Application of hydroponic systems for the treatment of source-separated human urine

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Abstract

Hydroponic systems are widely used for the treatment of nutrient rich wastewaters. In this study, a hydroponic system was applied as the final treatment stage of source-separated human urine after urea hydrolysis, induced-struvite precipitation and ammonia stripping in tropical conditions (Singapore). The results showed that water spinach grew efficiently in the pretreated urine with 1:50 dilution ratio at the growth rate 0.68 cm/d, leaf number 2.27 pieces/d, shoot dry mass 0.33 g, water content 93.86%, and nitrogen and potassium conversion rate 0.46 and 0.51 mg/mg, respectively. This hydroponic system removed 58-66% chemical oxygen demand (COD), 41-49% total nitrogen (TN) and up to 47% total suspended solid (TSS), indicating sufficient urine stream polishing. Nitrification was observed when COD reduced by 60%, possibly because of oxygen competition between nitrobacteria for nitrification and microbes for COD degradation. The kinetic study revealed that zero-order model provided best fitting for COD and ammonia-nitrogen (NH$_4^+$-N) removal, while second-order model was more suitable for TN removal.

Key-words: Hydroponic systems; Urine treatment; Water spinach; Dilution ratios; Nitrification
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>TN</td>
<td>Total Nitrogen</td>
<td>Least Significant Difference</td>
</tr>
<tr>
<td>TP</td>
<td>Total Phosphorus</td>
<td>Plant Growth Rate</td>
</tr>
<tr>
<td>TSS</td>
<td>Total Suspended Solid</td>
<td>Plant Leaf Number</td>
</tr>
<tr>
<td>NH₄⁺-N</td>
<td>Ammonia-Nitrogen</td>
<td>Plant Dry Mass</td>
</tr>
<tr>
<td>NO₃⁻-N</td>
<td>Nitrate-Nitrogen</td>
<td>Plant Water Content</td>
</tr>
<tr>
<td>NO₂⁻-N</td>
<td>Nitrite-Nitrogen</td>
<td>Root Dry Mass</td>
</tr>
<tr>
<td>PO₄³⁻-P</td>
<td>Phosphate-Phosphorus</td>
<td>Shoot Dry Mass</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved Oxygen</td>
<td>Dilution Ratios</td>
</tr>
<tr>
<td>EC</td>
<td>Electrical Conductivity</td>
<td>Food and Agricultural Organization</td>
</tr>
<tr>
<td>DI</td>
<td>De-Ionized</td>
<td>National Environmental Agency</td>
</tr>
<tr>
<td>ICP-OES</td>
<td>Inductively Coupled Plasma-Optical Emission Spectroscopy</td>
<td>Inductively Coupled Plasma-Mass Spectroscopy</td>
</tr>
</tbody>
</table>
1. Introduction

Domestic wastewater is a misplaced resource rather than the waste to be discarded. Human urine is characterized as the main contributor of nutrients to domestic wastewater with 85% of nitrogen, 50% of phosphorus, and 55% of potassium but only 1% of the total volume (Larsen and Gujer, 1996). The installation of source-separated toilet diverts urine from faeces making it more effective to recover and reuse nutrients (Larsen et al., 2001). Urine is an inexpensive source that may contribute to plant nutrition or enhance soil fertility (Salminen et al., 2001). However, the untreated urine may pose many environmental problems as well as odor issues.

Researchers have applied several phytoremediation technologies to treat human urine. A step-by-step procedure of green algae, zooplankton and plants cultivation was applied for nutrient recycling from human urine (Adamsson, 2000). Kirchmann and Pettersson (1995) have demonstrated the feasibility of using human urine in agriculture. Urine occupies equal fertility value with industrial fertilizers (Pradhan et al., 2007). Constructed wetlands with upflow macrophyte were successfully applied to remove ammonia in the diluted urine system (Farahbakhshazad and Morrison, 1997). New concept of forward osmosis treatment technology demonstrated good rejection of 50-80% for ammonium in hydrolyzed urine and >90% for phosphate and potassium in both fresh and hydrolyzed urine (Zhang et al., 2014). In addition, systematic investigations on urine management regarding urea hydrolysis, induced-struvite precipitation and ammonia stripping were conducted in our previous studies in order to recover phosphorus and nitrogen from human urine (Liu et al., 2014, 2013; Zhang et al., 2013). However, very limited studies reported further urine polishing to reach the sewer discharge standard.
The hydroponic system is a new technique of cultivating plants in a soilless environment to avoid nuisances of weeds, soil-borne diseases and land limitations. Domestic wastewater and anaerobic digestion effluent of food and vegetable wastes have been used as feeding nutrient solutions in hydroponic systems (Krishnasamy et al., 2012; Vaillant et al., 2003). Silverbeet, tomato, radish, lettuce and water spinach were commonly employed as targeted hydroponic plants (Endut et al., 2010; Krishnasamy et al., 2012; Lorena, 2012). In a source-separated sanitation concept, the rich nutrients in urine can be used for plant growth, and plants serve as biofilters for urine polishing (Endut et al., 2010).

