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Urine pretreatment enhances energy recovery by boosting medium-chain fatty acids production from waste activate sludge through anaerobic fermentation

Huan Liu, Xuan Li *, Zehao Zhang, Jibin Li, Ting Zhou, Zhenyao Wang, Qilin Wang *

Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Ultimo, NSW 2007, Australia

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ABSTRACT

Medium-chain fatty acids (MCFAs) represent a promising form of energy recovered from waste activated sludge (WAS) through anaerobic fermentation. However, the low WAS degradability hindered the MCFAs generation from WAS. In this study, urine pretreatment of 5 – 25 wt% was applied to enhance the MCFAs production in anerobic WAS fermentation with ethanol as an electron donor. Urine pretreatment (5 – 25 wt%) dose-dependently improved the MCFAs production by 0.8 – 3.3 times (from 2.4 to 4.4 – 12.8 g chemical oxygen demand (COD)/L) and enhanced the MCFAs selectivity by 0.8 – 3.5 times (from 12.2 % to 21.4 % – 54.4 %). Urine pretreatment (5 – 25 wt%) improved WAS degradation by up to 54.8 % and enhanced electron transfer efficiency from substrates (i.e. WAS and ethanol) to MCFAs by up to 18.6 %, which contributed to the improvement in MCFAs production. According to model-based analysis, the MCFAs production potential (P_m) rose from 2.71 ± 0.09 g COD/L with 0 % urine pretreatment to 4.51 – 13.11 g COD/L with 5 – 25 wt% urine pretreatment, while the lag phase (λ) decreased from 5.30 d to 4.67 – 3.08 d, which may be the reason for the improved MCFAs production. Additionally, urine pretreatment significantly facilitated each step involved in the MCFAs generation, i.e. solubilization, hydrolysis, acidification, and chain elongation, by up to 600 %, 51 %, 17 %, and 42 %, respectively. This study for the first time reported an innovative method, urine pretreatment, enhances energy recovery by MCFAs from WAS through anaerobic fermentation, which potentially brings wastewater treatment plants economic and environmental profits.

1. Introduction

Climate change impacts have underscored the need for an alternative energy source to fossil fuel [1–3]. Waste activated sludge (WAS), the final recipient of organic compounds in sewage treatment plants (STPs), has been recognized as a valuable carbon resource for renewable energy recovery [4–6]. WAS is ubiquitously and substantially produced in STPs and the annual production of WAS is estimated to be 9–12 million tonnes/year in Australia [4,7]. WAS also contains the concentrated organic content (total chemical oxygen demand (TCOD) of 30–100 g/L), balanced nutrient composition and specific microbial communities, which makes it a promising resource for the renewable energy in biological processes. Various carbon forms of energy recovery from WAS through biological processes have been explored [6,8,9], such as methane generated in anaerobic digestion [6,8–12], short-chain fatty acids (SCFAs) and medium-chain fatty acids (MCFAs) generated in anaerobic fermentation [13,14]. Among these products, MCFAs are recognized as the ideal products. MCFAs are saturated fatty acids containing 6 – 12 carbon atoms [15–17], including caproic acid (C6), heptanoic acid (C7), caprylic acid (C8), pelargonic acid (C9), decanoic acid (C10), undecylic acid (C11), and lauric acid (C12). Compared to methane (CH_4) and SCFAs (C2 – C5, i.e. acetate, propionate, butyrate, and valerate), MCFAs have higher energy density, stability, and easier availability in separation. Furthermore, MCFAs are extensively applied as biofuel precursors [18–20]. Besides, the current MCFAs production heavily relies on the pyrolysis of plant oil, incurring high product prices (e.g. 3000–5000 USD per ton) but limited production capacity (satisfying only 1.2–1.4 % of the market's demand) [21–24]. Thus, an economic and applicable alternative to promote MCFAs production is highly needed. These characters make MCFAs an important and promising option for energy recovery from WAS.

However, MCFAs generation from WAS through anaerobic

* Corresponding authors.
E-mail addresses: Xuan.Li@uts.edu.au (X. Li), Qilin.Wang@uts.edu.au (Q. Wang).

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fermentation encounters certain challenges stemming from low MCFAs production (i.e. the concentration of MCFAs in fermentation liquid) [25]. Low degradability of WAS is a major challenge that limits the MCFAs generation from WAS [12]. Furthermore, MCFAs produced from WAS usually have a low product selectivity (i.e. the percentage of MCFAs among all the products in the fermentation liquid), which leads to the generation of undesired products and difficulties in MCFAs separation [26,27]. Therefore, several strategies have been developed to improve the production and selectivity of MCFAs in anaerobic WAS fermentation, such as utilization of additives and pretreatment technologies [27–30]. Ferroferric oxide addition at 20 g/L enhanced MCFAs production and selectivity by 155 % and 67 %, respectively [31]. The thermal hydrolysis pretreatment improved the MCFAs production and selectivity by around 128 % and 27 %, respectively [27]. However, these strategies cause extra expenditure on chemical and energy investment, leading to additional economic burdens for STPs.

Urine is inherently present in sewage as human waste, which contains a high pH of 9.4–9.5 and a high concentration of total ammonia nitrogen of 4330 – 11600 mg N/L [32,33]. This makes urine a valuable source that is rich in free ammonia content. Previous studies have extensively demonstrated that free ammonia pretreatment can enhance the degradability of WAS [12,34–36]. Our previous study also revealed that urine pretreatment improved the degradability of WAS and boosted methane production through anaerobic digestion [37]. As a readily available human waste, urine can be separated from the sewage stream through separate urine collection systems that extensively developed worldwide [32,38–40]. Compared to free ammonia pretreatment, urine is more readily available and urine pretreatment is safer for handling since it does not require additional alkali. Compared to other strategies, such as thermal and advanced oxidation pretreatment [27,30], urine pretreatment does not require additional energy or chemical inputs and does not introduce external pollutants. These characters make urine pretreatment a sustainable, environmental-friendly, and cost-effective approach. However, the effects of urine pretreatment on MCFAs production from WAS in anaerobic fermentation are still unclear.

This study investigated the viability and effect of urine pretreatment used in MCFAs production from anaerobic WAS fermentation for the first time. The MCFAs generation tests were conducted in laboratory-scale batch experiments with a series of urine pretreatment (0–25 wt% of urine and WAS mixture, 24 h), where 0 wt% of urine addition worked as a control. The effects of urine pretreatment on MCFAs production and selectivity, products distribution, WAS degradation, and electron transfer efficiency from substrates to products in anaerobic WAS fermentation were evaluated. Based on the modified Gompertz model, the maximum MCFAs production potential (P_m), maximum MCFAs production rate (R_m) and lag phase (λ) were predicted. Furthermore, the effects of urine pretreatment on solubilization, hydrolysis, acidification and chain elongation (CE) processes were examined for revealing potential mechanisms during MCFAs generation. Overall, this investigation facilitates the application of urine pretreatment on efficient energy recovery from WAS through MCFAs generation.

2. Materials and methods

2.1. Sludge origin and properties

The WAS used in this study was sourced from the secondary clarifier of a biological STP (Australia), whose sludge retention time (SRT) was 15 days. The inoculum was collected in a lab-scale WAS fermentation reactor operated for the production of MCFAs, with a SRT of 9 days [29]. Detailed properties of the WAS and inoculum can be found in Table S1.

2.2. Urine pretreatment

A series of urine ratio (0–25 wt% urine/urine + WAS) was used in the pretreatment. The stored urine was obtained from the urine diversion toilets located at Building 11 of the University of Technology Sydney. The urine characteristics were provided in Table S2. For the pretreatment, 375 mL of WAS was evenly distributed among 5 covered bottles, with each bottle containing 75 mL of sludge. The stored urine was placed into the pretreatment reactors to achieve 0 %, 10 %, 15 %, 20 %, and 25 % of urine and WAS mixture, where 0 % urine pretreatment worked as a control group. The mixture (WAS or urine + WAS) was stirred at room temperature (23 ± 2 °C) for 24 h during the pretreatment. Afterwards, distilled water was added to reach 100 mL in each bottle, resulting in an identical volatile solids (VS) of pretreated WAS for the subsequent MCFAs generation tests.

