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Adaptation of Urine Source Separation in Tropical Cities: Process Optimization and Odor Mitigation

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Source-separating urine from other domestic wastewaters promotes a more sustainable municipal wastewater treatment system. This study investigated the feasibility and potential issues of applying a urine source-separation system in tropical urban settings. The results showed that source-separated urine underwent rapid urea-hydrolysis (ureolysis) at temperatures between 34–40°C, stale/fresh urine ratios greater than 40%, and/or with slight fecal cross-contamination. Undiluted (or low-diluted) urine favoured ureolysis; this can be monitored by measuring conductivity as a reliable and efficient indicator. The optimized parameters demonstrated that an effective urine source-separation system is achievable in tropical urban areas. On the other hand, the initial release of CO₂ and NH₃ led to an elevated pressure in the headspace of the collection reservoir, which then dropped to a negative value, primarily due to oxygen depletion by the microbial activity in the gradually alkalized urine. Another potential odor source during the ureolysis process was derived from the high production of volatile fatty acids (VFA), which were mainly acetic, propanoic, and butyric acids. Health concerns related to odor issues might limit the application of source separation systems in urban areas; it is therefore vital to systematically monitor and control the odor emissions from a source separation system. As such, an enhanced ureolysis process can attenuate the odor emissions.

Implications: Urine source separation is promising to improve the management of domestic wastewater in a more sustainable way. The work demonstrates the achievability of an effective urine source-separation system in tropical urban areas. The installation of urine-stabilization tanks beneath high-rise buildings lowers the risk of pipe clogging. Conductivity measurement can be utilized as a reliable process indicator for an automated system. However, urine hydrolysis raises a strong potential of odor emission (both inorganic and organic), which might limit the application of
source separation systems in urban areas. An enhanced ureolysis process could shorten and attenuate the odor emissions.

INTRODUCTION
The increasing awareness of energy crisis, water shortage and resource scarcity have led to the development of the innovative sanitation system based on the concept of “source separation” (Langergraber and Muellegger, 2005; Larsen et al., 2009). Relying on the distinct chemical contents and concentrations, yellow water (urine), brown water (faeces) and grey water can be collected and treated separately to seek “waste-to-resource” and “waste-to-energy” purposes (Cordell et al., 2009; Love et al., 2009). In particular, the highly-concentrated urine stream contributes about 85% of nitrogen, 50% of phosphorus and 55% of potassium to the domestic wastewater but only 1% of the total volume (Larsen and Gujer, 1996; Meinzinger and Oldenburg, 2009). It is, therefore, acceptable to seek separate urine collection, which may significantly decrease the discharge of nutrients into water bodies and prolong the longevity of existing wastewater treatment plants (Wilsenach and Van Loosdrecht, 2004). Besides, the separate treatment of micropollutants contained in urine appears as an additional merit of the source separation system (Winker et al., 2008).

It is known that urine undergoes a series of intensive biological, physical, and chemical transformation immediately after its excretion (Hoglund et al., 2002). After sufficient storage, source-separated urine can be essentially stabilized as stale urine with a strong buffering capacity. Ureolysis, one of the most crucial parts, occurs with ease due to the existence of ubiquitous urease-positive bacteria, leading to subsequent mineral precipitation and ammonia volatilization at elevated pH-value (Larsen et al., 2003; Udert et al., 2006). Mineral precipitation might cause serious blockage in the urinary pipe. In case of a leakage, a strong odor is released during urea hydrolysis, thereby contributing another critical concern to urine separation systems in an urban setting. An enhanced urine stabilization process is, therefore, expected to not only minimize the storage volume required prior to any further treatment, but also shorten the duration of odor emission. Due to strict regulations of odor control in many countries, a systematic odor assessment (recording, characterizing and evaluating) should be done before any sanitation system is applied (Schlegelmilch et al., 2005).
Over the last decades, an increasing number of ecological sanitation systems (pilot or full scale) have been implemented all over the world based on the urine separation concept (e.g. No-Mix technology) (Hoglund et al., 1998). These systems have been applied in rural areas rather than in densely-populated cities with shortage of water and resources, like Singapore. They are still subject to numerous issues regarding social acceptance, infrastructure investment, technical availability, local regulations, etc. (Lienert and Larsen, 2010). These issues may impede the application of source separation system in urban areas. Currently, investigation on the feasibility and efficiency of a community-scale decentralized sanitation system in urban sectors is carried out. The main purpose is to achieve smart domestic waste management with water conservation, and resource/biomass energy recovery. Under this aspect, this study aims to obtain an overall understanding of urine transformation (or urine hydrolysis) during its separation, storage and transport. A schematic diagram of the urine source separation system is illustrated by Figure 1.