To our knowledge, there is limited research on using urine as a medium for hydroponic cultivation. Direct use of hydrolyzed urine is phytotoxic due to its high ammonia concentration. Ammonium ion tends to convert into ammonia gas when the pH value surpasses 7.5 (Demuynck et al., 1984). Dissolved ammonia is more toxic to plants than ammonium ion (Hageman, 1984). The objective of this study was to engage the hydroponic system to polish urine after partial nutrient recovery, to meet sewer discharge standard. Optimum urine dilution ratio for maximum plant growth and pollutant removal with microbial relevant mechanisms were both under consideration. Bench-scale experiments were conducted under tropical conditions and commercial nutrient solution was applied for comparison.

2. Materials and methods

2.1. Urine collection

Fresh urine was collected from 30 voluntary adults and mixed in a sterile plastic bottle. Urease enzyme (~1 mg) was added to 15 L urine to enhance urine hydrolysis giving final
pH value 9.3 (Zhang et al., 2013). Afterwards, 0.2 L seawater per liter urine was added to recover phosphorus by induced-struvite precipitation, while the remaining phosphorus (~16 mg/L) was used for plant cultivation (Liu et al., 2013). Air striping process was implemented until ~800 mg/L ammonia remained in the urine (Liu et al., 2014). Table 1 shows the differences between the pretreated urine (which was the feed solution for the hydroponic system), concentrated nutrient solution (which was commercially used for plant cultivation after dilution), and the FAO wastewater standards for crop cultivation (FAO, 1992).

Table 1: Characteristics of undiluted urine after pretreatment, concentrated commercial nutrient solution, and FAO wastewater quality standards for crop production.

<table>
<thead>
<tr>
<th>Parameters*</th>
<th>Pretreated Urine (before dilution)$^1$</th>
<th>Concentrated nutrient solution$^2$</th>
<th>FAO Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.45</td>
<td>5.89</td>
<td>6.5-8</td>
</tr>
<tr>
<td>EC(µs/cm)</td>
<td>31350</td>
<td>337200</td>
<td>700$^a$</td>
</tr>
<tr>
<td>DO</td>
<td>2.15</td>
<td>8.47</td>
<td>N.A.</td>
</tr>
<tr>
<td>COD</td>
<td>4120</td>
<td>3400</td>
<td>N.A.</td>
</tr>
<tr>
<td>TSS</td>
<td>3150</td>
<td>2000</td>
<td>N.A.</td>
</tr>
<tr>
<td>TN</td>
<td>1210</td>
<td>33320</td>
<td>N.A.</td>
</tr>
<tr>
<td>NH$_4^+$-N</td>
<td>881</td>
<td>4300</td>
<td>N.A.</td>
</tr>
<tr>
<td>NO$_3^-$-N</td>
<td>9.04</td>
<td>27600</td>
<td>5$^a$</td>
</tr>
<tr>
<td>NO$_2^-$-N</td>
<td>0.33</td>
<td>0.8</td>
<td>N.A.</td>
</tr>
<tr>
<td>PO$_4^{3-}$-P</td>
<td>16</td>
<td>8780</td>
<td>N.A.</td>
</tr>
<tr>
<td>K</td>
<td>1667</td>
<td>44525</td>
<td>N.A.</td>
</tr>
<tr>
<td>Ca</td>
<td>17.8</td>
<td>14020</td>
<td>N.A.</td>
</tr>
<tr>
<td>Na</td>
<td>4207</td>
<td>673</td>
<td>207$^b$</td>
</tr>
<tr>
<td>Mg</td>
<td>32.65</td>
<td>10595</td>
<td>N.A.</td>
</tr>
<tr>
<td>Zn</td>
<td>0.77</td>
<td>8.71</td>
<td>2.0$^c$</td>
</tr>
<tr>
<td>Fe</td>
<td>0.419</td>
<td>519.8</td>
<td>5.0$^c$</td>
</tr>
<tr>
<td>B</td>
<td>2.435</td>
<td>372</td>
<td>0.7$^a$</td>
</tr>
<tr>
<td>Mo</td>
<td>0.074</td>
<td>0.076</td>
<td>0.01$^c$</td>
</tr>
<tr>
<td>Mn</td>
<td>0.003</td>
<td>192</td>
<td>0.20$^c$</td>
</tr>
<tr>
<td>Cu</td>
<td>0.6125</td>
<td>6.3</td>
<td>0.20$^c$</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>5170</td>
<td>100900</td>
<td>N.A.</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>7955</td>
<td>N.D.</td>
<td>365$^b$</td>
</tr>
</tbody>
</table>
Notes:

1. The unit is mg/L unless specified.
2. N.D. means not detectable.
3. N.A. means data are not available.
4. \(a\) Guideline of no restriction on use for irrigation.
5. \(b\) Guideline of less than moderate restriction on use for irrigation.
6. \(c\) Threshold levels of trace elements for crop production.
7. 1 Urine parameters before dilution (dilute ratio 1:10, 1:20, 1:30 and 1:50 for practical usage).
8. 2 Concentrated nutrient solution parameters (dilute ratio 1:200 for practical usage).

2.2. Plant selection

The cultivation of indigenous plant species in hydroponic systems is favorable since these plants require less management and acclimatize quickly in native climate conditions. Vital criteria for plant selections in hydroponic systems are specified: a) adaptability to hydroponic systems; b) availability in local context; c) relatively short life circle. Preliminary experiments were carried out to evaluate several kinds of plants such as leaf lettuce (\textit{lactuca sativa}), water spinach (\textit{ipomoea aquatica}), lawn (\textit{hydrocotyle sibthorpioides}), and golden pothos (\textit{epipremnum aureum}). However, only water spinach grew sufficiently in the hydroponic system and thus it was selected as the cultivation plant. Water spinach was nourished from seeds to plantlets in a farm in Singapore for two weeks until it reached to an approximate length of 8 cm.

2.3. Experimental design

Bench-scale hydroponic experiments were conducted in a transparent PVC tank with 6 mm thickness and 800 mm \(\times\) 600 mm \(\times\) 100 mm (length \(\times\) width \(\times\) height) dimension (Fig. 1). The tank was divided into three troughs. Three covers with 5 holes (D = 120 mm) covered each trough in order to reduce odor. The holes were packed with plastic pots which were filled with light-expanded clay aggregates as an environmental friendly material for
supporting plant roots to grow in the hydroponic system for nutrient uptake.

Fig. 1. Schematic diagram of the hydroponic system.

Experiments were carried out in pretreated urine with four different dilution ratios (1:10, 1:20, 1:30 and 1:50) and five replicates of pots were situated. The dilution ratio 1:5 was not engaged because there was complete death of plants after one day of implantation in the preliminary experiment. De-ionized (DI) water was used to dilute urine to designed ratios. For comparison purposes, unplanted trough with 1:10 diluted urine was served as blank, while commercial nutrient solution was served as control experiment. The main components of the nutrient solution are listed in Table 1. Compared with the commercial nutrient solution, pretreated urine contained lower concentrations of many nutrition elements (e.g. \( \text{NO}_3^-\)-N, \( \text{PO}_4^{3-}\)-P, K, Ca, etc.), however they were sufficient for plant growth. The concentrated nutrient solution was diluted to manage vegetable growth according to the instructions as 5 mL solution per liter DI water. The effective volume of each trough was kept at 5 L to ensure sufficient contact of plant roots within the medium. There was no additional water, urine or nutrient solution during cultivation. Each plastic pot possessed nine plantlets of water spinach with their root systems fixed in sponge to provide adequate liquid contact and stability of the whole plant.
Liquid samples were collected every three days, while plant biomass samples were obtained at the beginning and end of each experiment. The experiments were performed outdoor for 21 days under tropical conditions. The temperature was between 24-32 °C daily, while photosynthesis occurred under natural sunlight.

