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Photoinactivation of jack bean (*Canavalia ensiformis*) urease in fresh human urine using dichromatic low-pressure UV irradiation

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ABSTRACT

In source-separating sanitation systems, inhibiting urease activity prevents enzymatic urea hydrolysis and volatilisation of ammonia when urine is concentrated by evaporation. This study tested UV-based photoinactivation as a novel alternative to existing methods of inactivating urease that require dosing urine with acid, base or oxidants. The enzymatic activity of jack bean (*Canavalia ensiformis*) urease in water, synthetic fresh urine and real fresh urine was investigated, with and without a 15 W low-pressure UV lamp that emitted 185 nm and 254 nm radiation. In UV-free controls, urea was hydrolysed at a rate of 3.2×10^{-3} mmol mg_{urease}^{-1} min^{-1}, 3.3×10^{-3} mmol mg_{urease}^{-1} min^{-1} and 2.0×10^{-3} mmol mg_{urease}^{-1} min^{-1} in water, synthetic urine and real urine, respectively. In the presence of UV, no urease activity was detected in any matrix. A UV irradiation time of 1.3 and 3.3 min was needed for inactivating urease in water and synthetic urine, respectively, whereas an irradiation time of 71 min was needed for inactivating urease in real urine. Overall, the electrical energy demand for photoinactivation of urease in real human urine was estimated to be 29.1 kWh m^{-3}. Photolysis and photo-oxidation of amino acid residues at the active site of the enzyme were likely reasons for inactivation. Organic metabolites in real urine affected photoinactivation by (i) absorbing radiation between 190 nm and 400 nm, which reduced incident radiant flux; and (ii) scavenging hydroxyl radicals, which impeded oxidative damage to the enzyme. Overall, the findings demonstrate the feasibility of on-site treatment using a low-pressure UV lamp for inactivating urease in freshly excreted urine.

1. Introduction

Urease is a nickel-based metalloenzyme that hydrolyses urea to ammonia and carbamic acid [27]. Urease is produced by various bacteria, fungi, algae, plants and some invertebrates [35]. These organisms produce urease either to maintain a source of cellular nitrogen or to modify the pH of their surrounding microenvironment for survival [29]. Urea, the most favourable substrate for urease, is the most widely used nitrogen fertiliser globally [34]. In the human body, urea is produced by the liver due to nitrogen metabolism, filtered by the kidneys and excreted in urine [16]. Human urine contributes 80 % of the nitrogen (N) and 50 % of the phosphorus (P) load in domestic wastewater [41]. Recovering plant-essential nutrients excreted in urine and recycling them as fertiliser could be one approach to improve the circularity of sanitation systems [22,24]. While urine has been used historically as a fertiliser [2], the practice is now also attracting attention within the water treatment sector [1], since separation and local treatment of urine could improve existing wastewater treatment plants by increasing BOD: N ratio and reducing emissions of N_2O and CO_2 related to nitrification, denitrification and COD oxidation [4].

Several technologies are being developed globally to concentrate the nutrients present in urine [24]. Many of these technologies rely on keeping the main proportion of nitrogen as urea, making it necessary to inactivate urease, which is ubiquitous in sanitation systems that collect urine separately [38]. By inhibiting urease activity, these technologies limit loss of volatile nitrogen when urine is concentrated to remove water. Inactivation of urease can be reversible or irreversible. In reversible inactivation, urease regains catalytic activity once the inhibitor is removed and the microenvironment surrounding the enzyme is restored [28]. In irreversible inactivation, inhibitors cause changes to the enzyme that are not relieved by removal of the source. These can include chemical modification of the active site [18], conformational changes to the enzyme [20], or other structural modifications that cause the enzyme to cease functioning. In freshly excreted human urine,

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reversible inactivation of urease can be achieved by addition of acid to pH 3 [32]or base to close to pH 11 [31]. For irreversible urease inactivation, fresh urine must be dosed with oxidants such as hydrogen peroxide [3] and peroxydisulphate [26], heated to temperatures above 95 °C or brought to pH > 13 [13]. Research within soil science suggests that heavy metals [30] and urea analogues such as methyl urea and thiourea [27] can competitively inhibit urease [21]. According to Svane et al. [36], there are 71 commercially available chemical compounds with anti-ureolytic properties. However, not all compounds can be used safely in sanitation systems or are effective urease inhibitors in human urine. For instance, fluoride can bind to the active site of urease [11], but precipitates in urine due to the presence of calcium (Ca) [32].

Instead of dosing urine with chemicals, UV-based photoinactivation was tested in this study as a novel alternative to inactivate urease in fresh human urine. Irradiation with UV light has been shown to be effective in degrading micropollutants such as pharmaceuticals [10] and inactivating microorganisms such as *E. coli* and bacteriophages [14] and enzymes such as alpha-amylase, catalase and urease [9]. Irradiation with UV can inactivate urease by direct photolysis and/or photo-oxidation of photosusceptible functional groups present in the active site of the enzyme [25]. UV lamps that emit light radiation of wavelengths shorter than 200 nm into water also produce hydroxyl radicals (•OH) [43] that can react with amino acid residues present in the enzyme [8,40].