Figure 1 here

It is known that urine hydrolysis could be affected by many factors, including temperature, mixing ratio of stale to fresh urine and the amount of urease enzyme (Kabdasli et al., 2006; Zhao et al., 2008). The potential effects of other important factors have received less attention such as faecal contamination. The objective of this study is to comprehensively determine the influence of multiple parameters on the urine hydrolysis process, including temperature, dilution ratio, stale/fresh urine ratio, brown water (BW) cross-contamination, and infection of urease-positive bacteria. The characteristics of spontaneous precipitates formed during the hydrolysis process were monitored. In order to provide a detailed description on potential odor emissions, the interaction of urine hydrolysis with the emission of inorganic and organic gases was evaluated as well. Lastly, the feasibility and potential issues of urine source-separation system applied in a tropical urban setting were investigated.

EXPERIMENTAL

Experimental Variables
Lab-scale experiments were performed to investigate the effects of temperature, dilution ratio, volumetric ratio of stale urine to fresh urine (S/F urine ratio), BW cross-contamination and initial infection of urease-positive bacterial on urine hydrolysis process. The investigations on these influencing factors were conducted in several independent runs. Fresh urine was engaged in all experimental runs except for the second one which was meant to investigate the impact of S/F urine ratio. Fresh human urine was collected from a group of 30 healthy adults in a voluntary basis and well mixed in sterile plastic bottles. The ranges of initial pH, conductivity and NH₄⁺-N concentration of the undiluted fresh urine were between 7.0 - 7.3, 16.6 - 22.7 mS cm⁻¹ and 371 - 630 mg L⁻¹, respectively. In all batches, a quantitative aliquot (200 mL) of urine sample was added into each glass bottle (250 mL), covered tightly to simulate a relatively anoxic environment representing real conditions.

In run 1, the effect of temperature on urine hydrolysis at 4, 18, 23, 28, 34, 40, 50 °C was investigated. The temperatures from 18 to 34 °C were carefully selected with an increment of ~5 °C, representing the typical room temperatures with/without air-conditioning, and nocturnal/diurnal/peak ambient temperatures in tropical urban environment. Refrigeration temperature (4 °C) and extremely high temperature (50 °C, with additional heating supply) were also investigated for comparison purpose. Three dilution ratios were also examined (undiluted, dilution 1:2, and dilution 1:5) to simulate the influence of flushing water. The dilution ratios were selected to match the flushing volume of several water-saving urinals, such as waterless system, eco-friendly non-mix toilet, and traditional low-water urinals (Larsen et al., 2009; PUB, 2006).

In run 2, stale urine was mixed with fresh urine at different ratios (v/v) (0%, 10%, 20%, 40%, 60% and 80%) to investigate the effects of S/F urine ratio on urine hydrolysis process at the optimal temperature observed from the first run (34 °C). The stale urine (pH, conductivity and NH₄⁺-N concentration was 9.0, 31.1 mS cm⁻¹, 3800 mg L⁻¹, respectively) was prepared by storing the fresh urine at room temperature (24 °C) for several weeks. The selection of S/F urine ratios here and BW/fresh urine ratios in run 3 were arbitrarily determined mainly taking into accounts of the potential mixing behaviors from the non-mix toilet system.
Runs 3 and 4 were carried out simultaneously with special control on bacterial contamination. Both runs were conducted in a UV sterilization cabinet where all materials and devices, except the fresh urine samples, were disinfected by UV light with a peak wavelength of 254 nm for at least 5 min. In run 3, fresh BW was taken from a conventional toilet and quantitatively introduced into fresh urine since at source-separating toilet cross-contamination with BW tends to occur. In order to further investigate the effects of initial infection of urease-positive bacteria on urine stabilization, the fresh urine sample was centrifuged and the bottles were autoclaved in run 4. The sterilization pretreatment was done before the experiments to rule out any other bacteria source from air or device. t-Tests and one-way analysis of variance (ANOVA) were applied at the significance level of 0.05 to analyze the differences between variables. A P value of <0.05 was considered significant.