2.3. Batch MCFAs generation tests

The impacts of urine pretreatment (0–25 wt%) on MCFAs generation through anaerobic WAS fermentation were investigated by batch tests. Briefly, 54 mL of inoculum (acclaimed fermented sludge) and 44 mL of pretreated WAS as described in section 2.2 were added to each test bottle for anaerobic fermentation. The test bottles (160 mL each) used for batch tests had a working volume of 100 mL. In each bottle, 0.72 g of absolute ethanol (Sigma, Germany) was added as the electron donor and 1 g of sodium 2-bromoethanesulfonate (Sigma, Germany) was added as the methane inhibitor. This dosage is widely applied in laboratory anaerobic fermentation systems for MCFAs generation in previous studies [31,41]. In this study, we used a methane inhibitor to suppress the substrates converted to methane. For real applications, a lower dosage of methane inhibitor might be more economically appealing. The potential of using a lower dosage of methane inhibitor requires future investigations. Afterwards, pH in test bottles was adjusted to approximately 5.5 by HCl (Sigma, Germany). Prior to sealing the bottles, a 15-minute nitrogen flushing was performed to create an anaerobic atmosphere in the headspace. The test bottles were subsequently placed at a homothermic incubator (37 ± 0.8 °C). A Blank was processed using 54 mL of inoculum mixed with 44 mL of distilled water as a substitute of WAS to estimate the MCFAs production solely from the inoculum. The batch tests were triplicated and lasted for 30 days until the MCFAs generation in bottles was negligible. The concentrations of SCFAs, MCFAs and alcohols and soluble chemical oxygen demand (SCOD) of the fermentation liquid were measured at the end of MCFAs generation tests and shown as g chemical oxygen demand (COD)/L. The conversion between product mass and COD values is detailed in the supporting information (Text S1). After all the tests, the sludge from each text bottle was collected as the inoculum, which was utilized in the subsequent tests (i.e. hydrolysis, acidification and CE tests).

It should be noted that this study serves as a proof-of-concept investigation, demonstrating the efficacy of urine pretreatment for MCFAs generation through batch experiments. Further investigations should be conducted systematically to determine the optimal urine dosage and pretreatment duration. Due to the existence of inoculum ($VS_{inoculum}$: VS_{WAS} = 1:1) in experiments, the VS removal and sludge dewaterability in this study cannot be evaluated as the inoculum would mask the actual VS removal and dewaterability of fermented WAS. Future long-term continuous systems are highly recommended to evaluate the detailed impacts of urine pretreatment on the MCFAs generation through anaerobic fermentation, such as the VS removal and dewaterability of fermented WAS.

2.4. Model-based analysis of MCFAs production

The impacts of the urine pretreatment (0 %–25 %) on the MCFAs production potential and kinetics in anaerobic fermentation of WAS with ethanol were analysed and indicated by MCFAs production potential (P_t), MCFAs production rate (R_m) and the lag phase time (λ) using the modified Gompertz model (Eq.1) [42]. The aim of the model analysis is to quantize the P_t, R_m, and λ, which can quantitively assess the promotion extent of urine pretreatment [42,43].

$$P(t) = P_m \times \exp(-\exp(R_m \times e \times (\lambda - t)/R_m + 1)) \quad (1)$$

where P(t) represents the cumulative product production at time t (g COD/L); P_m represents the maximum product production potential at the end of fermentation (g COD/L); R_m represents the maximum product production rate (g COD/L/d); e is the mathematical constant and equal to 2.71828; λ is the lag phase of the fermentation (d), indicating duration that microorganisms adapted new environment before their growth and reproduction; *t* is the fermentation time (d). Among these parameters, P_m, R_m and λ are obtained by the simulation based on the experimental results using Origin (Origin 2021, US).

2.5. Calculation of MCFAs selectivity, WAS degradation and electron transfer efficiency

(1) WAS degradation is calculated using Eq. (2)

$$WAS\,degradation(\%) = SCOD\,released\,from\,WAS \times V_{test}/(TCOD_{WAS} \times V_{WAS}) \times 100\% \quad (2)$$

SCOD released from WAS = SCOD released from mixed sludge - SCOD released from inoculum

SCOD released from mixed sludge= ($SCOD_{detected}$ + $SCOD_{biogas}$) - ($SCOD_{WAS}$ + $SCOD_{inoculum}$ + $SCOD_{urine}$) - $SCOD_{ethanol}$.

SCOD released from inoculum = ($SCOD_{detected\text{-}blank}$ + $SCOD_{biogas\text{-}blank}$) - $SCOD_{inoculum}$ -$SCOD_{ethanol}$.

where $SCOD_{detected}$ is the SCOD of the fermentation liquid at the end of MCFAs generation tests (g/L); $SCOD_{biogas}$ is calculated by the COD of measured total biogas in the tests divided by V_{test} (g/L); V_{test} is the total volume of fermented sludge in each test bottle (100 mL); $SCOD_{WAS}$ is the SCOD of the WAS before the test started (g/L); $SCOD_{urine}$ is the SCOD introduced by urine (g/L); $SCOD_{inoculum}$ is the SCOD of the inoculum sludge in the blank before the test started; $SCOD_{ethanol}$ represents the COD equivalent of ethanol used in the tests; $SCOD_{detected\text{-}blank}$ is the SCOD of the fermentation liquid at the end of the Blank; $SCOD_{biogas\text{-}blank}$ represents the COD equivalent of total biogas from the Blank (g/L). $TCOD_{WAS}$ represents the total COD of pretreated WAS used in the tests. V_{WAS} is the volume of added WAS after pretreatment (44 mL).

(2) MCFAs selectivity is obtained by using Eq. (3).

$$MCFAs\,selectivity(\%) = SCOD_{MCFAs}/SCOD_{detected} \times 100\% \quad (3)$$

where $SCOD_{MCFAs}$ is the MCFAs concentration shown as COD at the end of MCFAs generation tests (g COD/L).

(3) The electron transfer efficiency represents the extent of electron transferred from the substrates (including WAS and ethanol) to products (including fatty acids and alcohols) in the MCFAs generation tests. The electron transfer efficiency is obtained by using Eq. (4).

$$Electron\,transfer\,efficiency = Products(mmol\,e^-)/Substrates(mmol\,e^-) \times 100\% \quad (4)$$

The electron equivalent of in each product is detailed in the supporting information (Text S2).

2.6. Impacts of urine pretreatment on the solubilization, hydrolysis, acidification and CE processes of anaerobic WAS fermentation

Solubilization tests: Solubilization tests were conducted following the same procedure as the MCFAs generation tests. The released concentrations of polysaccharide and protein from urine pretreated WAS/WAS after a 12-hour tests were measured to reveal the solubilization extent of WAS [44,45].

Hydrolysis tests: Hydrolysis tests were performed to assess the breakdown extents of protein and polysaccharide. In this hydrolysis tests, bovine serum albumin (BSA) with an average molecular weight of 67 000 and dextran with an average molecular weight of 23 800 were employed as model protein and polysaccharide substrates, respectively. The extent of hydrolysis was determined by measuring the removal of substrate [44,45].

To conduct the hydrolysis tests, inoculum (25 mL) and synthetic sewage (75 mL) were placed into test bottles, resulting in a sludge (inoculum) concentration of ~ 1.5 g/L. BSA and dextran were then added to achieve the concentrations of 6.0 and 1.5 g/L in the test bottles, respectively. The inoculum was obtained from test bottles at the end of MCFAs generation tests described in Section 2.3. Prior to inoculation, sludge was washed with the synthetic sewage three times to eliminate any residual matters. The composition of synthetic sewage is provided in the Text S3. The performance of hydrolysis process was indicated by the removals of BSA and dextran after 2 days. Other experimental conditions remained consistent with the experimental procedure of MCFAs generation tests.

Acidification tests: The conversion of monosaccharide and amino acid compounds into SCFAs was involved in the acidification process. In this study, glucose was utilized as a model monosaccharide compound, while L-alanine served as the model amino acid compound in acidification tests. The experimental procedure was as same as hydrolysis tests except that the substrates were restored by 1.2 g/L glucose and 3.8 g/L L-alanine. The extent of acidification was determined by the removals of substrates [44,45].

CE Tests. During the CE process, SCFAs (mainly acetate) are utilized to generate MCFAs. The model substrates were 6.9 g/L ethanol and 3.0 g/L acetate in this study [30]. Apart from the changes in model substrates, all experimental procedures remained consistent with the hydrolysis test. The effectiveness of the CE process was assessed by measuring the MCFAs production after 9 days.

2.7. Analytical methods

The total chemical oxygen demand (TCOD), SCOD, VS and total solids (TS) were determined by using standard methods [46]. The concentrations of carbohydrates, including fatty acids (C2 – C8) and alcohols, were obtained by using gas chromatography (7820A, Agilent, US) and calculated as g COD/L. C2 – C8 indicated acetate, propionate, butyrate, valerate, caproate, heptanoate and caprylate in this study. Biogas production amount was measured daily using a customer-designed manometer [47]. Gas chromatography (7820A, Agilent technology, USA) was used to analyse the biogas compositions. The concentrations of polysaccharides and glucose were measured through anthrone colorimetric method [48]. The concentrations of protein were measured by the Lowry − Folin method [49]. The concentration of L-alanine were measured by the Pico-tag method [50].

2.8. Statistical analysis

The significance of difference between 0 % urine pretreatment (the control group) and tests with 5 % – 25 % urine pretreatment was indicated by p value obtained by *t*-test. The p-value less than 0.05 were considered statistically significant. Correlations between urine ratio in the pretreatment and the parameters were analysed by Pearson's correlation coefficient using Python 3.7 (Scipy Libraty). Pearson's correlation coefficient (R value) of − 1, 0, and 1, indicates the linear correlation between two group of variables is negative, no correlation, and positive, respectively.

