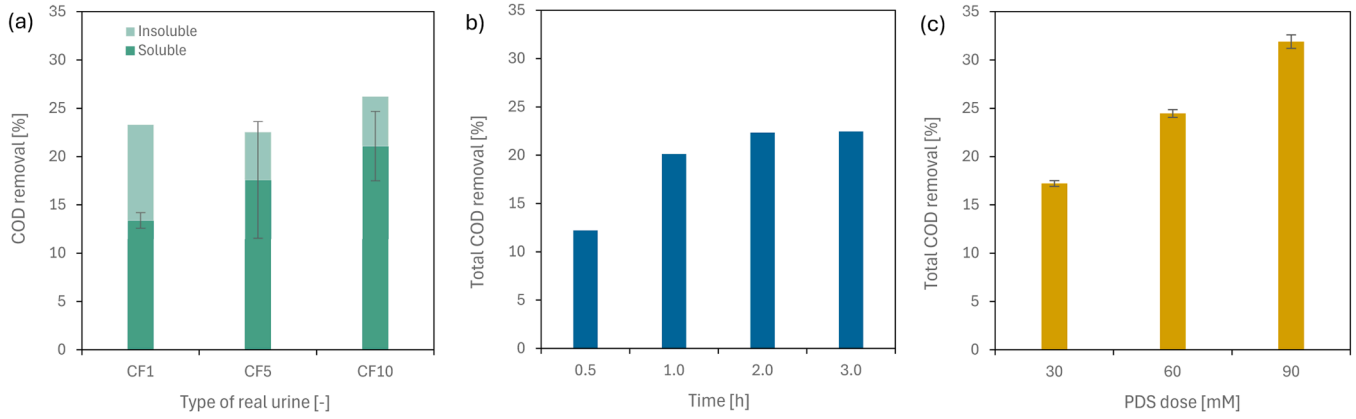

Fig. 2. Removal of chemical oxygen demand (COD,%) from (a) different types (CF1, CF5 and CF10) of real human urine acidified to pH 3.0 and treated with 60 mM peroxydisulphate at 90 °C for 1 h, (b) unconcentrated real (CF1) urine acidified to pH 3.0 and treated with 60 mM peroxydisulphate at 90 °C for different periods and (c) unconcentrated real (CF1) urine acidified to pH 3.0 and treated with different peroxydisulphate doses (mM) at 90 °C for 1 h. Error bars when shown represent standard deviation of mean values ($n = 2$).

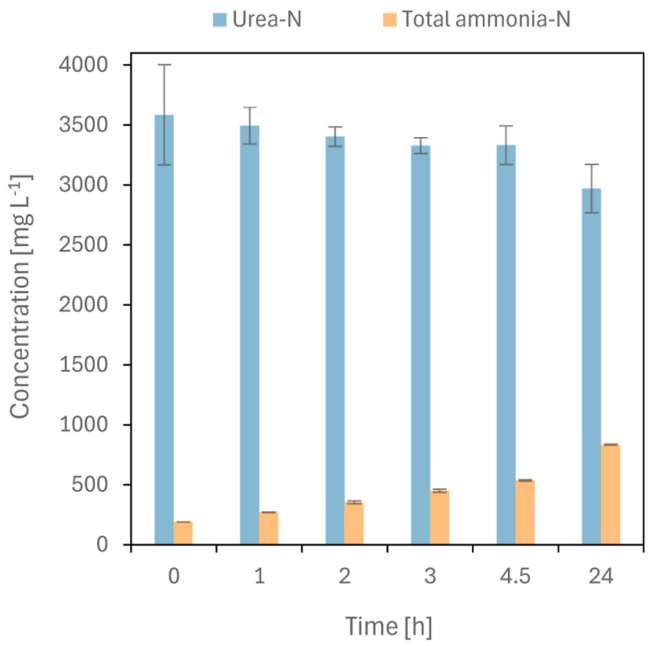

Fig. 4. Change in concentration (mg L^{-1}) of urea nitrogen and total ammonia nitrogen over time (h) in real unconcentrated (CF1) urine acidified to pH 3.0 and treated at 90 °C in the absence of peroxydisulphate. Error bars show standard deviation of mean values ($n = 2$).

chemical hydrolysis of urea was linear (R^2>0.99) up to 3 h of treatment.