In addition, a set of experiments were conducted to monitor headspace pressure variation in the urine-loaded glass bottles using Oxitop pressure sensors (WTW, Germany). Additional experiments were conducted to determine gas emissions, in which the bottle headspaces were first rinsed with N2 to simulate anaerobic conditions. All the aforementioned experiments including runs 1~4 were carried out in duplicates to verify the reproducibility of the testing procedure. The duplicate experiments showed very slight difference between them.

**Analytical Methods**

The suspension pH value, conductivity and total ammonium (NH4+-N) concentration were monitored for five consecutive days in each experiment. The pH value and conductivity were measured using a D-54 pH/Conductivity meter (Horiba, Japan). Ammonium concentration was colorimetrically measured based on the salicylate method (Method 10205) using a DR 2800 spectrophotometer (Hach, USA). All reagents and chemicals used in the experiments were of analytical grade. At least two parallel replicates were engaged throughout the whole study for quality assurance.

Spontaneous precipitation occurred during urine hydrolysis. Therefore, after hydrolysis completed, the stale urine was centrifuged at 10000 rpm for 15 min to separate the precipitates.
formed. The collected precipitates were rinsed with deionized water and ethanol (99%), and then dried at 60 °C overnight. X-ray diffraction (XRD) analysis of the precipitates was carried out using a model DMS 2000 system (Scintag Inc., USA). Fourier transform infrared spectroscopy (FTIR, Perkin Elmer PE 1600, USA) and scanning electron microscopy (SEM, Zeiss Evo 50, Germany) were also used to further characterize the spontaneous precipitates. The concentrations of VFA in urine were determined using gas chromatography with a flame ionization detector (GC-FID, Agilent). A selective capillary GC column (DB-FFAP) was applied for good separation of nine VFA species including acetic, propionic, iso-butyric, n-butyric, iso-valeric, n-valeric, iso-caproic, n-caproic and heptanoic acids. For each analysis, 1.0 mL sample was taken and filtered through a 0.20 μm Whatman glassfibre filter, then mixed with 0.2 ml phosphoric acid (85%) and finally stored at 4 °C until analysis.

The inorganic gas composition in headspace (CO₂, N₂, CH₄, H₂ and H₂S) was determined using gas chromatography with a thermal conductivity detector (GC-TCD, Agilent) and a selective packed column (HP-PLOT Q). The amount of NH₃ generated during the process was directly calculated based on the assumption that the headspace gases mainly consisted of N₂, CO₂ and NH₃, while other compositions can be safely neglected in terms of volume. The measured pressure was also used to correct all the calculations to STP condition (standard temperature and pressure, 298.15 K and 1.0 atm).

**RESULTS AND DISCUSSION**

**Factors Influencing Urine Hydrolysis Process**

*Effects of Temperature and Dilution Ratio.* Figure 2 presents the pH, conductivity, and NH₄⁺-N concentration profiles during urine hydrolysis at different temperatures and dilution ratios. Fresh human urine contains nitrogen mainly in the form of urea, with relatively low ammonium content. The enzyme urease generated by urea-hydrolyzing bacteria catalyzes the hydrolysis of urea to ammonia and bicarbonate (Larsen et al., 2003). As seen in Figure 2a, the temperature increment had a dynamic effect on urea hydrolysis process. The optimal temperature for rapid urea hydrolysis process in undiluted urine was between 34_C and 40_C, at which the pH, conductivity, and NH₄⁺-N concentration of urine solution reached their maximum values in less than 2 days.
The results indicate that at higher temperatures a more rapid urea hydrolysis rate could be achieved. Moreover, the extremely high temperature (50°C) significantly inhibited the growth of urease-positive bacteria in urine and ultimately the entire hydrolysis process. The variations of conductivity and pH value followed the same trend as NH₄⁺-N concentration. These results are in accordance with other similar studies on urine hydrolysis (Larsen et al., 2003; Zhao et al., 2008).

With a dilution ratio of 1:2, the pH-value of urine solution also needed around two days to reach its highest level. However, both conductivity and NH₄⁺-N concentration reached their highest values within five days (Figure 2b). The asynchronous increase implied a slower urea hydrolysis process in diluted urine. The major reaction in fresh urine is urea hydrolysis, which enzymatically converts urea into CO₂ (weak acid) and ammonia (weak base). The generated CO₂ and NH₃ will contribute to a strong buffer solution of hydrolyzed urine (pH~9.0). Dilution of urine led to a lower substrate (urea) concentration, and therefore retarded the reaction rate of urea hydrolysis and the formation of buffer solution. As showed in Fig. 2b, dilution did affect pH, but to a lower extent than conductivity and ammonium concentration.