2.4. Analysis

2.4.1. Urine

Ammonia-nitrogen (NH₄⁺-N), nitrate-nitrogen (NO₃⁻-N), nitrite-nitrogen (NO₂⁻-N), and phosphate-phosphorus (PO₄³⁻-P) concentrations were determined by chromogenic reactions based on salicylate, diazotization, ascorbic acid and dimethylphenol method, respectively, detected by a portable DR2800 spectrophotometer (Hach, USA). Samples were digested for chemical oxygen demand (COD) and total nitrogen (TN) determination and measured by DR2800 spectrophotometer (Hach, USA). Total suspended solid (TSS) was determined by filtration through 47µm glass fiber filter, followed by drying to constant weight at 103-105°C. Dissolved oxygen (DO), electrical conductivity (EC) and pH were measured by portable DO (YSI ProODO, USA), EC (Mettler Toledo, Switzerland) and pH meter (Accumet ® Basic AB15), respectively. Micronutrient and macronutrient elements, including potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), boron (B), copper (Cu), manganese (Mn), zinc (Zn), iron (Fe) and molybdenum (Mo), were determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES, Perkin Elmer, UK) for concentrations more than 200 µg/L and Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) for concentrations less than 200 µg/L. Sulfate and chloride ions were measured by ion chromatography (ICS-1100, Dionex)
2.4.2. Plant

At the beginning of the experiment, wet and dry mass of shoots and roots of similar size plants were weighted by an electronic balance (KERN ABS). Leaf number and plant height were manually measured periodically. At the end of the experiment, plants were harvested and plant size and appearance was evaluated. Shoots were cut at the sponge rim and roots were taken out from the sponge, and then wet mass was determined. Dry mass was determined after drying the plants at 85°C for 72h in an oven (Memmert, Germany). Dried biomass was digested in a mixture of nitric acid and hydrogen peroxide (ratio = 7:2) using a high performance microwave digestion system (ETHOS One, Milestone) and the digested solution was analyzed by ICP-OES or ICP-MS. The content of nitrogen in plant dry mass was determined by an elemental analyzer (CHNS/O 2400, Perkin Elmer, USA).

2.5. Statistical methods

One-way analysis of variance (ANOVA) and least significant difference (LSD) test were applied for significance test of difference in plant growth characteristics (i.e. growth rate, element content, etc.) affected by the initial urine dilution ratios. A specified level of type I error $\alpha$ is equal to 0.05 ($p \leq 0.05$) which means $p$ value less than 0.05 indicates significant difference.

3. Results and discussion

3.1. Plant development

Plant growth rate (PGR) and plant leaf number (PLN) are good indicators of plant development. The relationship between PGR and PLN with initial $\text{NH}_4^+$-N concentration in
the hydroponic system is shown in Fig. 2A. NH$_4^+$-N is selected for comparison due to its
dual characters, a necessary nutrient for plant growth and its potential phytotoxic effect at
high concentrations. Both PGR and PLN increased with the decrease of initial NH$_4^+$-N
concentration. Maximum PGR 0.68±0.07 cm/d and PLN 2.27±0.28 pieces/d occurred when
urine had 1:50 dilution ratio. These results were comparable with PGR 0.84±0.04 cm/d and
PLN 2.37±0.31 pieces/d using the commercial nutrient solution. However, at lower dilution
ratio, plant inhibition occurred probably due to high NH$_4^+$-N concentration and/or salinity.
The inhibition effect is also discussed by other researchers (Shin et al., 2005).

Plant dry mass (PDM) and plant water content (PWC) are commonly used to evaluate
the plant development. PWC is an indicator to measure plant growth conditions as
transpiration plays an important role in plant health. A slight reduction in PWC could
influence both growth and physiological functions, such as photosynthesis and respiration
(Hatfield, 1997). Fig. 2B presents PDM and PWC at different dilution ratios after 21 days
cultivation. The shoot dry mass (SDM) showed an increasing trend from 0.22 g to 0.33 g
with the increase of dilution ratios, while the SDM of plants cultivated in the nutrient
solution was comparable with that in urine with 1:50 dilution ratio. On the contrary, there
was no significant difference in the root dry mass (RDM) of plants cultivated in urine with
different dilution ratios. PWC had the similar increasing trend as PDM ranged from 91.56%
to 93.86%. The PWC value in urine with 1:50 dilution ratio was similar to that in the
nutrient solution, indicating comparable plant growth effects.
Fig. 2. A. Relationship between plant growth rate (PGR) and plant leaf number (PLN) after 21 days cultivation with initial NH$_4^+$-N concentration in the hydroponic system. B. Relationship between plant dry mass (PDM) and plant water content (PWC) after 21 days cultivation with different dilution ratios in the hydroponic system. Values of PGR, PLN, PDM and PWC are given by mean ± standard deviation (n = 5). Labels of same letters (a, b, c) or digitals (1, 2, 3, 4, 5) are not significantly different at p≤0.05 level. (■ plant growth rate, □ plant leaf number, □ shoot dry mass, □ root dry mass, ▲ water content, NS: nutrient solution).