However, previous studies on photoinactivation of enzymes have only used simple matrices, such as distilled water [9] and pyrophosphate buffer solution [23], whereas human urine usually contains hundreds of metabolic breakdown products [6]. Even in distilled water, it has been shown that urease inactivation is more effective with a 222 nm excimer lamp compared to a 254 nm mercury lamp [9]. Considering all these aspects, this study aimed at evaluating the photoinactivation of urease in real human urine using a dichromatic (185 nm and 254 nm) low-pressure UV lamp. The specific objectives were to (i) evaluate kinetics of enzymatic urea hydrolysis in Milli-Q water, synthetic fresh urine containing no urea, and real fresh urine, with and without UV irradiation, and (ii) determine irradiation time and electrical energy demand required for inactivation of urease in all matrices.

2. Methodology

2.1. Materials

A stock solution of jack bean (*Canavalia ensiformis*) urease was prepared by dissolving 3.5 g of lyophilized urease (activity of $\geq$ 5 U mg^{-1}; Merck, Germany) in 200 mL of 50 % (v/v) glycerol/Milli-Q water solution. Synthetic fresh urine was prepared following the recipe of Ray et al. [32] (see Supplementary Information (SI), Table S1), but no urea was added to avoid enzymatic urea hydrolysis during UV treatment.

First-morning real fresh urine (Table S2 in SI) was collected from eight volunteers (male and female, aged 20–40 years) using 500 mL high-density polyethylene bottles. The urine donations were pooled, mixed and stored at 4 °C for < 4 h, and then allowed to reach room temperature (20 ± 2 °C) before use in the experiment.

2.2. Photoreactor set-up

The UV treatment was conducted in a cylindrical photoreactor (diameter 3.7 cm, length 40 cm, and volume of 430 mL) equipped with a 15 W dichromatic low-pressure mercury lamp with a UV fluence of 0.43 μW m^{-2} (GPH303T5VH-4, Heraeus, Germany). According to the manufacturer [15], the lamp emits light at a relative intensity of 100 % at 254 nm and 8 % at 185 nm. The lamp was surrounded by a synthetic quartz sleeve that can transmit light predominantly with wavelength of 185 nm and 254 nm. A peristaltic pump (Masterflex, Fisher Scientific, USA) recirculated fluid in the photoreactor at a rate of 40 mL min^{-1}. The pump and the reactor were connected using UV-resistant Tygon tubing (4.8 mm ID) with a total volume of 76 cm^3, which was fitted with a shut-off valve fitted so that treated samples could be withdrawn.

2.3. Experimental procedure

Photoinactivation of urease was evaluated in three different matrices: Milli-Q water (hereafter referred to as water), synthetic fresh human urine without any urea, and real fresh human urine. Before the start of the treatment, the lamp was switched on for 10 min to attain constant UV fluence. Then, 300 mL of real urine, urea-free synthetic urine or water were added to the reactor and the peristaltic pump was switched on. Once circulation started, 8.6 mL of urease stock solution were added to the reactor, so that the initial concentration of the enzyme was 500 mg L^{-1} (or 2500 AU L^{-1}), and the treatment began. Samples (25 mL) were withdrawn from the column after an irradiation time of 0.4, 1.3, 3.3, 7.1, 16.5, 35 and 71 min. Before each sampling, the first 2 mL were withdrawn from the shut-off valve and discarded. Electrical conductivity (EC) and temperature were measured immediately after samples were withdrawn and thereafter at intervals of 0.5, 1, 2, 4, 8, 12 and 24 h. In experiments involving water and synthetic urine, 7.5 mL of a 10 M solution of urea were added to each sample on withdrawal before taking the EC and temperature readings. The urease enzyme in all matrices received irradiation intermittently, as the solution passed between UV reactor and the recirculation pipes during the UV treatment. Sampling times were adjusted to consider the time spent by the solution in the recirculation pipes. The experiments were conducted without any attempt to either remove or add O_2.

As controls, urease activity was determined in the three matrices in the absence of UV light. For this, 100 mL of real fresh urine or synthetic fresh urine (including urea) were placed in a 125 mL beaker and spiked with 2.86 mL of urease stock solution, so that the initial concentration of the enzyme was 500 mg L^{-1}. The beakers were then covered and mixed at room temperature (20 ± 2 °C) on a magnetic mixer at 100 rpm. In the case of water, 2.5 mL of 10 M urea solution were added before spiking with urease. Temperature and EC of the solutions were monitored 1, 2.5, 5, 10 and 30 min after spiking, and thereafter at 1, 2, 4, 8 and 12 h. At these same time points, samples were taken for determination of concentration of total ammonia nitrogen. Since the solution temperature increased during UV treatment (due to warming of the bulb), additional control experiments were conducted to develop empirical relationships between EC and temperature for the three matrices.