The same observation was demonstrated at even higher dilution ratio of 1:5 (Figure 2c). At this dilution ratio, urea hydrolysis required more than five days to be completed although the initial urea concentration was five times lower. It is remarkable that high water usage for urine flushing may lead to an increase of precipitates with low solubility because of the presence of water hardness (Larsen et al., 2003). This, in turn, increases the risk of pipe blockage issue in source separation systems. The treatment of highly-diluted urine increases the treatment cost, which
concurrently may also reduce nutrients recovery efficiency. The water-saving purpose can be achieved based on novel separated toilet design with high urine-diversion efficiency. Furthermore, low ammonia concentrations in highly-diluted urine can result in slow inactivation of pathogenic microbes (Vinneras et al., 2008). Hence, source-separated urine with less or even no dilution is favorable for a quick ureolysis process and subsequent inactivation of contained microorganisms.

**Effect of Stale/Fresh Urine Ratio.** Figure 3a shows the variations of NH$_4^+$-N concentration using different stale/fresh urine ratios at the favorable temperature (34 °C). It seems that urine hydrolysis in fresh urine can be significantly enhanced by adding stale urine because of the introduction and growth of urease-positive bacteria. The mixture of a higher ratio of stale urine exhibited a more active urea hydrolysis process. The urea hydrolysis took 3-4 days in a controlled experiment with fresh urine. The slightly slower hydrolysis rate compared to run 1 was likely due to less initial infection of urease-positive bacteria in fresh urine. By addition 10-20% of stale urine into fresh urine, the time needed to complete urea hydrolysis can be reduced to less than two days. The optimal mixing ratio of stale-fresh urine was at 40%, beyond which the process could be completed in less than one day. Assuming a constant influent, however, an even higher mixing ratio (> 40%) would probably enlarge the volume of urine hydrolysis tank.

**Figure 3 here**

**Effect of Urease-Positive Bacteria.** Urine contamination by feces is very possible in source separation systems (Hoglund et al., 1998). As illustrated in Figure 3b, urine hydrolysis was significantly enhanced by adding merely 1% (v/v) BW into fresh urine due to the introduction of urease-positive bacteria and urease enzymes that exist in BW. Some of these bacteria, like Proteus, belong to the family of Enterobacteraceae. Proteus are nonpathogenic enteric bacteria that lack of the ability to ferment lactose, but are characterized by fast urease activity (Stuart et al., 1945). Stuart’s urea broth was analyzed to confirm the existence of urease-positive bacteria in fresh BW (data not shown). In addition, other enterobacteria are also detected for their weaker urease-positive activities using Christensen’s urea agar (Christensen, 1946). The enhancement of urine hydrolysis by minor cross-contamination from brown water was therefore confirmed. However, urine hydrolysis was observed to slow down significantly when adding higher portion of BW (Figure 3b). As the microbial pathogens in urine are mainly derived from fecal cross-contamination (WHO, 2006), the complicated microbial community contains a wide range of species. The urease-positive
bacteria might not have the advantage competing with others in the early intense microbial competition stage (Brooks and Keevil, 1997; Sears, 2005), which resulted in a slower hydrolyzing process. However, the enteric pathogens quickly died off due to overexposure to ammonia (Vinneras et al., 2008). As such, the urease-positive bacteria became the dominators in the hydrolyzing urine.

As expected, the urine hydrolysis process in run 3 was much slower compared to those in other runs. The process was lagged due to the unavailability of urease-positive bacteria from the sterile environment. Nevertheless, complete urea hydrolysis was achieved in less than 10 days. Larsen et al. (2003) indicated that 14% of urea hydrolysis activity could be due to free urease that presents in fresh human urine. In run 4, the bacteria cells and large organic molecules in the fresh urine were settled down via centrifugation. The results (Figure 3c) show that urea hydrolysis in pretreated urine was much slower than in the urine contaminated with 1% BW. However, no significant effect ($p > 0.05$ for all conditions at the significance level of 0.05) on urea hydrolysis was observed either by autoclaving pretreatment of the bottles (NN versus AN, NC versus AC) or by centrifuge of the urine sample before experiments (NN versus NC, AN versus AC).