Treatment with peroxydisulphate reduced the concentration of chloride by 2.6% (±3.4%) in real CF1 urine and by 25% (±8%) in synthetic CF1 urine. Treating real urine with higher CF resulted in higher chloride oxidation (Fig. 3). A follow-up experiment treating unconcentrated synthetic urine with peroxydisulphate showed that degradation of urea was twice as more in urine containing 16- and 32-fold higher concentration of chloride. It should be noted, however, that the colorimetric assay used for determining chloride concentration was based on the mercury (II) thiocyanate method and had standard deviation of 4% and minimum detection limit of 40 mg Cl^- L^{-1}.

The concentration of free, total, and combined chlorine in real CF1 urine measured immediately after treatment with peroxydisulphate was 0.38 (±0.05), 0.41 (±0.11) and 0.03 (±0.05) mg L^{-1}, respectively. In experiments without any gas headspace, the concentration of free, total, and combined chlorine in real CF1 urine measured immediately after treatment with peroxydisulphate was 1.12 (±0.17), 1.22 (±0.06) and 0.1 (±0.23) mg L^{-1}. The observed increase in total chlorine content did not correspond to the amount of chloride oxidised in urine by the treatment. Based on speciation assumptions for chlorine, almost all the chlorine produced during urine oxidation was in the form of free/active chlorine (HOCl and Cl_2), since the pH of urine varied between 1.2 and 3.0 during treatment.

In terms of changes in physicochemical properties, treatment with peroxydisulphate decreased the pH of CF1 urine, CF5 urine and CF10 urine from 3.0 to 1.76, 1.27 and 1.17, respectively. In addition, the EC of the urine increased from 15.08 to 33.6 mS cm^{-1} for CF1 urine, 63.2 to 119 mS cm^{-1} for CF5 urine and 103.9 to 150.8 mS cm^{-1} for CF10 urine. These changes are attributed to the breakdown of peroxydisulphate to hydrogen ions and sulphate ions following activation.

3.3. Fate of organic metabolites in real urine

In acidified fresh urine, 211 organic metabolites (OMs) were detected and quantified by targeted metabolomics (Table S4 in SI). In terms of diversity, these metabolites represented eight superclasses, 24 classes and 46 subclasses of chemicals according to the Human Metabolome Database, which classifies metabolites using the ClassyFire chemical taxonomy (Djoumbou Feunang et al., 2016). The majority of the metabolites had low molecular weight (<200 g mol^{-1}) and most belonged to the superclass organic acids and their derivatives (Fig. S3 in SI). Metabolites belonging to the superclass lipids and lipid-like molecules ($n = 14$) were the only chemical species in urine with high molecular weight (>500 g mol^{-1}). Of the 56 metabolites not detected in urine, half were lipid and lipid-like molecules.

The ΣOMs concentration in untreated urine, not including urea, was 1.43 g L^{-1}. The major metabolites on a mass basis were creatinine (38%), hippuric acid (8%), glucose (6%), creatine (6%), guanidinopropionic acid (4%), histidine (3%), uric acid (3%), phenylacetylglutamine (2%), taurine (2%) and 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA) (2%) (Table S4 in SI). Peroxydisulphate treatment reduced the ΣOMs concentration in treated urine to 0.82 g L^{-1} and resulted in ΣOMs degradation of 43% and total organic carbon removal of 56%. When considering only the 13 predominant metabolites that contributed most to ΣOMs concentration in urine, the treatment resulted in ΣOMs degradation of 50%, varying between 21% for creatinine and 99% for phenylacetylglutamine (Fig. 5a). Treatment of CF10 urine resulted in slightly higher ΣOMs degradation than treatment of CF1 urine, but the difference was not statistically significant ($p > 0.1$) for metabolites in any of the eight chemical superclasses. Following treatment with peroxydisulphate, the concentration of 54 metabolites in urine increased, but for 41 of these metabolites the increase was less than 1%. The metabolites with the largest increases were succinic acid, methylamine, dimethylamine and allantoin (Fig. 5b).

Amongst the different chemical superclasses (Fig. 6), median OMs degradation ranged from 50% for lipids and lipid-like molecules to 98% for phenylpropanoids and polyketides. There was high degradation (>70%) of metabolites belonging to chemical classes such as carboxylic acids and derivatives ($n = 48$), organonitrogen compounds ($n = 9$), phenols ($n = 9$), indoles and derivatives ($n = 7$) and benzene and substituted derivatives ($n = 7$). There was low degradation (<30%) of metabolites belonging to chemical classes such as organic sulfonic acids and derivatives ($n = 1$), purine nucleosides ($n = 3$), keto acids and derivatives ($n = 2$) and sphingolipids ($n = 2$). Metabolites belonging to the chemical classes fatty acyls ($n = 44$), carboximidic acids and derivates ($n = 2$), hydroxy acids and derivates ($n = 4$), organooxygen compounds ($n = 4$) were moderately degraded (30–70%).