3. Results

3.1. Effects of urine pretreatment on the productions of MCFAs, SCFAs and alcohols

The detected products were MCFAs (i.e. caprylate, heptanoate, and caproate), SCFAs (i.e. vaterate, butyrate, and propionate, acetate),

alcohols (i.e. octanol, heptanol, and butanol) and the unknown (Fig. 1). The unknown represented the difference between the detected SCOD and the sum total SCOD of detected products. Biogas produced in the MCFAs generation tests was at negligible level, accounting for only 0.01 % – 0.21 % of the input SCOD (released SCOD from WAS and $SCOD_{e\text{-}thanol}$). Ethanol was used up in all tests and the production of MCFAs came into a steady level towards the end of tests, indicating the MCFAs generation process was completed.

Urine pretreatment enhanced the total MCFAs production and the production of major MCFAs product, caproate, in the MCFAs generation tests (Fig. 1). The MCFAs production was significantly improved from 2.4 g COD/L with 0 % urine pretreatment to 4.4, 7.4, 9.9, and 12.8 g COD/L with 5 %, 10 %, 15 %, 25 % urine pretreatment ($p = 0.03$, 0.01, 0.002, 0.01), respectively. Compared to the control (0 % urine), MCFAs productions were improved by 0.8, 2.1, 3.1, 4.3 times with 5 %, 10 %, 15 %, and 25 % urine pretreatment, respectively. The productions of the major MCFAs product, caproate, was improved from 1.6 g COD/L in the control to 3.0, 5.4, 7.9 and 11.0 g COD/L with 5 %, 10 %, 15 %, and 25 % urine pretreatment, respectively. The highest MCFAs production of 12.8 g COD/L and the highest caproate production of 11.0 g COD/L were achieved under 25 % urine pretreatment, which were 4.3 and 6.9 times of that in the control (2.4 and 1.6 g COD/L), respectively. The MCFAs and the caproate productions exhibited significant and strong linear correlations with the urine ratio in pretreatment ($R = 0.98 – 0.99$, $p = 0.001 – 0.003$). Other MCFAs compounds included caprylate and heptanoate. The caprylate production was increased from 0.5 g COD/L in the control to 0.8 – 1.5 g COD/L with 5 % – 25 % urine pretreatment. The heptanoate productions were comparable and ranged from 0.3 to 0.9 g COD/L under 0 % – 25 % pretreatment.

On the contrary, 5 % – 25 % urine pretreatment decreased the total SCFAs production and the production of the major SCFAs product, butyrate. The SCFAs production was 12.8 g COD/L with the control (0 % urine), but decreased to 10.8, 8.4, 8.1, and 6.0 g COD/L with 5 %, 10 %, 15 %, 25 % urine pretreatment, respectively. Compared to the control, the SCFAs productions were relatively decreased by 15.6 %, 34.3 %, 36.7 %, and 53.1 % with 5 %, 10 %, 15 %, and 25 % urine pretreatment, respectively. The production of major SCFAs product, butyrate, decreased from 8.7 g COD/L in the control to 6.9, 4.8, 4.3, and 2.9 g COD/L with 5 %, 10 %, 15 %, and 25 % urine pretreatment, respectively. The reductions of SCFAs and butyrate production were significant when urine ratio was over 5 % ($p = 0.007 – 0.01$). The decrease of SCFAs and butyrate production were negatively correlated with the growth of urine ratio in pretreatment ($R = -0.97$ and -0.96, $p = 0.007$ and 0.04, respectively). Similarly, the productions of propionate decreased from 1.3 g COD/L in the control to 1.1 – 0.7 g COD/L with 5 % – 25 % urine pretreatment. The productions of acetate decreased from 0.7 g COD/L in the control to 0.6 – 0.2 g COD/L with 5 % – 25 % urine pretreatment. The productions of vaterate were comparable under 0 % – 25 % urine pretreatment (2.17 – 2.64 g COD/L). The overall productions of alcohols, including heptanol, octanol and butanol, were at a constant level of 3.8 – 4.5 g COD/L without significant difference between the control and the tests with 5 % – 25 % urine pretreatment ($p = 0.09 – 0.72$).

The products distribution of MCFAs, SCFAs, alcohols and the unknown were altered by urine pretreatment in the MCFAs generation tests (Fig. 2A). In the control (0 % urine), SCFAs were the most abundant products with a proportion of 62.5 %, whereas MCFAs only accounted for 11.9 %. Urine pretreatment of 5 %, 10 %, 15 %, and 25 % improved the MCFAs proportions to 21.1 %, 35.4 %, 43.3 % and 54.0 %, making MCFAs the most abundant production under 15 % – 25 % urine pretreatment. On the contrary, the proportions of SCFAs were decreased to 52.0 %, 40.2 %, 35.5 %, and 25.2 % with 5 %, 10 %, 15 %, and 25 % urine pretreatment, respectively. Urine pretreatment of 5 % – 25 % exhibited a dose-dependent effect on the increase of MCFAs' proportion ($R = 0.98$, $p = 0.004$), while causing a decrease in the proportion of SCFAs. Additionally, the proportion of alcohols maintained at a comparable level (19.2 % – 20.8 %) under 0 % – 10 % urine pretreatment, but decreased to 14.9 % – 15.6 % with 15 % – 25 % pretreatment. This indicates urine pretreatment decreased the proportion of alcohol when urine ratio was above 15 % – 25 %.

Zooming in on specific products, the predominant products were caproate, butyrate, vaterate and heptanol, collectively accounting for 73.8 % – 76.4 % in all the MCFAs generation tests with 0 % – 25 % urine pretreatment (Fig. 2B). The proportion of the main MCFAs products, caproate, significantly increased from 7.8 % with the control (0 % urine) to 14.2 %, 25.7 %, 34.7 %, and 46.3 % with 5 %, 10 %, 15 %, and 25 % urine pretreatment ($p < 0.05$), respectively. On the contrary, the proportion of the predominant SCFAs product, butyrate, reduced from 42.3 % in the control to 33.4 %, 23.1 %, 19.1 %, and 12.3 % with 5 %, 10 %, 15 %, and 25 % urine pretreatment ($p < 0.05$), respectively. However, productions of vaterate and heptanol were similar among all the tests, accounting for 9.2 % – 11.8 % and 9.2 % – 15.7 %, respectively. Overall, the proportions of caproate in MCFAs generation tests raised with the growth of urine ratio in the pretreatment, whereas the proportion of butyrate decreased along with the growth of urine ratio.

3.2. *Urine pretreatment promotes WAS degradation and MCFAs selectivity*

Urine pretreatment promoted the WAS degradation in the MCFAs generation tests, (Fig. 3). WAS degradation was slightly improved from 27.7 % in the control (0 % urine) to 30.2 % and 31.6 % with 5 % and 10 % urine pretreatment, respectively. While the WAS degradation was further significantly improved to 46.9 % and 54.8 % with 15 % and 25 % urine pretreatment ($p = 0.01 – 0.02$), respectively. Compared with the

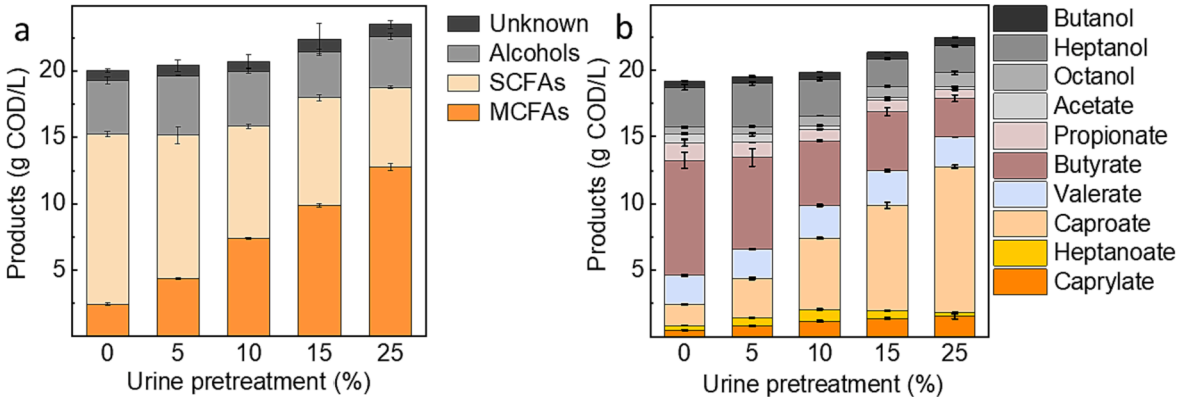

Fig. 1. Products in the MCFAs generation tests with 0% – 25% urine pretreatment. (a) Products categorized as MCFAs, SCFAs, alcohols and the unknown; (b) Products categorized as specific products. Error bars represent the standard error. Some error bars are too small to be visually seen in the figure.