**Conductivity as Process Indicator**

From the engineering perspective, it is essential to find out a suitable process indicator, which can be easily installed in an automated urine source-separation system. The indicator should be reliable, simple and inexpensive. From this point of view, multiple physico-chemical parameters were studied. In particular, the correlation among pH-value, conductivity and corresponding NH$_4$-N concentration within the urine hydrolysis experiments (Runs 1, 2 and 3) is shown in Figure 4. The pH-value quickly increased to 9.0 under partial urea hydrolysis while the corresponding NH$_4$-N concentration was still low (1500 mgL$^{-1}$). This indicated the formation of a strong ammonium-bicarbonate buffer in the partially-hydrolyzed urine. In contrast, conductivity increased gradually and linearly with NH$_4$-N concentration in the undiluted urine. They appeared to be proportional to each other with high correlation coefficient ($r^2 = 0.977$, $p < 0.0001$). The total ammonium concentration of the undiluted urine during ureolysis can be estimated by eq 1.

\[ C = 280.6k - 4778 \]
where C is total ammonium concentration (mg L\textsubscript{-1}) and k is electrical conductivity (mS cm\textsubscript{-1}). Hence, with a conductivity value around 40 mS cm\textsubscript{-1}, the final NH\textsubscript{4}\textsuperscript{+}-N concentration will be around 6000–7000 mg L\textsubscript{-1}.

Figure 4 here

In case of diluted urine, the conductivity and corresponding ammonium concentration also showed a very good correlation (Figure 4). The precise correlation between these two factors was likely due to the continuous conversion of organic urea into both ammonium and carbonate, leading to a gradual increase of ion strength and ultimately the solution’s conductivity. The fluctuation was probably due to the conductivity measurement without temperature adjustment, ammonia volatilization, CO\textsubscript{2} loss, phosphate precipitation and other potential chemical and biological reactions. In overall, the results indicate that conductivity can act as a better indicator for urine hydrolysis process compared to pH-value. Other studies have also reported conductivity as a time-saving and cost-efficient indicator for phosphate monitoring in wastewater treatment (Maurer and Gujer, 1995; Udert et al., 2011). Thus, conductivity seems to be a simple but more reliable indicator to monitor the progress of urine hydrolysis process.

**Spontaneous Precipitation and Pipe-Clogging Prevention**

Small amount of precipitates appeared at the initial stage of hydrolysis. That was likely due to the insoluble uric acid and urate in slightly acidic fresh urine, but they became readily soluble and degradable at alkaline conditions in stale urine (Kirchmann and Pettersson, 1995). Significant precipitates were then generated in all experiments when the pH-value of urine increased above 8.0. The production of spontaneously precipitated solids was around 0.53 g (TSS) per liter of undiluted urine. Human urine generally contains abundant soluble phosphate, about 30\% of which can naturally precipitate as insoluble minerals (Larsen et al., 2003). Over time, the natural precipitates tend to accumulate in the urinary pipes and may lead to serious blockage.

The spontaneous precipitates produced in human urine were characterized using SEM, XRD and FTIR techniques (Figure 5). Although many intermediate precipitates could be produced during
urine hydrolysis process, crystalline struvite (MgNH₄PO₄·6H₂O) and hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂) were estimated as the final dominant minerals (Larsen et al., 2003). The XRD pattern and SEM image confirmed the formation of well-crystallized struvite in urine. The characteristic XRD peaks of the spontaneous precipitates were well matched with those of pure struvite. However, the high noise peaks and heterogeneity of SEM morphology indicated a low extent of struvite crystallization and simultaneous precipitation of other solids (e.g. hydroxyapatite, magnesium phosphate). This was probably due to the effects of complex matrices. Additionally, the strong characteristic absorption bands at around 570 cm⁻¹, 1010 cm⁻¹ and 1430 cm⁻¹ of the FTIR spectrum were corresponding to ν₁(P-O) P-O bend, ν₃ (PO₄³⁻) antisym str and NH₄⁺ (Banks et al., 1975), respectively, further demonstrating the formation of crystalline struvite.