2.4. Physical and chemical analyses

Solution pH was measured using an Accumet AE150 (Fisher Scientific, USA) pH meter attached to an electrode (13–620-AE6, Fisher Scientific, USA), while EC and temperature were measured using a Cond 340i multimeter (WTW, Germany) connected to a TetraCon 325 probe (WTW, Germany). The measured values for EC were corrected to compensate for the increase in temperature during UV treatment and are reported for a reference temperature of 25 °C, i.e. EC_{25}.

Colorimetric analysis using Spectroquant® test kits (Merck KGaA, Darmstadt, Germany) and a photometer (NOVA 60 A, Merck KgaA, Germany) was conducted to determine the concentration of total ammonia nitrogen (TAN), total nitrogen, and chemical oxygen demand (COD). The procedures used are described in detail in previous studies [39]. The concentration of P, potassium (K), Ca and magnesium (Mg) was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) using an Avio® 200 spectrophotometer (PerkinElmer, USA), prior to which samples were digested with 65 % HNO_3 and diluted with Milli-Q water. The UV absorbance of samples withdrawn during the experiments was measured between 190 nm and 400 nm on a Lambda 365 UV–vis spectrophotometer with 1 cm optical path length (Perkin-Elmer, USA). Background absorbance was measured using Milli-Q water (5 ppb TOC). Urine samples were diluted 10-fold before recording the absorbance.

2.5. Enzyme kinetics

Based on data collected in the control experiments, empirical equations were developed relating enzyme activity to measured EC values (Fig. S1 in SI). These equations were then used to estimate urease activity in the three matrices during UV treatment. This follows from Ray et al. [32], who showed that urea hydrolysis in human urine can be characterised by measurement of EC. The activity of urease (mmol TAN mg urease^{-1} min^{-1}) was estimated as:

$$Enzymatic\ activity(EA) = \frac{C_{TAN}}{MM} \times \frac{1}{X \times t} \quad (1)$$

where C_{TAN} is concentration of total ammonia nitrogen (mg L^{-1}) in solution, MM is molar mass of ammonia (mg mmol^{-1}), X is concentration of urease (mg L^{-1}) and t is time (min). Experimentally determined concentration of TAN was plotted against time and fitted to pseudo zero-order kinetics to determine the rate constant for enzymatic urea hydrolysis, calculated as:

$$C_t = k \times t + C_0 \quad (2)$$

where C_0 and C_t is concentration of TAN (mmol mg$_{urease}^{-1}$) initially and at any time t (min), respectively, and k is the rate constant (mmol TAN mg$_{urease}^{-1}$ min^{-1}) for enzymatic urea hydrolysis.

Relative enzymatic activity (REA, %) was calculated as the ratio of enzymatic rate constant in presence of UV (k_{UV}, mmol TAN mg$_{urease}^{-1}$ min^{-1}) to enzymatic rate constant in absence of UV (k_C, mmol TAN mg$_{urease}^{-1}$ min^{-1}) for all three matrices:

$$REA = \frac{k_{UV}}{k_C} \times 100 \quad (3)$$

Real fresh urine naturally contained urea, some of which was hydrolysed inside the column during UV treatment. No urea hydrolysis occurred during UV treatment of synthetic urine and water, as urea was added to these matrices only after treatment. Therefore, when calculating REA for experiments with real urine, hydrolysis occurring inside the column was ignored and k_{UV} was determined by measuring production of TAN after UV treatment.

2.6. Data analysis

Two-way analysis of variance (ANOVA) at 95 % confidence interval was performed to examine the influence of matrix and UV irradiation time on urease activity. Residual analysis was performed to test for the assumptions of the ANOVA. Outliers were assessed by the box plot method, normality was assessed using Kolmogorov-Smirnov test and homogeneity of variances was assessed using Levene's test. Pairwise comparison of group levels of main effects was analysed using Tukey's post-hoc test. There were no extreme outliers, residuals were normally distributed ($p > 0.05$) and there was homogeneity of variances ($p > 0.05$) for all matrices. All tests were performed using the statistical software R (version 4.1.2) and RStudio version 2022.02.

3. Results

In the absence of UV, enzymatic hydrolysis of urea occurred in all matrices. The concentration of TAN increased at a similar rate in synthetic urine and water, reaching equilibrium after 2 h. In real urine, it took 4 h for the concentration of TAN to reach equilibrium (Fig. 1). The rate constant for enzymatic urea hydrolysis was estimated to be 3.2 × 10^{-3} mmol mg$_{urease}^{-1}$ min^{-1}, 3.3 × 10^{-3} mmol mg$_{urease}^{-1}$ min^{-1} and 2.0 × 10^{-3} mmol mg$_{urease}^{-1}$ min^{-1} for water, synthetic urine and real urine, respectively (Fig. 1, Table S3 in SI).