4. Discussion

The peroxydisulphate dose and temperature required to activate more than 90% of peroxydisulphate in real human urine were significantly higher than the levels required for synthetic human urine. This indicates that use of synthetic urine for investigating the activation of precursors, such as peroxydisulphate, in advanced oxidation processes may not be suitable. This finding also supports the viewpoint of a recent perspective article which advocates for the increased use of real urine in decentralized sanitation research (Simha et al., 2024). The synthetic urine used in experiments contained major inorganic salts, urea and water, but lacked >200 organic metabolites that were detected in real urine. Real urine also contains inorganic metabolites, including transition metals such as iron, copper, cobalt and manganese (Vinnerås et al., 2006), that can catalyse peroxydisulphate activation by redox cycling (Anipsitakis and Dionysiou, 2004). The concentrations of these were not measured in this study. However, transition metals can be chelated by organic compounds in urine, e.g. caffeic acid, impeding peroxydisulphate activation (Maurya and Devasagayam, 2010). Antioxidants in urine, such as glucose and ascorbic acid, can inhibit peroxydisulphate activation by reducing peroxydisulphate to sulphate ions (Uzunboy et al., 2021). According to Kolthoff and Miller (1951), thermal decomposition of peroxydisulphate ions in aqueous solution is acid-catalysed, with the rate of reaction decreasing with increasing ionic strength. This explains the lower activation of peroxydisulphate in concentrated urine