Figure 5 here

Potential Odor Emissions

*Elevated Headspace Pressure.* Figure 6 presents the typical changes of relative headspace pressure during regular and enhanced urine hydrolysis processes (at 34 °C). For hydrolysis enhancement, 2 mL stale urine was added. The gases (CO₂ and NH₃) released during the initial step of the process led to an increase in headspace pressure in both experiments. However, during the enhanced hydrolysis process, the pressure suddenly dropped to negative value as a result of complete urea hydrolysis (final pH = 9.3). As showed in Table 1, both CO₂ and NH₃ were released from urine solution into the headspace during ureolysis process, supposedly leading to an elevated headspace pressure. In fact, the system arrived at a negative headspace pressure (about -180 hPa) at the end of ureolysis process (Figure 6). This was most likely due to the O₂ consumption by microbial activity rather than the CO₂ absorption by the alkalized urine. Comparing the two experiments, it seems that the regular experiment without enhancement underwent a much slower hydrolysis process (final pH = 7.1) because of the absence of initial bacteria loading, meanwhile, its headspace pressure remained at a level of more than 50 hPa (for seven days). The positive pressure compared to the ambient pressure indicated a high potential of gas emission from source-separated urine.
during hydrolysis. It is a fact that a strong unpleasant odor appeared especially during the first few days of urine storage. Apparently, a slower urine hydrolysis process may induce more odor emissions.

Figure 6 here

Inorganic Gas Emissions. Table 1 shows the composition of inorganic headspace gases monitored at different stages of urine hydrolysis at 34 °C. Urea gradually decomposes into CO₂ and NH₃ during urine storage. Since the headspace of Oxitop bottles were rinsed with N₂ before experiments and isolated as anaerobic spaces, the data presented in Table 1 showed both CO₂ and NH₃ emissions from urine solution, which led to a noticeable increase of headspace pressure (above 200 hPa). Due to the absence of oxygen in the headspace, the increasing pressure could be only due to the gas emissions from the hydrolyzing urine. After three days, the CO₂ generated together with the existing N₂ contributed about 92% (v/v) of the total headspace gas. The rest was mainly due to NH₃ emission. When the system reached a high relative pressure (~ 200 hPa), the corresponding concentration of NH₃ in the headspace measured was 9520 mg m⁻³, which was much higher than the theoretical equilibrium concentration of 2300 mg m⁻³ (STP), exceeding the odor threshold (Udert et al., 2006).

Table 1 here

Theoretically, the volatilization of CO₂ and NH₃ (as well as other trace volatile compounds) from urine is mainly dependent on two thermodynamic equilibriums: species dissociation equilibrium in aqueous solution and gas/liquid equilibrium at the water surface (Ni, 1999; Srinath and Loehr, 1974). Both equilibriums are significantly affected by temperature and pH conditions. At an elevated pH-value, the accumulated CO₂ in the headspace will be absorbed by the alkaline solution as bicarbonate and carbonate (HCO₃⁻, α₁=0.939 and α₂=0.059 at pH = 9.1 (Benjamin, 2002)), resulting in a decreasing amount of CO₂ in the headspace (day 9 in Table 1). Contrarily, the equilibrium NH₃ concentration in headspace will increase due to a higher distribution coefficient of free ammonia in stale urine (α₁ =0.420 at pH=9.1).
The gas composition analysis also showed that there was no H$_2$S, H$_2$ or CH$_4$ emission from source-separated urine during nine-day's storage, although urine contains high concentration of sulfate. It is also important to note that humidity and other volatile organic compounds (VOCs) generated can also contribute to the elevated headspace pressure to a certain amount. Even though NH$_3$ was the major odorous compound released during urine storage, its concentration calculated in headspace might be slightly overestimated. Other trace organic compounds can be of critical importance in view of their low odor thresholds, such as methyl mercaptan, ethyl mercaptan, methylamine and butyric acid (Vincent and Hobson, 1998). Further investigation on these odorous compounds should be conducted regarding to their occurrence, composition, concentration and emission properties.

*Potential Organic Odor Emissions.* The production of VFA during urine hydrolysis is presented in Figure 7. The total amount of VFA gradually increased until day 7 reflecting intense microbial activity during urine hydrolysis process. Acetic, propanoic and butyric acids were the three most abundant VFA fractions in urine at highest concentrations 54.3, 6.0 and 2.3 mM, respectively. The valeric acid was consumed during the entire process, while other compounds with longer carbon chain remained around 0.2 mM. However, it required more than one month to complete the urine hydrolysis process since this experiment was done in the absence of oxygen which led to the unavailability of initial urease-positive bacteria. Also, it was noticeable that most VFA produced during the ureolysis process were substantially degraded in a longer period (2~4 months).