In the presence of UV, the rate of enzymatic urea hydrolysis decreased in all matrices. Type of matrix had a significant influence ($p < 0.01$) on urease activity (Fig. 2). In synthetic fresh urine and water, no urea hydrolysis was observed after 1.3 min and 3.3 min of UV irradiation, respectively (Fig. 3). In real urine, the minimum irradiation time required to inhibit > 95 % enzymatic urea hydrolysis was 35 min (Fig. 4E). The relative loss of enzymatic activity in synthetic urine was faster and greater than for water (Fig. 5). The relative loss of enzymatic activity was slower for real urine, but the loss was greater at higher irradiation time and was significantly greater ($p < 0.01$) at an irradiation time of 35 min and 71 min (Fig. 5). Overall, the irradiation time required to reduce urease activity to below the detection limit in real fresh urine was 22-fold longer than the irradiation time required for treating water (3.3 min) and 52-fold longer than the irradiation time required for treating synthetic urine (1.3 min).

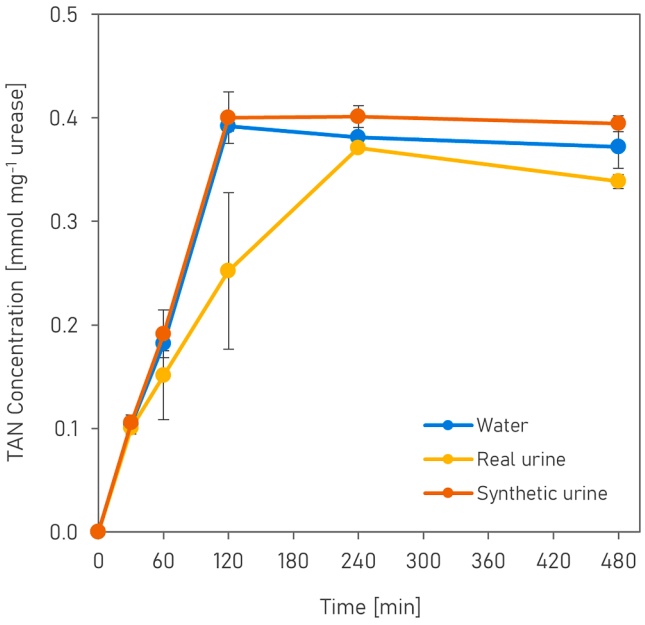

Fig. 1. Concentration of total ammonia nitrogen (TAN, mmol mg$_{urease}^{-1}$) in untreated real fresh urine, synthetic fresh urine and water over time (min) after spiking with 500 mg urease L^{-1} (2500 AU L^{-1}). Error bars show standard deviation ($n = 3$). Water and synthetic urine were both spiked with 10 g/L of urea, real urine contained natural urea.

In real urine, enzymatic urea hydrolysis also occurred during UV treatment. For instance, the concentration of TAN in real urine immediately after an irradiation time of 16.5 min was 32 % higher than the concentration initially present. At a higher irradiation time, the temperature of urine inside the column was higher (35 ± 1 °C) and the extent of urea hydrolysis was greater compared to the UV free control (22 ± 1 °C) (Fig. 4, Fig S3 in SI). After 0.4 min of irradiation, enzymatic activity in UV treated real urine was similar to the enzymatic activity in real urine in absence of UV for a similar duration (p > 0.05) (Fig. 4A). After 71 min of irradiation, enzymatic activity of real urine during UV treatment (0.003 mmol mg$_{urease}^{-1}$ min^{-1}) was higher than the enzymatic activity of real urine in absence of UV (0.002 mmol mg$_{urease}^{-1}$ min^{-1}). However, no enzymatic activity was detected in real urine after 71 min of UV treatment (Fig. 4F).

The UV–vis spectroscopy results revealed that urease absorbed light in the wavelength range 190–240 nm, with high absorbance between 190 nm and 200 nm (Fig. 2). Urea also absorbed UV radiation between 190 nm and 220 nm, with peak absorbance at 196 nm. Following UV treatment, the intensity and range of light absorbance increased in water and synthetic urine samples spiked with urease, suggesting that UV-absorbing degradation products were formed. The absorbance curves of untreated and UV treated real fresh urine were similar and extended over a large wavelength range (190–330 nm) (Fig. 2).