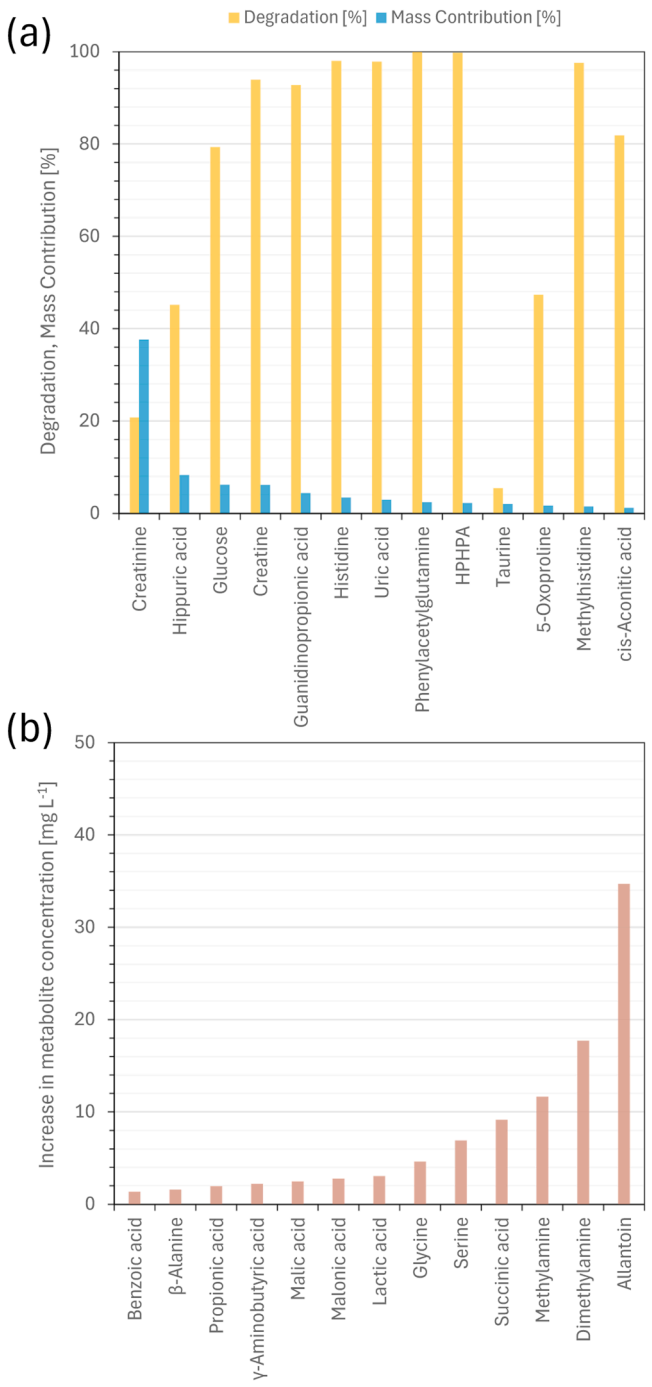

Fig. 5. (a) Degradation (%) of 13 organic metabolite (OMs) species in unconcentrated real (CF1) urine acidified to pH 3.0 and treated with 60 mM peroxydisulphate at 90 °C for 1 h (mass contribution of these metabolites to ΣOMs concentration in urine is also shown) and (b) increase in concentration (mg L^{-1}) of metabolite species in unconcentrated real (CF1) urine acidified to pH 3.0 and treated with 60 mM peroxydisulphate at 90 °C for 1 h.

(CF5 and CF10) compared with unconcentrated urine (CF1). The amount of peroxydisulphate activated in real acidified CF1 urine was similar irrespective of whether the urine was treated at 90 °C for 1 h or 80 °C for 4 h. Since the difference in energy demand between the two treatments is low (<0.015 kWh L^{-1} urine), other factors must determine the optimal treatment conditions.

There was no loss of total nitrogen when real CF1 urine was acidified to pH 3.0 and treated at 90 °C without peroxydisulphate. However, urea was degraded to ammonia by chemical hydrolysis. The rate constant for urea degradation in these conditions was 1.9×10^{-6} s^{-1}, which is lower than estimated by Shaw and Bordeaux (1955) for decomposition of urea at 90 °C in 0.05 M H_2SO_4 (1.2×10^{-5} s^{-1}). However, it is similar to the rate constant reported by Randall et al. (2022) for chemical hydrolysis of urea in real urine alkalised to pH 13.0, but treated at a significantly lower temperature of 70 °C (1.32×10^{-6} s^{-1}). Therefore, nitrogen recovery is more favourable from acidified urine treated at high temperatures than from alkalised urine, especially since low pH also retains nitrogen by preventing volatilisation of ammonia formed due to hydrolysis.

More than 90% of total nitrogen was recovered and about 25% of COD was removed when real CF1 urine was treated with heat-activated peroxydisulphate. Approximately 3% of the total nitrogen loss was due to degradation of organic urine metabolites containing nitrogen in their chemical structure (n = 124). Treating concentrated real urine with heat-activated peroxydisulphate did not lead to greater nitrogen losses or significantly improve COD removal. However, degradation of urea was significantly higher in concentrated urine, which could be due to three main reasons. First, the rate of urea degradation by chemical hydrolysis was likely higher in concentrated urine than in unconcentrated urine. According to Shaw and Bordeaux (1955), degradation of urea increases with ionic strength. The EC, a proxy for ionic strength, of CF10 urine after treatment was 5-fold higher than that of CF1 urine after treatment. Second, it is possible that chloride ions in urine scavenged sulphate radicals to form chloride radicals, which are strong oxidants (E^0 = 2.13 V) that can react with organic compounds with rate constants in the range 10^6–10^9 M^{-1} s^{-1} (Lei et al., 2021). The two amine groups and carbonyl group in urea are susceptible to attack by chloride radicals, which could lead to the formation of chloramines. However, the low content of combined Cl_2 in real CF1 urine after treatment (<0.1 mg L^{-1}) suggests this was not the major pathway for nitrogen loss, unless some of the chloramines decomposed to trichloramine (NCl_3), which cannot be detected by the test kits we used for determining free and total chlorine (LaKind et al., 2010). Conversely, chloride radicals can recombine (k = 1.0×10^8 M^{-1} s^{-1} according to Magazinovic et al. (2004)) to form chlorine gas (Lutze et al., 2015), particularly in acidic conditions such as those in our study (pH 1.2–3.0). According to Blatchley III and Cheng (2010), chlorine and urea can also react to form *N*-chlorourea at pH 3.0. Third, our results showed that oxidation of chloride ions was significantly higher in both synthetic urine and concentrated real urine treated with peroxydisulphate, compared to that in unconcentrated real urine. The lowest chloride oxidation (2%) was observed when real CF1 urine was treated with peroxydisulphate. These observations were supported by a follow-up experiment treating unconcentrated synthetic urine with peroxydisulphate, which showed that the degradation of urea was significantly higher in urine containing higher initial concentration of chloride. In summary, the results showed that nitrogen recovery and COD removal were consistent across all urine types tested, regardless of concentration factor, while chloride oxidation was minimised in acidified (pH ≤3.0) unconcentrated (CF1) urine treated with heat-activated peroxydisulphate. Further research using specific analytical methods, as outlined by Zhang et al. (2023), is required for a comprehensive understanding of formation and potential risks associated with chlorinated byproducts in unconcentrated, acidified urine treated with sulphate radicals.