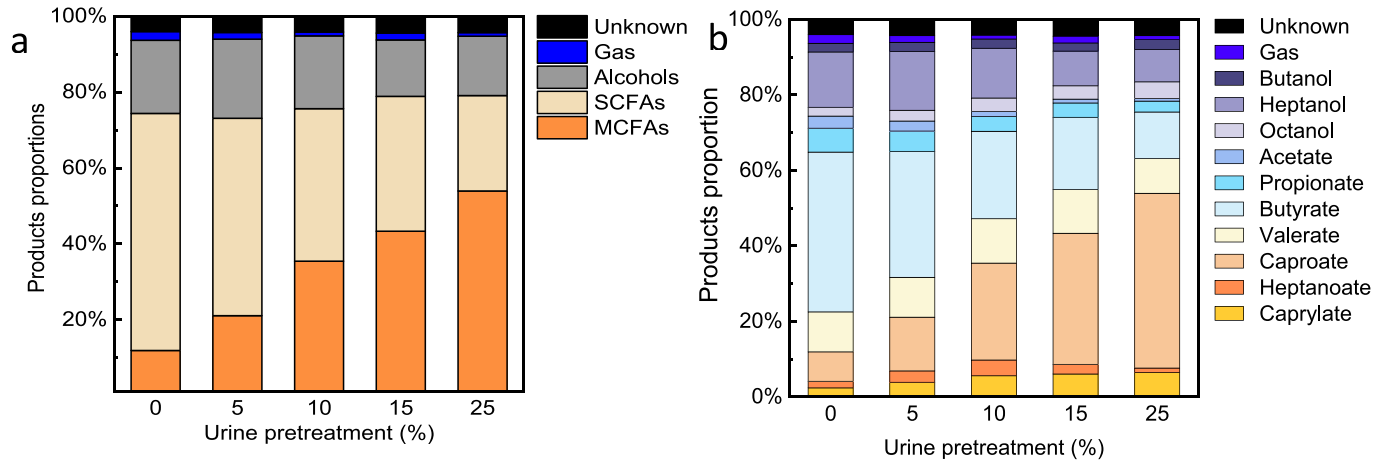

Fig. 2. Distribution of MCFAs, SCFAs and alcohols in the products of MCFAs generation tests: (a) Proportions of MCFAs, SCFAs, alcohols and unknown products based on the $SCOD_{input}$ (SCOD released from WAS + $SCOD_{ethanol}$); (b) Proportions of specific products based on the $SCOD_{input}$ (SCOD released from WAS + $SCOD_{ethanol}$).

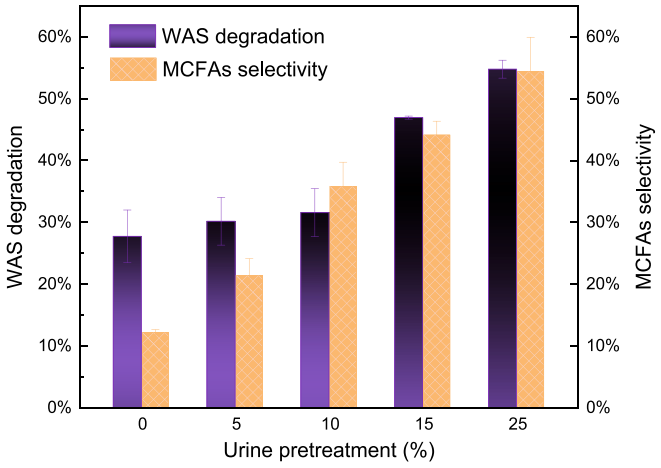

Fig. 3. WAS degradation and MCFAs selectivity in the MCFAs generation tests with 0% – 25% urine pretreatment. Error bars represent the standard error. Some error bars are too small to be visually seen in the figure.

control, the WAS degradation was improved by 10.8 % – 54.8 % due to 5 % – 25 % urine pretreatment. The highest WAS degradation (54.8 %) was achieved with 25 % urine pretreatment, nearly doubling the WAS degradation of the control (27.7 %). WAS degradation had a linear and positive correlation with the urine ratio of 0 % – 25 % ($R = 0.95$, $p = 0.01$), indicating a dose-dependent relationship between the WAS degradation and the urine ratio in pretreatment.

Additionally, urine pretreatment also contributed to the improvement in MCFAs selectivity (Fig. 3). The MCFAs selectivity was 12.2 % in the control group (0 % urine) during MCFAs generation tests. Urine pretreatment of 5 %, 10 %, 15 % and 25 % significantly enhanced the MCFAs selectivity to 21.4 %, 35.8 %, 44.1 % and 54.4 % ($p = 0.008$ – 0.04), respectively. Such an improvement was positively correlated with the urine ratio ($R = 0.98$, $p = 0.004$) in the pretreatment. The highest MCFAs selectivity was observed at 25 % urine pretreatment, representing 3.5-fold increase compared to the control.

Nevertheless, WAS and the electron donor (ethanol) provided substrates for MCFAs production in both the control and tests with urine pretreatment. Ethanol consumption exceeded 80 % on Day 14 in all tests, while it was almost depleted on Day 26 in all tests (Fig. S1). The complete consumption of ethanol is commonly observed in anaerobic fermentation of WAS for MCFAs productions [26,29–31]. The COD conversion from WAS to MCFAs was dramatically improved by urine pretreatment (Fig. S2). Compared to the control (0 % urine pretreatment), the 5 %-25 % urine pretreatment improved the WAS-contributed COD in MCFAs from 0.41 g COD/L to 0.78–3.58 g COD/L and improved the WAS conversion to MCFAs from 3.4 % to 6.5 %-29.9 % (Fig. S2).

3.3. *Effects of urine pretreatment on MCFAs production potential, rate and lag phase*

The modified Gompertz model was applied to evaluate the maximum product production potential (P_m), maximum production rate (R_m) and lag phase (λ) in control (0 % urine) and under 5 % – 25 % urine pretreatment (listed in Table 1). The measured total MCFAs production (Fig. 4), heptanoate and caprylate production data (Fig. S3 and Table S4) well fitted the model. The total MCFAs production included caproate, heptanoate, and caprylate production, which was represented through caproate production as caproate could act as substrate for both heptanoate and caprylate formation. Key important parameters, i.e. estimated P_m and R_m and λ, were listed in Table 1.

The maximum MCFAs and caproate potential (P_m) significantly increased from 2.71 ± 0.09 g COD/L in the control (0 % urine) to 4.51 ± 0.10, 7.66 ± 0.15, 9.64 ± 0.21, and 13.11 ± 0.29 g COD/L with the 5 %, 10 %, 15 %, and 25 % pretreatment ($p < 0.05$), respectively (Table 1). The P_m of MCFAs positively increased with the ratio of urine in the pretreatment, with the highest improvement in P_m of MCFAs (383 %) achieved under 25 % urine pretreatment. Similarly, urine pretreatment of 5 %, 10 %, 15 %, and 25 % significantly increased the R_m of MCFAs from 0.14 ± 0.01 g COD/L/d in the control, to 0.35 ± 0.02, 0.65 ± 0.05, 1.06 ± 0.11, and 1.03 ± 0.08 g COD/L/d, respectively ($p < 0.05$). The R_m of MCFAs was achieved under 15 % urine pretreatment, which was improved by 657 % compared to the control. However, lag phage (λ) R_m of MCFAs decreased from 5.30 ± 0.38 d in the control to 4.67 ± 0.42, 4.48 ± 0.41, 3.95 ± 0.48, 3.08 ± 0.45 d with 5 %, 10 %, 15 %, and 25 % urine pretreatment, respectively, while the decrease was only significant with 25 % urine pretreatment. The minimum lag phase was only 3.08 ± 0.45 with 25 % urine pretreatment. In general, the urine ratio was positively correlated with P_m and R_m of MCFAs ($R = 0.91$ – 0.99, $P = 0.005$ – 0.03), but negatively correlated to the λ ($R = -0.99$, $P = 0.0005$). Similarly, higher urine ratio in pretreatment (5 % – 25 %) contributed the higher P_m, R_m of caprylate and shorter λ, but this trend did not apply to heptanoate (Fig. S1). The simulated P_m of caprylate was enhanced from 0.65 ± 0.10 in the control to 0.90 – 1.58 g COD/L with 5 % – 25 % urine pretreatment and the simulated R_m of caprylate was improved from 0.03 g COD/L/d in the control to 0.06 – 0.11 g COD/L/d with 5 % – 25 % urine pretreatment (Table S4). The simulated λ of

Table 1
Simulated maximum MCFAs production potential (P_m), production rate (R_m) and lag phase (λ) at different urine ratio (with standard errors).