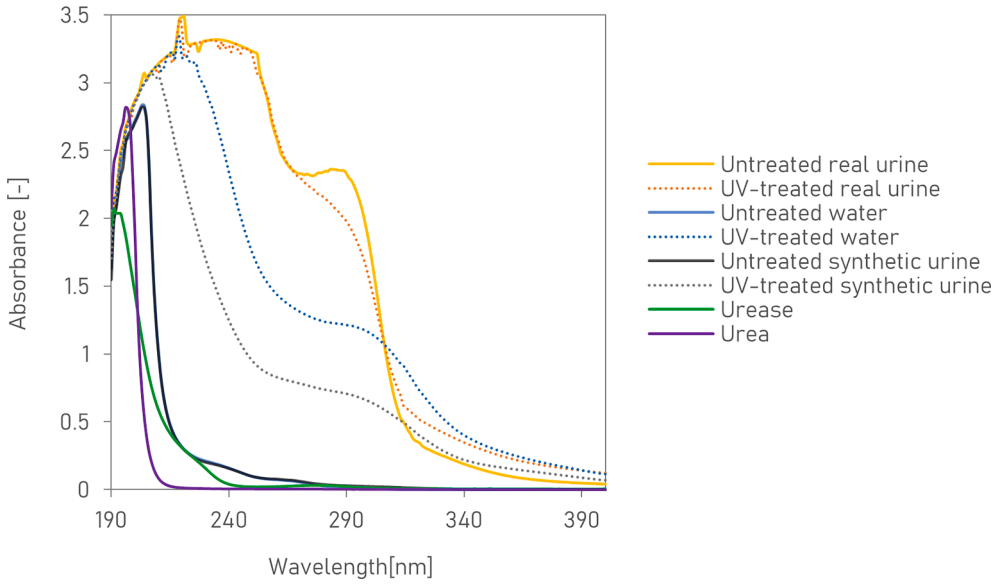

Fig. 2. Ultraviolet light absorbance of untreated and treated samples of water, synthetic urine (without urea) and real urine spiked with 500 mg urease L^{-1}. The treated samples received UV irradiation from a 15 W dichromatic low-pressure mercury lamp with a fluence of 0.43 μW m^{-2} for 71 min. All the samples were diluted 10-fold with Milli-Q water prior to absorbance measurements, except for solutions of urea (10 g/L) and urease (500 mg/L) in water which were diluted 100-fold.

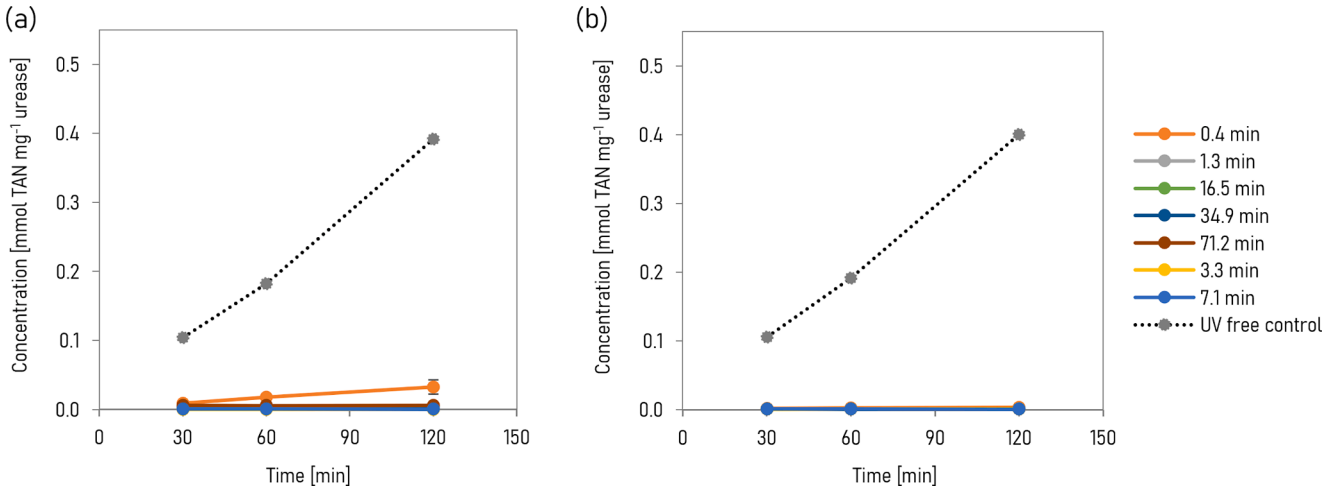

Fig. 3. Concentration of total ammonia nitrogen (TAN, mmol mg^{-1}_{urease}) over time (min) after receiving different levels of UV irradiation in (a) water and (b) synthetic fresh urine (without urea) containing 500 mg urease L^{-1} (or 2500 AU L^{-1}). The UV treatment was done using a 15 W dichromatic (185 nm and 254 nm) low-pressure mercury lamp with a fluence of 0.43 μW m^{-2}. Urea (10 g/L) was added to both water and synthetic urine after the UV treatment. Concentration of TAN in untreated water and synthetic urine after addition of 500 mg urease L^{-1} and 10 g/L urea is shown as dotted black lines, for comparison. Error bars show standard deviation (n = 3).