The dissociation constants ($pK_a$) of VFA compounds are generally low. For instance, the $p$Ka of acetic acid is 4.75 (Benjamin, 2002). The VFA compounds produced during ureolysis process were mainly remained in their ionized forms. Nevertheless, the volatilization of VFA, especially with short carbon chains, was inevitable because of their high concentrations in urine and extremely low odor thresholds. The persistent and strong odor generated during the slow ureolysis process was also attributed to other odorous organic compounds at trace levels.
Moreover, a significant reduction of chemical oxygen demand (COD) (about 30%) was observed along with the ureolysis process. Since urea or its decomposed products (i.e. CO₂ and NH₃) does not contribute to COD, the COD reduction was likely due to the degradation of organic compounds by the microbes growing in urine. Fresh urine contains high concentration of creatinine, amino acids, uric acid and other organic compounds (Udert et al., 2006). In anoxic environment, these compounds can be degraded into soluble organic substances (e.g. amino acids and fatty acids) (Madsen et al., 2011). However, the main drawback is the high ammonium concentration simultaneously produced during urine hydrolysis, which can significantly inhibit the anaerobic digestion.

**Engineering and Administrative Aspects**

Based on the above batch experiments, it is inferred that the relatively higher ambient temperature in tropical or semitropical areas could benefit the urea hydrolysis process. On the contrary, extra energy is needed (e.g. from adjacent anaerobic digestion system) to maintain a favorable hydrolysis temperature when source separation systems are applied in relatively cold areas. Hoglund et al. (2002) also reported that human urine stored at 20 °C for at least six months can be considered safe to use as fertilizer for crops. When source separation systems are introduced in urban setting, such long storage period is impracticable because of land-scarcity constraints.

From an engineering perspective, mechanical stirring can be applied to enhance the mixing of incoming fresh urine with the existing stale/semi-stale urine in the urine stabilization tank, which benefits the reproduction and growth of activated urease-positive bacteria; this leads to rapid urea hydrolysis. The mixture also prevents potential nitrification and autotrophic denitrification in the tank. After hydrolysis, nitrogen in the urine (mostly as ammonium) can be easily recovered through various physico-chemical technologies, such as ammonia stripping (Gustin and Marinsek-Logar, 2011), struvite precipitation (Kabdasli et al., 2006; Ronteltap et al., 2010), selective ion exchange (Hedstrom and Amofah, 2008), or a combination among these technologies (Lind et al., 2000). The recovered nitrogen, either in the form of struvite or concentrated ammonium solution, can be further used for horticultural activities in cities. On the other hand, mechanical stirring in the urine stabilization tank will also increase stripping of urine-
derived VOCs and intensify the potential odor problems. Some preventive measures, such as a well-sealed and/or buried urine tank or odor control devices, might be necessary to prevent odor leakage to neighboring communities.

It is also important to note that although the cross-contamination with BW has certain positive effects on urea hydrolysis process, the recycling of source-separated urine could introduce a new route for pathogens transmission (Vinneras et al., 2008). The faecal indicators total coliforms, *E. coli*, clostridia and faecal streptococci were found in various amount in urine collection tanks and some of them only have a minor decrease in several months (Hoglund et al., 1998). Dilution, pH-value and temperature affect the survivability of the microorganisms in urine. For example, urine storage below 20 °C has a high risk of containing viable viruses. High ammonia concentration (2100 mg L⁻¹, pH 8.9) is recommended for rapid inactivation of enteric pathogens (Vinneras et al., 2008). At 20 °C, the time required for 90% inactivation for rotavirus and phage is 35 and 71 days, respectively (Hoglund et al., 2002). Therefore, further risk assessment on pathogens transmission via source separation systems is needed.

Nowadays, many source separation systems encounter costly maintenance issues mainly due to serious blockage in the urinary piping system (SWITCH., 2008). The installation of extra urine stabilization tank (as pretreatment) beneath densely-populated buildings may prevent further mineral precipitation in the downstream piping system. The struvite generated in the tank can be periodically collected for further use. However, early crystallization may inevitably take place in the inner surfaces of urinary pipes, which serves to connect source-separating toilets to the urine stabilization tank. Consequently, a suitable piping system design is needed to reduce or avoid early crystallization, such as proper pipe diameter, pipe material with smooth inner surface, piping system with less sharp turns, instant flushing after urination and regular clean-up using chemical detergents, and so on.

Generally, odor is generated from different stages of conventional end-of-pipe wastewater treatment plants during the treatment of domestic wastewater (Capelli et al., 2009). The source separation systems produce highly-concentrated wastewater streams (i.e. yellow water, brown water and grey water) posing higher potential of odor emissions. This becomes a critical concern and even constrains the application of source separation systems in urban areas. It is, therefore,
vital to systematically monitor and control the odor emissions from a source separation system. Additional attention should be paid to other trace organic odorants generated during the downstream treatment of source-separated urine.