Urine pretreatment	P_m (g COD/L)	Improvement in P_m	R_m (g COD/L/d)	Improvement in R_m	λ	Reduction in λ
0 % (control)	2.71 ± 0.09	/	0.14 ± 0.01	/	5.30 ± 0.38	/
5 %	4.51 ± 0.10	66 %	0.35 ± 0.02	150 %	4.67 ± 0.42	12 %
10 %	7.66 ± 0.15	183 %	0.65 ± 0.05	364 %	4.48 ± 0.41	15 %
20 %	9.64 ± 0.21	256 %	1.06 ± 0.11	657 %	3.95 ± 0.48	25 %
30 %	13.11 ± 0.29	383 %	1.03 ± 0.08	635 %	3.08 ± 0.45	42 %

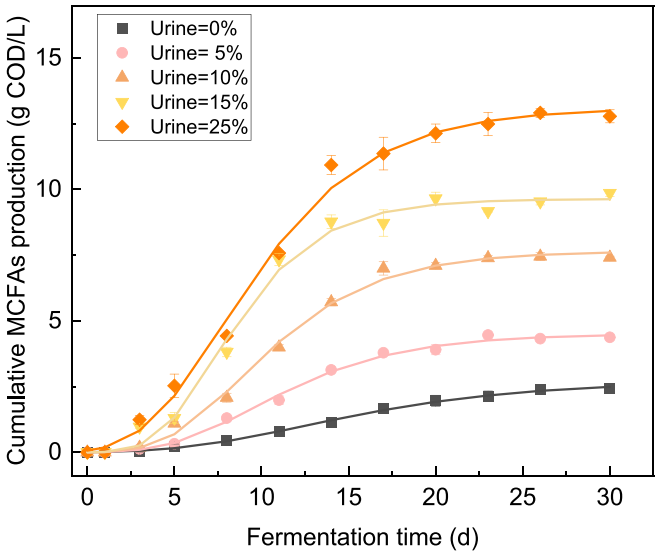

Fig. 4. Measured and simulated cumulative MCFAs production from anerobic WAS fermentation with 0% – 25% urine pretreatment.

caprylate was decreased by 19 % – 65 % from 8.67 d in the control to 6.98 – 3.05 d with 5 % – 25 % urine pretreatment. However, the P_m and R_m of heptanoate increased with 5 % and 10 % urine pretreatment compared to the control and reached the maximum with 10 % urine pretreatment, while decreased with 15 % – 25 % urine pretreatment (Table S4). On the whole, the results indicated that 5 % – 25 % urine pretreatment improved the P_m of MCFAs, accelerated the R_m of MCFAs and reduced the lag phase of MCFAs generation.

3.4. Urine pretreatment promotes the electron transfer efficiency

The electron transfer efficiency quantifies the proportions of electron shifted from substrates (i.e. WAS and ethanol) towards the detected products (i.e. MCFAs, SCFAs, alcohols and the unknown) in MCFAs generation tests. When an identical electron equivalent of substrates was provided in all the tests, urine pretreatment improved the overall electron transfer efficiency from substrates towards the detected products (Fig. 5a). In the control (0 % urine), electron transfer efficiency was 70.5 %, which was enhanced to 72.7 % with 5 % urine pretreatment. The electron transfer efficiency was significantly increased to 73.9 %, 79.4 %, and 83.6 % with 10 %, 15 %, and 25 % urine pretreatment ($p = 0.001 – 0.04$), respectively. The relative increments of electron transfer efficiency compared to the control were 3.1 % – 18.6 % due to 5 % – 25 % urine pretreatment. The electron transfer efficiency positively correlated with the urine ratio (0 % – 25 %) in pretreatment ($R = 0.97$, $p = 0.005$).

More importantly, urine pretreatment of 5 % – 25 % enhanced the electrons transferred from substrates to MCFAs and the enhancements became significantly when urine ratio was above 5 % ($p = 0.002 – 0.01$) (Fig. 5b). In the control group (0 % urine), the majority of electrons (47.3 %) were transferred to SCFAs, whereas only 9.0 % of the electrons were transferred to MCFAs. However, more electrons were transferred from substrates to MCFAs with 5 %, 10 %, 15 %, and 25 % urine pretreatment, accounting for 16.2 %, 27.5 %, 36.5 % and 47.4 %, respectively. While less electrons were transferred from substrates to SCFAs with 5 %, 10 %, 15 %, and 25 % urine pretreatment, which were 40.0 %, 31.2 %, and 30.0 %, and 40.2 %, respectively. The proportions of electrons transferred from substrates to MCFAs had a positive correlation with urine ratio of 0 % – 25 % in pretreatment ($R = 0.98$, $p = 0.001$), while those of SCFAs showed a negative correlation with urine ratio ($R = -0.97$, $p = 0.007$). These results indicated urine pretreatment dose-dependently facilitated electron transferring form substrates to MCFAs and decreased the electrons transferred towards SCFAs. The electrons transferred towards alcohols accounted for 12.6 % – 16.0 % in all the tests, showing similar levels without significant differences

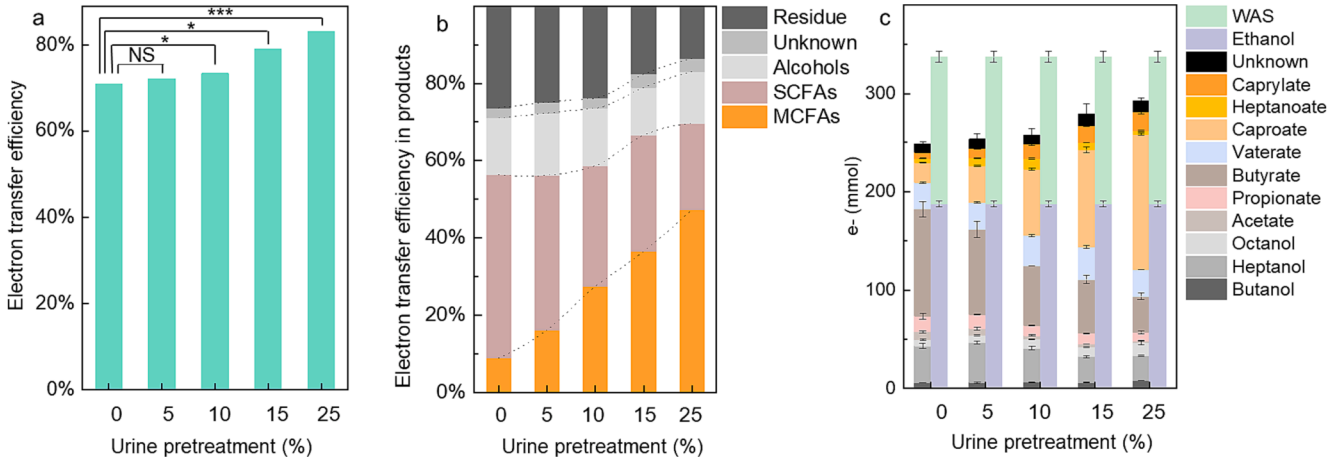

Fig. 5. The electron transfer efficiency in MCFAs generation tests with 0 % – 25 % urine pretreatment (a). The p-value of < 0.001, ≥0.01 and < 0.05, and > 0.05, are indicated by ***, *, and NS, respectively; (b) The proportions of electrons transferred to MCFAs, SCFAs, alcohols and unknown products; (c) The electron equivalent of products and substrates. Error bars represent the standard error. Some error bars are too small to be visually seen in the figure.

between the 0 % and 5 % – 25 % urine pretreatment (p = 0.97 – 0.99).

Additionally, the proportions of electron equivalent of residue decreased from 26 % in the control (0 % urine) to 25 %, 24 %, 18 %, and 13 % with 5 %, 10 %, 15 %, and 25 % urine pretreatment, respectively (Fig. 5b). Urine pretreatment promoted the electron transfer from WAS to MCFAs and reduced the remaining electrons in the WAS. This was consistent with enhanced WAS degradation observed under urine pretreatment (section 3.2). Furthermore, electrons equivalent of caproate increased from 19 mmol e^- to 137 mmol e^- along with the increase of urine ratio from 0 % to 25 % in the pretreatment (Fig. 5c). However, the electrons equivalent of butyrate decreased along from 108 mmol e^- to 37 mmol e^- along with the increase of urine ratio. These results indicated that more electrons transfer occurred in CE process when urine pretreatment was applied.

3.5. *Urine pretreatment promotes the solubilization, hydrolysis, acidification, and CE processes*

The effects of urine pretreatment on each step of MCFAs generation were further evaluated, including sludge solubilization, hydrolysis, acidification, and CE processes (Figs. 6-9). In solubilization tests, the concentration of released polysaccharide from WAS was only 24 mg COD/L with 0 % urine pretreatment (the control group), which was improved to 26 mg COD/L with 5 % urine pretreatment (Fig. 6a). The 10 %, 15 %, and 25 % urine pretreatment significantly promoted the release of polysaccharide to 50, 97, and 163 mg COD/L (p = 0.0006 – 0.001), respectively (Fig. 6a). Similarly, the concentration of released protein was improved from 73 mg COD/L in the control to 83, 162, 296, and 501 mg COD/L mg with 5 %, 10 %, 15 %, and 25 % urine pretreatment, respectively (Fig. 6b). These improvements were significant with 10 % – 25 % urine pretreatment (p = 0.0002 – 0.002). The highest levels of released polysaccharide and protein were achieved under 25 % urine pretreatment, which were approximately 6 times higher than that of the control (Fig. 6c). The concentrations of released polysaccharide were linear and positive correlated with the urine ratio (R = 0.97, p = 0.005). Correspondingly, the concentrations of released protein also showed a positive correlation with urine ratio (R = 0.98, p = 0.004). These were also supported by the improvement in WAS degradation through urine pretreatment (Section 3.2).