4. Discussion

The lower urease activity detected in untreated real urine compared with untreated synthetic urine and water can be attributable to several factors. Real urine contains trace amounts of heavy metals including cadmium (Cd), cobalt (Co), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) [19]. Tabatabai [37] showed that heavy metals commonly found in sewage sludge can effectively block active sites of urease in soil. Zaborska et al. [42] found that inhibition of urease by heavy metal ions is biphasic, with inhibition constant as low as 7.1 nM for copper. Heavy metal ions competitively bind slowly to the Ni-containing active site of urease [21]and modify/react with the group of amino acid residues such as histidine and tryptophan [28]. Real urine also contains thiols such as taurine, creatinine and cysteine [6]that can inhibit urease by directly interacting with the enzyme metallocentre [27].

Irradiation with UV affects urease activity in several ways. Urease contains amino acid residues that absorb radiation between 180 and 230 nm, e.g., tryptophan residues absorb radiation at 254 nm Beaven, G. t. and Holiday, E. [5]. Certain parts of the enzyme also absorb irradiation at 254 nm, notably the aromatic side chains of some amino acid residues and disulfide linkages, which may lead to irreversible chemical modifications. Therefore, the enzyme can be inactivated through direct photolysis of aromatic and disulphide bonds of amino acids and/or photo-oxidation of amino acids by deamination, decarboxylation or hydroxylation [9,25]. Low-pressure UV lamps emitting radiation of wavelengths shorter than 200 nm into water also produce hydroxyl radicals (•OH) in situ [43]. Hydroxyl radicals can react with amino acid residues, which can cause chemical modifications, structural modifications and/or conformational changes to the active site of the enzyme [8,40]. According to Mozhaev and Martinek [28], this occurs in two steps. First, amino acid residues susceptible to oxidation but usually found in the middle of the enzyme structure are exposed. Second, in