Metabolomic analysis revealed that treatment with heat-activated peroxydisulphate degraded 157 organic metabolites in urine, of which 67 metabolites (ΣOMs = <0.5 g L^{-1}) were degraded by >80%. Since there was low chloride oxidation despite high chloride concentration in CF1 urine (3.1 g L^{-1}) and a rate constant for reaction of sulphate radicals with chloride ions of 2.7×10^8 M^{-1} s^{-1} (Magazinovic et al., 2004), it can be inferred that: a) rate constants of sulphate radicals with urine metabolites degraded in this study were >10^8 M^{-1} s^{-1} and b) these organic metabolites were oxidised before chloride in acidified and non-hydrolysed urine treated with peroxydisulphate. In contrast, it has been shown that organic metabolites in enzymatically hydrolysed urine

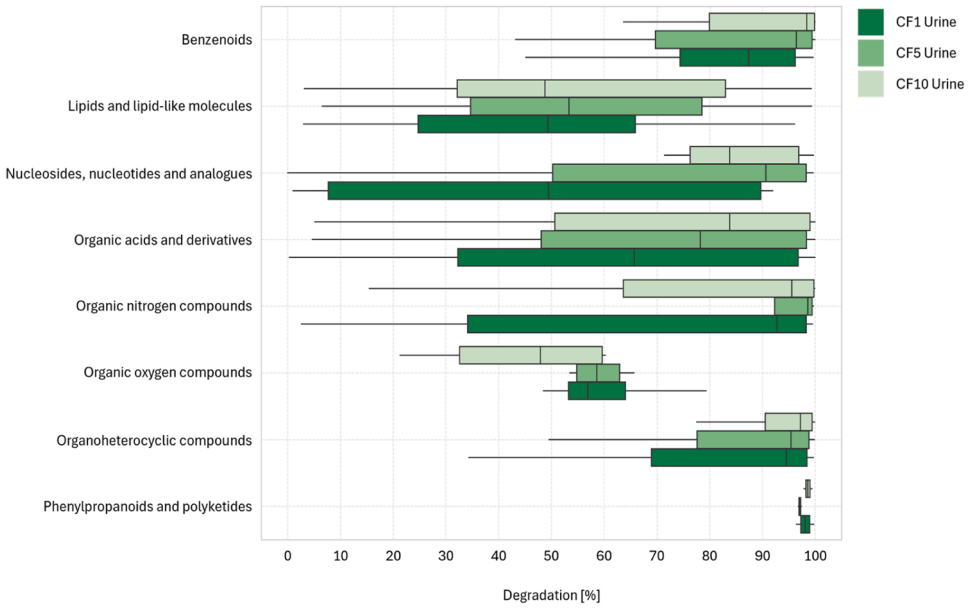

Fig. 6. Box plot showing degradation (%) of 211 metabolites in three types of real human urine (CF1, CF5, CF10) acidified to pH 3.0 and treated with peroxydisulphate at 90 °C for 1 h. The metabolites are grouped into eight chemical superclasses based on the ClassyFire chemical taxonomy. Each box displays the median degradation and interquartile range. Whiskers extend to 1.5 times the interquartile range and show range of metabolite degradation excluding outliers.

with pH adjusted to <4.0 are oxidised over ammonia in a two-chamber electrochemical cell with a boron-doped diamond anode, but that all chloride in urine is also oxidised, mostly to perchlorate (Jermakka et al., 2021). According to Zollig et al. (2015), inorganic and organic chlorinated byproducts form in hydrolysed urine during chlorine-mediated electro-oxidation. The degradation of organics over chloride, urea and ammonia in our study was likely because non-urea hydrolysed urine was treated at pH <3.0, where sulphate radicals ($SO_4^{\bullet-}$) are predominant, rather than pH >7.0, where $SO_4^{\bullet-}$ radicals are scavenged by OH^- (Oliveros et al., 2021).