The hydrolysis tests were conducted using model polysaccharide (dextran) and protein (BSA) (Fig. 7). The removal of dextran was significantly improved from 43 % in the control (0 % urine) to 48 %, 50 %, 60 % and 65 % with 5 %, 10 %, 15 %, and 25 % urine pretreatment, respectively (p = 0.01 – 0.04, Fig. 6a). The BSA removal was 30 % in the control, but it was improved to 33 % with 5 % urine pretreatment (Fig. 7b). The increase of urine ratio further significantly increased BSA removals to 38 %, 39 %, and 43 % under 10 %, 15 %, and 25 % urine pretreatment, respectively (p = 0.04 – 0.02). Both of the dextran and BSA removals were dose-dependently related to the urine ratio (R = 0.97 and 0.98, p = 0.009 and 0.005, respectively). The highest dextran and BSA removals were both achieved at 25 % urine pretreatment, which were improved by 51 % and 43 %, respectively (Fig. 7c).

Subsequently, the monosaccharides and amino acids were utilized by the acidogens to produce SCFAs. The removals of glucose (model monosaccharide) and L-alanine (model amino acids) represents the performance of acidogenesis process (Fig. 8). The removals of glucose exceeded 98 % without significant difference between the tests with 5 % – 25 % urine pretreatment and the control (0 % urine) (p > 0.05, Fig. 8a). Additionally, the L-alanine removal was increased from 64 % in the control to 67 % with 5 % urine pretreatment (Fig. 8b). With 10 % – 25 % urine pretreatment, the L-alanine removals were significantly improved to 70 % – 75 % (p = 0.007 – 0.02), which were 9 % – 17 % higher than the control (Fig. 8c). Comparatively, L-alanine removals (64 % – 75 %) were lower than glucose removals (>98 %), suggesting that L-alanine was more challenging for microorganism to degrade. Urine pretreatment promoted the removal of amino acids, and such an acceleration effect was positively correlated with the urine ratio (R = 0.94, p = 0.01).

The urine pretreatment also enhanced the MCFAs production in CE process. The urine pretreatment of 10 %, 15 %, and 25 % significantly improved the MCFAs concentrations from 7.6 g COD/L in the control (0 % urine) to 9.0, 9.5, and 11.8 g COD/L (p = 0.009 – 0.03), respectively (Fig. 9a). The generated MCFAs concentration (7.9 g COD/L) with 5 % urine pretreatment was comparable (p > 0.05) to that in the control (Fig. 9). The MCFAs concentrations exhibited a positive correlation (R = 0.99, p = 0.001) with the urine ratio. The maximum MCFAs concentration was 11.8 g COD/L achieved under 25 % urine pretreatment, representing a relative 42 % increase compared to the control (Fig. 9b).

Overall, urine pretreatment dose-dependently promoted each process the MCFAs generation from WAS, including solubilization, hydrolysis, acidogenesis, and CE processes. Urine pretreatment of 25 % achieved the highest extent of each process.

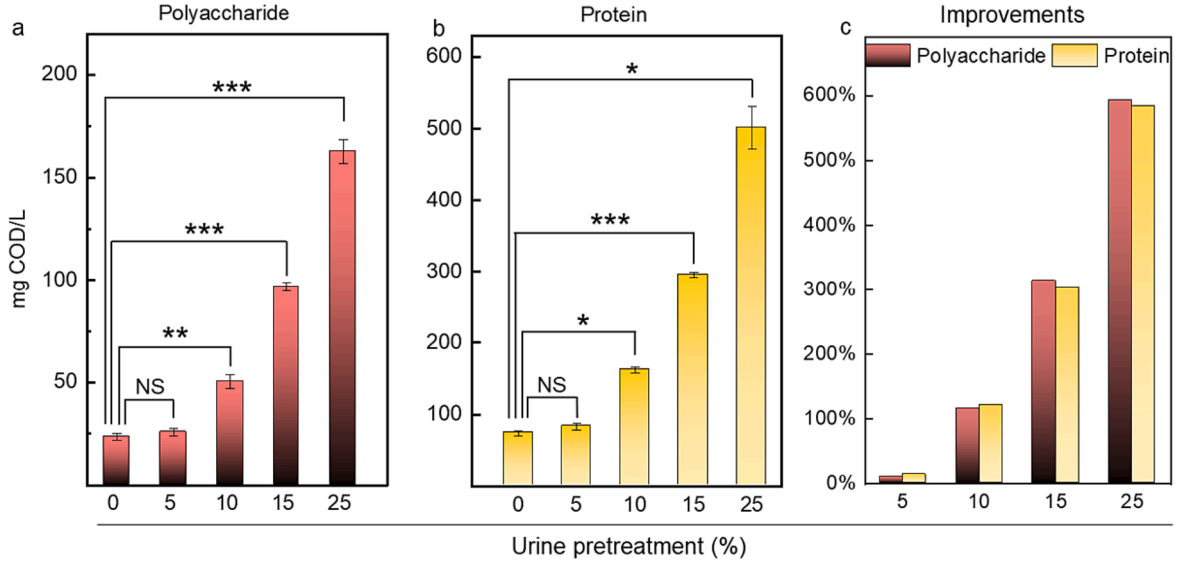

Fig. 6. The impacts of urine pretreatment on the solubilization process, indicated by the released polysaccharide level (a) and protein production level (b). The p-value of < 0.001, ≥0.001 and < 0.01, ≥0.01 and < 0.05, and > 0.05 is indicated by ***, **, *, and NS, respectively; (c) The relative improvements due to 5 % – 25 % urine pretreatment compared with that of the control. Error bars represent the standard error.

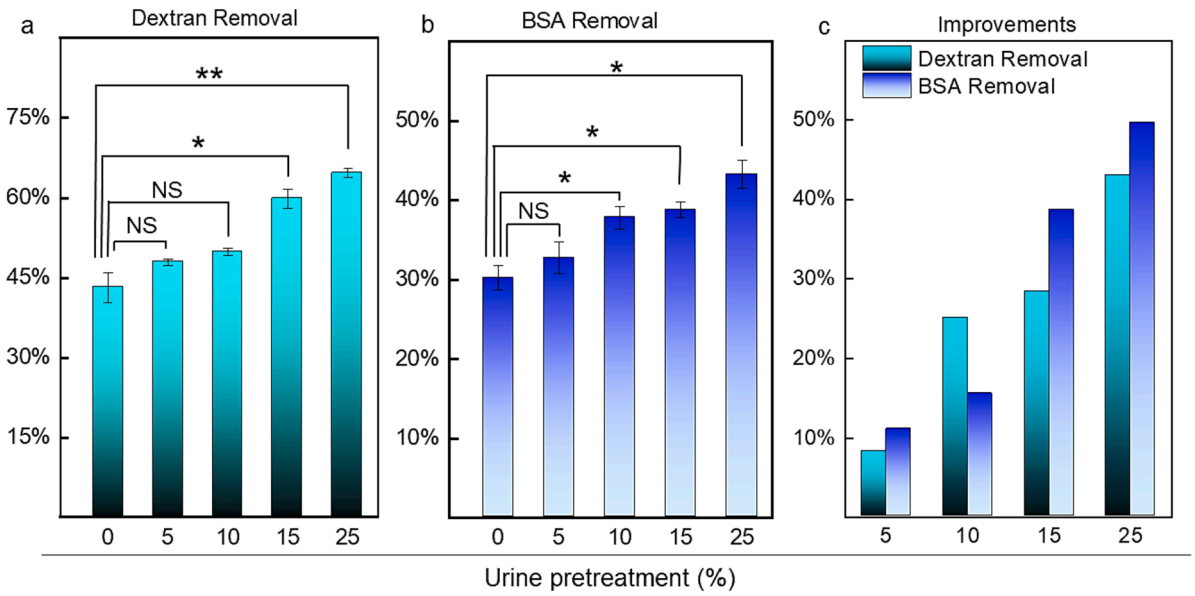

Fig. 7. The impacts of urine pretreatment on the hydrolysis process, indicated by the removals of dextran (a) and BSA (b). The p-value of < 0.01, ≥ 0.01 and < 0.05, and > 0.05 is indicated by **, *, and NS, respectively; (c) The relative improvements due to 5 % – 25 % urine pretreatment compared with that of 0 % urine pretreatment. Error bars represent the standard error.