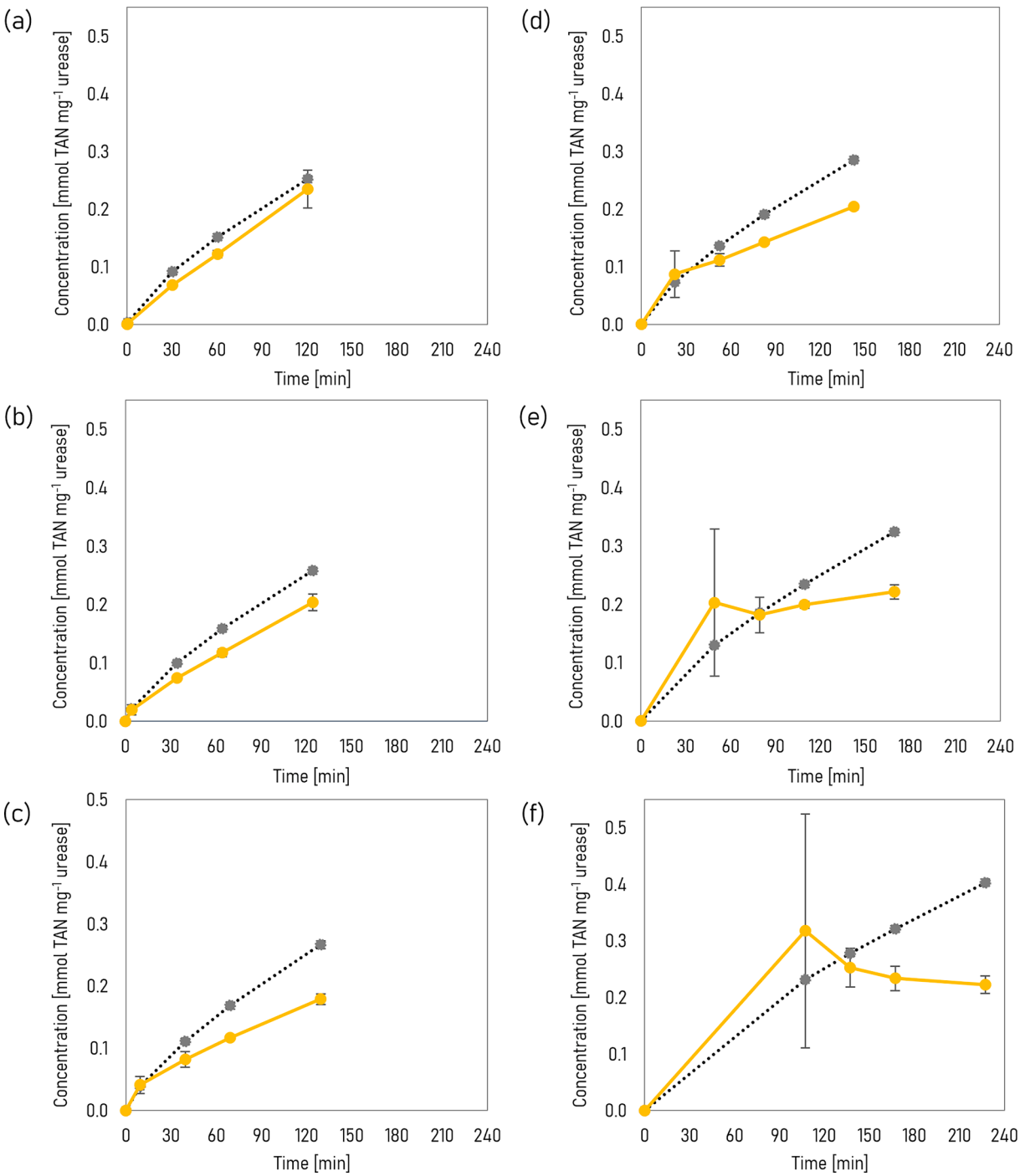

Fig. 4. Concentration of total ammonia nitrogen (TAN, mmol mg^{-1} urease) over time (min) after addition of 500 mg urease L^{-1} (or 2500 AU L^{-1}) to untreated real fresh urine (dotted black line) and real urine (solid yellow line) exposed to different levels of UV irradiation: (a) 0.4 min, (b) 3.3 min, (c) 7.1 min, (d) 16.5 min, (e) 35 min and (f) 71 min. The UV treatment was done using a 15 W dichromatic (185 nm and 254 nm) low-pressure mercury lamp with a fluence of 0.43 μW m^{-2}. Error bars show standard deviation ($n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

thiol side chain-containing amino acids such as cysteine, the sulfhydryl group (-SH) is oxidised to sulfenic acid (RSOH), sulfinic acid (RSO_2H) or a disulphide bond (RSSR), whereas aromatic amino acids such as tryptophan undergo oxidation by abstraction of a hydrogen atom from the indole core.

In our study, no urease activity was detected in water and synthetic urine at an irradiation time of 3.3 min using a dichromatic low-pressure lamp emitting 254 nm and 185 nm radiation. Clauß and Grotjohann [9] found that relative activity of urease in distilled water was > 85 % after irradiation with a low-pressure mercury lamp emitting 254 nm, whereas relative activity was reduced to 15 % after irradiation with a 222 nm KrCl-excimer lamp. Landen [23] investigated the photochemical behaviour of urease and showed that quantum yield for enzyme inactivation increased as wavelength decreased. For instance, the average quantum yield was 0.0008 for wavelength range 254–313 nm and 0.009 at 186 nm. Beaven, G.t. and Holiday, E. [5] also observed that the molar extinction coefficient (ε) of proteins increased as wavelength decreased, e.g. with ε for cysteine of 60 M^{-1} cm^{-1} at 230 nm and 10 M^{-1} cm^{-1} at 260 nm. Residues of cysteine are present in the mobile flap covering the active site of urease [17]. These findings suggest that the 185 nm

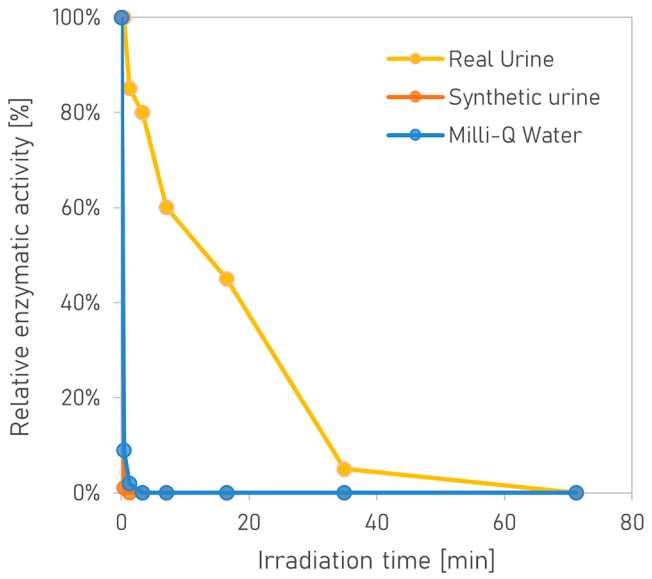

Fig. 5. Relative urease enzyme activity (%) in real fresh urine, synthetic fresh urine and water at different UV irradiation times (min). Relative activity was calculated as the ratio of rate constant for enzymatic urea hydrolysis in presence of UV (k_{UV}, mmol TAN mg_{urease}^{-1} min^{-1}) to rate constant for enzymatic urea hydrolysis in absence of UV (k_C, mmol TAN mg_{urease}^{-1} min^{-1}) for all three matrices.