**CONCLUSIONS**
In the ascent of global energy crisis, water shortage and resource scarcity, an innovative sanitation system based on the source separation concept has been intensively advocated to manage domestic wastewater in a more sustainable way. The present study evaluated the feasibility and potential limitations of applying urine source separation in a densely-populated tropical urban setting. A significantly enhanced ureolysis process could be achieved under optimized conditions (34~40°C, stale/fresh urine ratios >40%, slight faecal contamination and low dilution), and the entire process can be accurately monitored by measuring conductivity as a timesaving and cost-efficient indicator. The results demonstrated that an effective and automated urine source separation system could be achieved. Urine-stabilization tank beneath high-rise buildings could be adopted as a collector of spontaneous mineral precipitation. This design prevents pipe clogging for an integrated source-separating sanitation system. On the other hand, observations on the initial release of CO₂ and NH₃ indicated a strong potential of odor emission during urine storage. The production of high concentrations of volatile fatty acids (mainly acetic, propanoic and butyric acids) also contributed to the odor issues. To conclude, an enhanced ureolysis process could attenuate the odor emissions.

**ACKNOWLEDGEMENTS**
This study is supported by the National Research Foundation, Singapore; program number NRF-CRP5-2009-02, for the School of Civil and Environmental Engineering/Residues and Resource Reclamation Centre, Nanyang Technological University, Singapore.

**REFERENCES**


SWITCH. Cross-country assessment of the adoption, operational functioning and performance of urban ecosan systems inside and outside the EU; 018530 - SWITCH; Wageningen University, Department of Environmental Sciences, Urban Environment Group: The Netherlands, July 31, 2008.


### Table 1. Variation of volumetric proportion of headspace gases during urine hydrolysis at 34 °C

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 9</th>
<th>Control a (pH 9.3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure b (hPa)</td>
<td>0</td>
<td>114</td>
<td>238</td>
<td>197</td>
<td>135</td>
<td>-180</td>
</tr>
<tr>
<td>N₂</td>
<td>100%</td>
<td>81.6%</td>
<td>77.1%</td>
<td>76.4%</td>
<td>81.0%</td>
<td>85.0%</td>
</tr>
<tr>
<td>CO₂</td>
<td>0</td>
<td>10.8%</td>
<td>15.3%</td>
<td>16.5%</td>
<td>10.4%</td>
<td>1.0%</td>
</tr>
<tr>
<td>NH₃ c</td>
<td>0</td>
<td>7.5%</td>
<td>7.6%</td>
<td>7.1%</td>
<td>8.6%</td>
<td>14.0%</td>
</tr>
</tbody>
</table>

*Note: a control experiment conducted in bottle without N₂ purging, the final relative pressure in headspace was negative primarily due to microbial oxygen consumption; b relative pressure in headspace; c assume that the headspace gases mainly consist of N₂, CO₂ and NH₃.*
FIGURE CAPTIONS

Figure 1. Schematic diagram of a urine source separation system (YW: yellow water; BW: brown water. The present study focuses on the first steps in dash box)

Figure 2. Variation of pH-value, conductivity and NH$_4^+$-N concentration during urine hydrolysis at different temperatures and dilution ratios (a. no dilution; b. dilution 1:2; c. dilution 1:5)

Figure 3. Variation of NH$_4^+$-N concentration during urine hydrolysis with different stale-fresh urine ratios (a), faecal contamination (b) and pretreatments (c). (NN: normal bottle, normal urine; NC: normal bottle, centrifuged urine; AC: autoclave bottle, centrifuged urine; AN: autoclave bottle, normal urine; NN + 1% BW: normal bottle, normal urine with 1% brown water)

Figure 4. Correlation of pH-value (□), conductivity (■●▲) and corresponding NTT$_4^+$-N concentration during urine hydrolysis process

Figure 5. SEM image, X-ray diffraction pattern and FTIR spectrum of spontaneous precipitates generated from human urine (Standard XRD lines of pure struvite refers to JCPDS (No.15-0762))

Figure 6. Typical variation of relative pressure in headspace during regular (dotted line) and enhanced (continuous line) urine hydrolysis (pH-value was measured at the end of each experiment)

Figure 7. Production of volatile fatty acids during urine hydrolysis
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