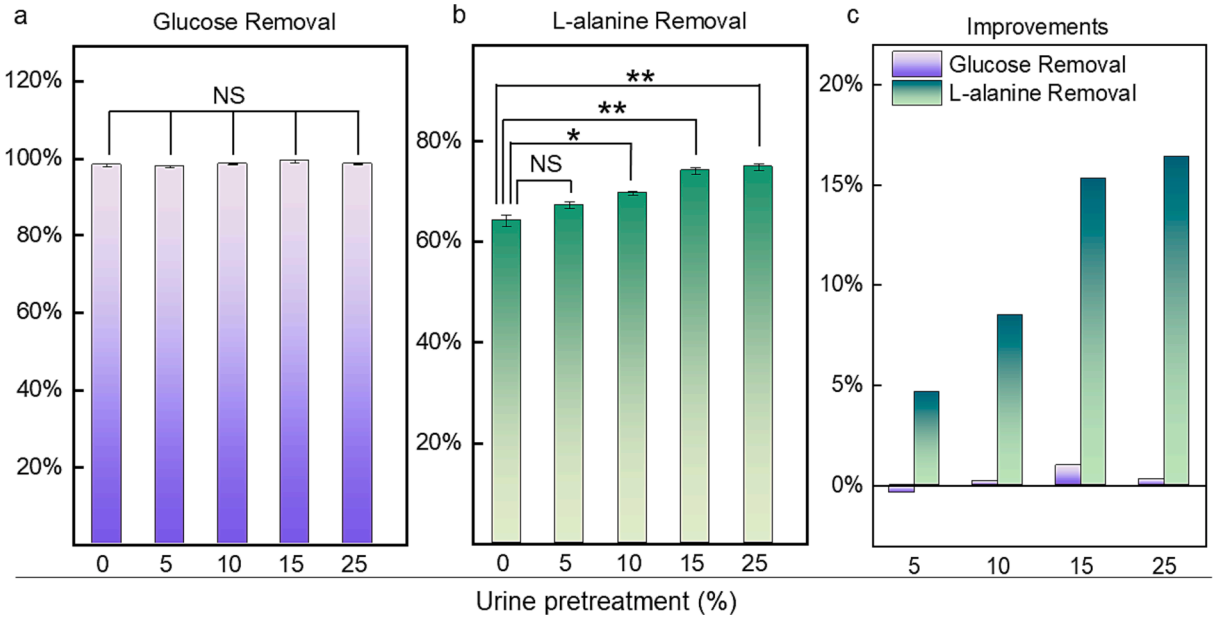

Fig. 8. The impacts of urine pretreatment on the acidification process, indicated by the removals glucose (a) and L-alanine (b). The p-value < 0.01, ≥ 0.01 and < 0.05, and > 0.05 is indicated by **, *, and NS, respectively; (c) The relative improvements due to 5 % – 25 % urine pretreatment compared with that of 0 % urine pretreatment. Error bars represent the standard error. Some error bars are too small to be visually seen in the figure.

4. Discussion

This study originally investigated the impact of urine pretreatment (5 % – 25 %) on MCFAs production in anaerobic WAS fermentation. Urine pretreatment of 5 % – 25 % dose-dependently improved the MCFAs production ($R = 0.99$, $p = 0.001$), but decreased the SCFAs production in MCFAs generation tests ($R = -0.97$, $p = 0.007$, section 3.1). This implies urine pretreatment promoted the conversation from SCFAs to MCFAs, facilitating the CE process for MCFAs generation. Furthermore, the urine pretreatment also significantly improved the MCFAs selectivity. The highest MCFAs production and selectivity, achieved with 25 % urine pretreatment, were 12.8 g COD/L and 54.4 %, respectively, which were 5.3 and 4.5 times of that with 0 % pretreatment, respectively.

The improved MCFAs production can be attributed to the improvements of WAS degradation and electron transfer efficiency from substrates to the products in MCFAs generation tests. Urine pretreatment of 5 % – 25 % improved the WAS degradation from 27.7 % in the control (0 % urine) to 30.2 % – 54.8 % in MCFAs generation tests. This is consistent with a previous study for anaerobic WAS digestion, where urine pretreatment increased WAS degradation from 25 % with 0 % urine pretreatment to 26 % – 34 % with 10 % – 30 % urine pretreatment [37]. Besides, the model-based analysis indicated that the maximum MCFAs production potential (P_m) extended by 66 % – 386 % due to the 5 % – 25

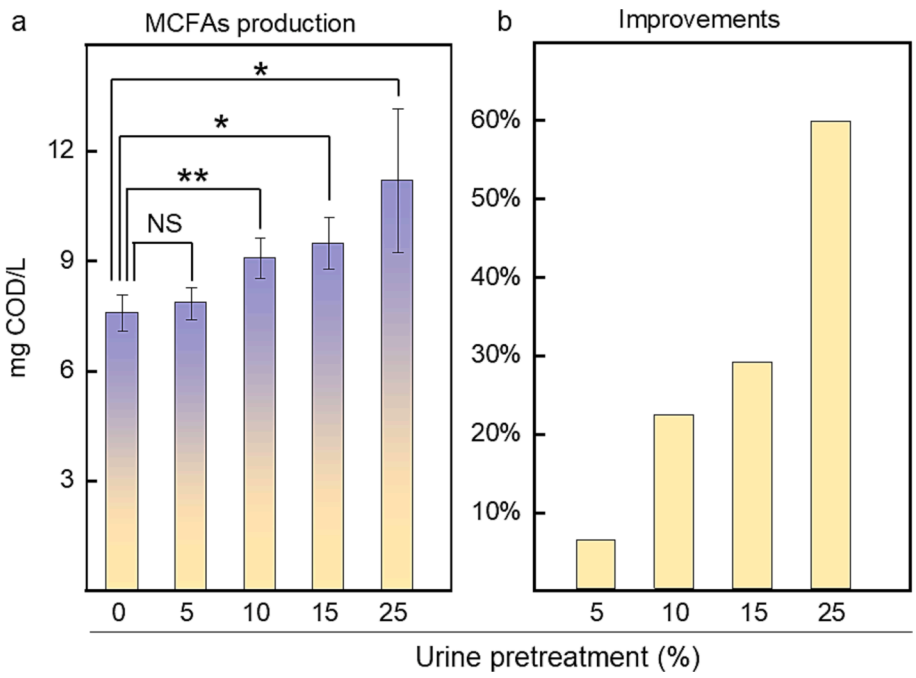

Fig. 9. The impacts of urine pretreatment on the CE process, indicated by the MCFAs production (a). The p-value of < 0.01, ≥ 0.01 and < 0.05, and > 0.05 is indicated by **, *, and NS, respectively; (b) The relative improvements because of 5 % – 25 % urine pretreatment compared with that of 0 % urine pretreatment. Error bars represent the standard error.

% urine pretreatment, which was also on account of the improvements in WAS degradation and MCFAs production. Additionally, the improvement in MCFAs production can also be attributed to the improved electron transfer efficiency from substrates to products. Urine pretreatment of 5 % – 25 % enhanced the electron transfer efficiency from substrates to products by up to 18.6 % in the MCFAs generation tests, where the maximum electron transfer efficiency of 83.6 % was achieved under 25 % urine pretreatment. The enhanced electron transfer efficiency is likely related to higher conductivity of urine, especially under high dosages. Urine possesses a high electric conductivity of 27 – 30 ms/cm [51,52], which is 10 – 100 times higher than that of WAS fermentation liquid with an electric conductivity of 0.3 – 2.6 ms/cm [53]. Generally, a high conductivity helps the electron transfer in fermentation system by providing a preferable conductive circumstance for the microbes [54]. Nevertheless, the underlying mechanisms through which urine pretreatment enhances electron transfer efficiency needs further systematic investigation.

Furthermore, urine pretreatment also promoted the key steps for anaerobic WAS fermentation (i.e. solubilization, hydrolysis, acidification, and CE processes). The soluble polysaccharide and protein levels in solubilization tests were increased by up to around 6 times (section 3.5) through 5 % – 25 % urine pretreatment. Besides, urine pretreatment promoted hydrolysis, acidification, and CE steps, by up to 51 %, 17 %, and 42 %, respectively. These promotion effects were dose-dependent on the urine ratio (5 % – 25 %) in pretreatment ($R = 0.95 – 0.99$, $p = 0.001 – 0.01$, section 3.5). These observations further support that urine pretreatment enhanced MCFAs generation from WAS in anaerobic fermentation.

These promoting impact of urine pretreatment on WAS degradation and each step of MCFAs generation are likely attributed to the free ammonia introduced by urine addition in the pretreatment. Previous research illustrated that free ammonia is able to disrupt the structure of WAS by breaking down extracellular polymeric substances and cell membranes [12,34–36,55], resulting in the improvement of WAS degradation. Free ammonia (210 – 680 mg NH_3-N/L) has been widely reported to improve the WAS degradation by 13 – 26 % in anaerobic digestion [56]. In this study, urine pretreatment of 5 % – 25 % introduced the concentrations of free ammonia at 8 – 287 mg NH_3-N/L (Table S3), which likely facilitated the degradation of WAS in MCFAs generation tests and enhanced solubilization, the hydrolysis, acidification, and CE steps. However, urine pretreatment is considered as a superior choice compared to free ammonia pretreatment. Although free ammonia pretreatment has been recognized as a sustainable technology for WAS digestion and fermentation [12,34–36,55], alkali might still be needed if the desired concentration of free ammonia is not attained [57]. Alkali requirement would lead to additional costs for its purchase and safety management. In such cases, urine pretreatment offers an alternative approach that can be readily obtained from urine separation systems in public centres. Furthermore, worldwide buildings are also developing urine-diverting toilet system, such as waterless urinal and NoMix for urine collection [39,40,58]. It should be noted that urine pretreatment does not introduce external pollutant to STPs because urine is an inherently present nitrogen source in sewage. Thus, urine pretreatment represents a promising and convenient method for enhancing MCFAs production in anaerobic WAS fermentation, serving as a prior alternative of free ammonia pretreatment.