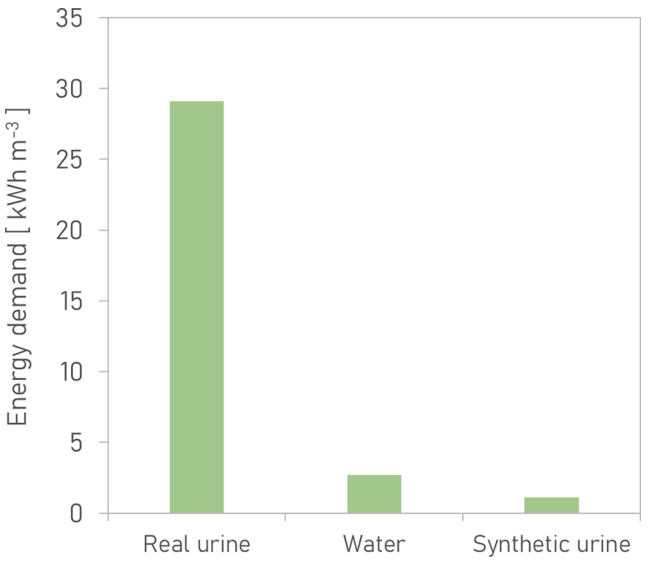

Fig. 6. Electrical energy demand (kWh m^{-3}) to reduce 95 % of urease activity in real urine, water, and synthetic urine by UV photoinactivation using a 15 W dichromatic (185 nm and 254 nm) low-pressure mercury lamp with a fluence of 0.43 μW m^{-2}. For all matrices, the initial concentration of urease is 500 mg/L (2500 AU L^{-1}).

radiation emitted by the UV lamp used in our study resulted in higher absorption of radiant flux by the enzyme, and thus higher urease inactivation than reported by Clauß and Grotjohann [9].

There was relatively faster urease inactivation in synthetic urine than in water. In contrast to Milli-Q water, the synthetic urine contained a range of cations and anions. While the role of inorganic cations such as Na^+ and K^+ in photo-oxidative damage of enzymes is not fully understood, there is evidence that they can participate in oxidation of proteins by altering dehydropeptides (peptides containing amino acid residues with a double bond on the side chain), which ultimately causes breakage of peptide bonds [33]. Inorganic anions can form radicals such as $SO_4^{\bullet-}$ and $CO_3^{\bullet-}$ during UV treatment of synthetic urine (Zhang et al. 2016) and these can react with amino acids present on the urease active site. For instance, the rate constant for reaction of $CO_3^{\bullet-}$ with cysteine and tryptophan at pH 7 is 4.6×10^7 and 7×10^8 L mol^{-1} s^{-1}, respectively (Neta et al. 1988). According to Duca et al. [12], photo-oxidation of organic compounds at 254 nm radiation is not affected by presence of nitrate (NO_3^-), bicarbonate (HCO_3^-) and sulphate (SO_4^{2-}), as they do not absorb light at that wavelength. However, HCO_3^- can scavenge hydroxyl radicals and NO_3^- can absorb 185 nm radiation and inhibit hydroxyl radical formation.

The electrical energy demand for inactivating urease in real urine was 25-fold higher than the electrical energy demand for inactivating urease in synthetic urine (Fig. 6). In contrast to synthetic urine, the real urine matrix is more complex and has higher UV light absorbance (Fig. 2). In addition to urea, real urine contains a range of hydrophilic organic metabolites, including amino acids, amino acid derivatives, carbohydrates, organic acids and lipids [7]. These metabolites absorb light radiation between 190 nm and 400 nm, which reduces the incident radiant flux available for enzyme inactivation. Organic metabolites in urine can also scavenge hydroxyl radicals and impede oxidative damage to the enzyme [8].

Overall, this study showed that UV photoinactivation of urease in water and synthetic urine, i.e. in the absence of urea, is very efficient, while it is considerably more challenging to inactivate urease in real fresh human urine using UV light. In real urine, several other metabolites such as urea and amino acids scavenge UV radiation, due to which the energy demand for photoinactivation is nearly 25-fold higher. However, there are other benefits of treating urine using UV, namely the combined reduction in enzymatic activity and degradation of a wide range of organic micropollutants [10].

5. Conclusions

This study determined the rate constant of enzymatic urea hydrolysis in water, synthetic urine and real urine. It also assessed the UV irradiation time and energy demand to inactive urease in these matrices. In UV-free controls, urea was hydrolysed at a rate of 3.2×10^{-3} mmol TAN mg_{urease}^{-1} min^{-1}, 3.3×10^{-3} mmol TAN mg_{urease}^{-1} min^{-1} and 2.0×10^{-3} mmol TAN mg_{urease}^{-1} min^{-1} in water, synthetic urine and real urine, respectively. After UV irradiation time of 1.3 min, 3.3 min and 71 min, there was no detectable urease activity in synthetic urine containing no urea, water and real urine, respectively. Urea and other organic metabolites present in real urine competitively absorbed UV radiation and scavenged free radicals, which meant that a higher UV irradiation time was required for urease inactivation in real urine compared with water or synthetic urine. Direct photolysis of aromatic and disulphide bonds of amino acids and photo-oxidation of amino acids such as cysteine, histidine and tryptophan are likely reasons for enzyme inactivation in all matrices. Overall, this study shows potential of UV for stabilising urea and treating freshly excreted urine collected in source-separating sanitation systems.

CRediT authorship contribution statement

Natnael Demissie: Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Prithvi Simha:** Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Anastasija Vasiljev:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Björn Vinnerås:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2024.149708.

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