The urine matrix is extremely complex (Bouatra et al., 2013) and developing reaction pathways for degradation of metabolites during advanced oxidation processes mediated by non-selective radicals like $SO_4^{\bullet-}$ can be challenging. Moreover, removal of total organic carbon from urine was only 56% in this study, indicating incomplete mineralisation and conversion of metabolites to intermediates and byproducts. Some metabolites were also partially oxidised (*e.g.* creatinine and hippuric acid). This complexity explains the absence of significant correlations (R^2 <0.4) between property descriptors of metabolites taken from the human urine metabolome database (Wishart et al., 2022) and degradation of different metabolites during treatment. However, based on urine metabolomic analysis and the literature, six main conclusions can be drawn: (i) uric acid was oxidised to allantoin (Kaur and Halliwell, 1990); (ii) glucose was oxidised to pyruvic acid (Doelle, 1975); (iii) histidine was converted to glutamic acid (Tabor et al., 1952); (iv) metabolites that contained nitrogen in their structure (e.g. creatinine and phenylacetylglutamine) were degraded to ammonium and nitrate (Singla et al., 2018), since their concentrations in urine increased after treatment (Table S4 in SI); (v) carboxylic acids, such as succinic acid, serine, glycine and malonic acid, and hydroxy acids, such as lactic acid and malic acid, were intermediate products that formed during oxidation of other metabolites, as their concentrations in urine increased after treatment. Formation of low-molecular-weight carboxylic acids, amino acids and alcohols has been observed in other studies evaluating oxidative degradation of organics (Gozmen et al., 2009; Sillanpaa et al., 2018). Degradation of organics in complex matrices occurs sequentially, in order of decreasing molecular size, according to Tercero Espinoza et al. (2009); and (vi) urine metabolites belonging to the superclasses benzenoids, organic nitrogen compounds, organoheterocyclic compounds, phenylpropanoids and polyketides were generally more oxidisable than metabolites belonging to organic oxygen compounds, lipids and lipids-like molecules, and organic acids and derivates (Fig. 6).

5. Conclusions

This study demonstrated that heat-activated peroxydisulphate can selectively oxidise organic metabolites over urea and chloride in unconcentrated and non-hydrolysed real urine acidified to pH 3.0. Initial investigations revealed that peroxydisulphate activation in urine was significantly influenced by peroxydisulphate dose, treatment temperature and type of urine used (synthetic versus real and concentrated versus unconcentrated). Optimal treatment parameters for achieving >90% peroxydisulphate activation in real unconcentrated urine were peroxydisulphate dose 60 mM, temperature 90 °C and reaction time 1 h. Further investigations revealed that treating real unconcentrated urine with peroxydisulphate resulted in degradation of over 150 organic metabolites and ΣOMs degradation of >40%, but less than 10% loss of total nitrogen and minimal chloride oxidation. Concentrated urine showed lower peroxydisulphate activation, higher urea degradation and higher chloride oxidation than unconcentrated urine. Further research is needed on chloride chemistry during peroxydisulphate treatment of urine, to achieve a complete mass balance for chlorine.

Urea was not oxidised by peroxydisulphate but underwent chemical hydrolysis to ammonia, which was then recovered due to the low urine pH. The rate constant for chemical hydrolysis of urea to ammonia at 90 °C and pH 3.0, in the absence of peroxydisulphate, was 1.9×10^{-6} s^{-1}. Organic metabolites from five superclasses (benzenoids, organic nitrogen compounds, organoheterocyclic compounds, phenylpropanoids and polyketides) were generally found to be more oxidisable than those belonging to the remaining three superclasses represented (organic oxygen compounds, lipids and lipid-like molecules, and organic acids and derivatives). The rate constant for the reaction between sulphate radicals and oxidisable organic metabolites was estimated to be $>10^8$ M^{-1} s^{-1}.

Overall, the results suggest that heat-activated peroxydisulphate treatment is more suitable for treating unconcentrated acidified urine than concentrated acidified urine. The treatment can be integrated with urine recycling technologies that involve heating to recover resources

from urine. Since it is effective in selectively oxidising organic urine metabolites, the findings hold promise for developing urine technologies where treatment goals are influenced by organic metabolites.

CRediT authorship contribution statement

Ali Peter Mehaidli: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rupasri Mandal:** Writing – review & editing, Software, Resources, Methodology, Investigation, Formal analysis. **Prithvi Simha:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, 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. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.121751.

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