Several chemical pretreatment methods have been reported to improve the production of MCFAs and medium-chain carboxylates (mainly MCFAs) in anaerobic WAS fermentation [6,20], such as pretreatment of combined Fenton and persulfate oxidation, and CuO nanoparticles addition [41]. The combined Fenton and persulfate oxidation pretreatment improved the production and selectivity of medium-chain carboxylates by 46 % and 69 %, respectively [59]. Additionally, CuO nanoparticles (2.5 mg/g-TS) improved the production and selectivity of medium-chain carboxylates by 37 % and approximately 18 %, respectively [41]. However, combined Fenton and persulfate pretreatment, and CuO nanoparticles required additional expenditure, which limit their extensive application. In contrast, urine pretreatment is considered as an economical option as urine is easily and readily available. Therefore, urine pretreatment is a sustainable and cost-effective method for enhancing MCFAs generation from WAS through anaerobic fermentation.

In addition, various resources have been employed for MCFAs generation through anaerobic fermentation, such as whey wastewater,

brewing wastewater and CO_2. Acid whey wastewater has been reported to produce caproate with a maximum production of 10.4 g/L [60]. The additional supply of CO_2 promoted the MCFAs production from brewing wastewater to 7.98 g/L [61]. However, these carbon resources are seasonal or case-dependent, posing challenges to the continuous production of MCFAs. On the contrary, WAS is constantly generated in large amounts in the STPs worldwide (e.g. 60–110 tons/d for a STP with 400,000 population equivalent), offering a stable and ubiquitous carbon resource [62]. Furthermore, WAS contains open-culture microbial communities and balanced nutrient compositions, making it suitable for microbial activities in fermentation systems [16,20]. Compared to WAS, these carbon resources (i.e. whey and brewing wastewater) require additional nutrient elements for microbe growth. The superiority of WAS recognizes it as a suitable carbon resource for MCFAs generation.

Urine pretreatment improving MCFAs production through anaerobic fermentation offers an alternative method to recover energy from WAS. The economic and environmental analysis were conducted to present the benefits of 25 % urine pretreatment in a theoretical STP severing 400,000 people (Table S4). Given that this theoretical STP has 100,000 tonnes influent per day, the requirement of urine amount around 25,000 L per day under the 25 % urine pretreatment with the highest MCFAs production of this study. This requirement of daily urine amount can be easily covered by only 3–4 % of the served population [63]. Urine can be collected in buildings with urine separation system and public moving toilets for urine collection. The collected urine is then used for urine pretreatment on WAS and producing valuable products (i.e. MCFAs, SCFAs, and alcohols) in WAS fermentation (Fig. 10). Compared to the STPs without pretreatment, the application of 25 % urine pretreatment would bring extra economic of around $3,044,000 annually from the products (Table S4). And the improved WAS degradation will reduce the volume of dewatered sludge by 28 % and thus decrease the expenditure on sludge transfer and settlement by $245,000 each year (Table S4). The total economic benefit would be approximately $33,000,000 considering the cost of urine pretreatment reactor and its associates ($8,500 per year). Previously, 30 % urine pretreatment used on anaerobic WAS digestion for methane generation is estimated to save $267,000 per year for a theoretical with same scale. Compared to methane generation, the implement of urine pretreatment on MCFAs generation from WAS brings approximately 10 times higher economic potential ($3,044,000 *vs* $267,000) for the same theoretical STP.

Besides, urine pretreatment could also deliver environmental benefit via reducing carbon footprint of valuable products generation. For example, the carbon emission for caproate production in WAS fermentation is reduced due to urine pretreatment. Without pretreatment, the carbon footprint of caproate is 7.3 kg CO_2-eq/ kg caproate through anaerobic WAS fermentation [64]. With 25 % urine pretreatment, the carbon footprint of caproate is estimated to decrease to 1.1 kg CO_2-eq/ kg caproate. Therefore, the CO_2 emission is estimated to reduce by approximately 10,000 tonne/year in this theoretical STP even if the environmental pay off of other products was not included. The environmental benefit of urine pretreatment indicated by reduced carbon emissions in MCFAs generation is around 10 times of methane generation in anaerobic WAS digestion (Fig. 10). Thus, urine pretreatment applied on anaerobic WAS fermentation presents economic and environmental merits, acting as a promising and advanced approach for STPs in energy recovery.

5. Conclusion

This study explores the impact of urine pretreatment (5 % – 25 %) on MCFAs generation from WAS though anaerobic fermentation with ethanol as an electron donor. The key findings allow us to draw the following main conclusion:

- Urine pretreatment of 5 % – 25 % does-dependently enhanced the MCFAs production by up to 4.3 times.
- Urine pretreatment improved WAS degradation by up to 97.8 %, supporting the improvement in MCFAs production. Urine pretreatment also improved the MCFAs selectivity by up to 3.5 times.
- Urine pretreatment enhanced the MCFAs P_m and R_m by up to 383 % and 657 %, respectively, while reduced the λ by up to 42 %.
- Urine pretreatment improved the electron transfer efficiency by up to 18.6 % and promoted the electrons transferred from substrates to MCFAs, facilitating the MCFAs generation in anaerobic WAS fermentation.
- Urine pretreatment of 5 % – 25 % does-dependently boosted the solubilization, hydrolysis, acidification, and CE steps by up to 600 %, 51 %, 17 %, and 42 %, respectively, ultimately leading to a substantial increase in the production of MCFAs.

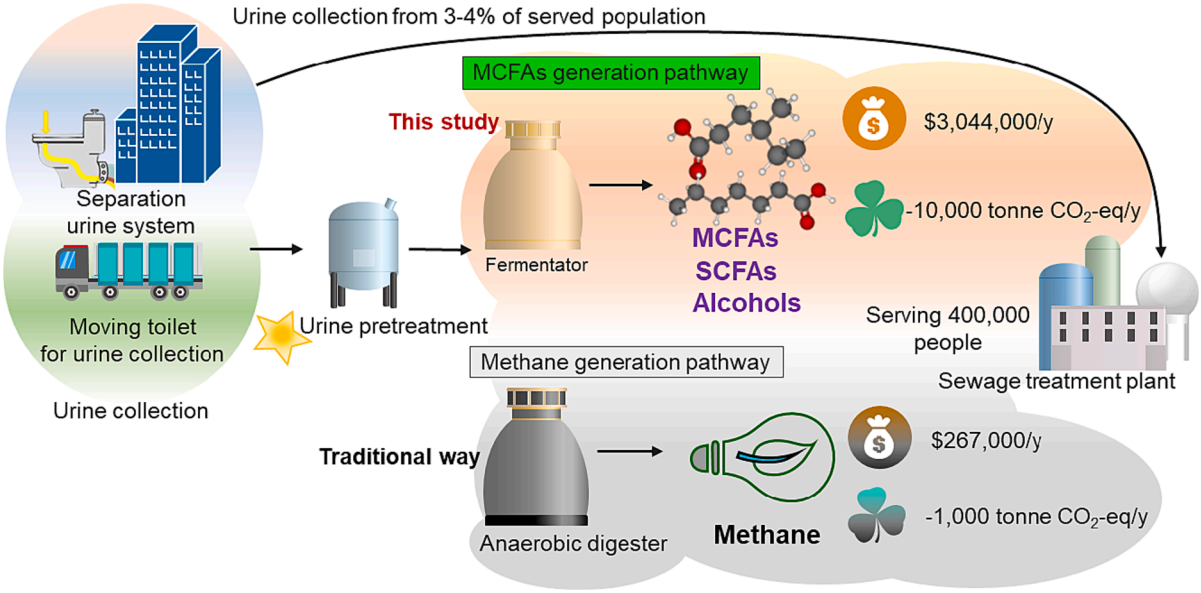

Fig. 10. Economic and environmental benefits of urine pretreatment to boost MCFAs production in anaerobic WAS fermentation, with comparation to methane generation through anaerobic WAS digestion.

- Urine pretreatment is a promising approach to improve the MCFAs production in anaerobic WAS fermentation, offering potential economic and environmental advantages in energy recovery for STPs.

CRediT authorship contribution statement

Huan Liu: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Xuan Li:** Formal analysis, Writing – review & editing. **Zehao Zhang:** Writing – review & editing. **Jibin Li:** Writing – review & editing. **Ting Zhou:** Writing – review & editing. **Zhenyao Wang:** Writing – review & editing. **Qilin Wang:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, 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.2024.148842.

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