3.2. Plant appearance

Plant appearance is a strong indicator of growth conditions, of which leaf color is a valuable characteristic of plant nutrition and health (Murakami et al., 2005). The
appearance of the plants after 21 days cultivation in urine at different dilution ratios and in
the nutrient solution is shown in Fig. 3. Plants in the nutrient solution and urine with 1:50
dilution ratio exhibited a splendid green color, while the color descended to gloomy yellow-
green with decrease of dilution ratios, thus plants can be distinguished in healthy and pale
groups. Other characteristics, such as height of shoot and leaf number, are described in
section 3.1.

Fig. 3. Appearance of water spinach after 21 days cultivation in urine at different dilution
ratios (left to right: dilute ratio 1:10, 1:20, 1:30, 1:50, nutrient solution).

3.3. Nutrient accumulation in plant dry mass

Nutrient elements accumulated in plant dry mass were measured to evaluate plants
growth. Elements can be divided into two categories based on their quantities. N, P, K, Na,
Ca and Mg are macronutrient elements with content more than 1000 mg/kg, and B, Cu, Mn,
Zn, Fe and Mo are micronutrient elements with content less than 1000 mg/kg. Fig. 4
demonstrates macronutrient and micronutrient element accumulation in SDM and RDM.
Fig. 4. Macronutrient and micronutrient element accumulations in shoot dry mass (SDM) and root dry mass (RDM) of plants in urine at different dilution ratios. Values are given by means ± standard deviations (n = 5). Labels of same letters (a, b, c, d) or same digitals (1, 2, 3, 4) indicate no significant differences.
3, 4) are not significantly different at p≤0.05 level (nutrient solution is not comparable due to its specific compositions). The unit of macronutrients (N, P, K, Na, Ca and Mg) is in % and the unit of micronutrients (B, Cu, Mn, Zn, Fe and Mo) is in mg/kg. (■ content in SDM, □ content in RDM, NS: nutrient solution).

3.3.1. Macronutrient elements

N, P and K are three major nutrient elements for plant growth. N content in both SDM and RDM showed a significant decrease (p ≤ 0.05, ANOVA), from 4.91±0.16% and 5.63±0.11% to 4.12±0.11% and 3.19±0.01%, respectively, with the increased dilution ratios. Similar trend was also obtained in P content. Plants in nutrient solution presented the highest N and P content, which could be attributed to the higher initial TN and TP concentration in the solution. On the contrary, K content showed opposite tendency since accumulation increased in higher dilution ratios. This might be caused by salt-induced mineral perturbations as Na concentration decreased with increasing dilution ratios. Mühling and Läuchli (2002) have mentioned that K deficiency can cause decrease of crop productivity due to high Na concentration.

Conversion rate is the main indicator of measuring the utilization efficiency. Nitrogen conversion rate (N mass utilized by plants to N mass reduced in the hydroponic system) showed an increase trend with the increase of dilution ratios, from 0.07 to 0.46 mg/mg. The potassium had the same trend with the range 0.02-0.51 mg/mg. In terms of nutrient solution, only 0.22 and 0.27 mg/mg for N and K were obtained (Table 2). This suggests that only part of N and K was removed by plant uptake. The remaining nitrogen might be removed by other mechanisms such as nitrification-denitrification (Flite et al., 2001) and anoxic
ammonia oxidation (Mulder et al., 1995), which could occur under different operation conditions, including oxygen supply, pH, plant type and so on.

Table 2
Nitrogen and potassium conversion rate of plants, and final pollutant removal efficiency in urine at different dilution ratios.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1:10</th>
<th>1:20</th>
<th>1:30</th>
<th>1:50</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen conversion rate (mg/mg)</td>
<td>0.07</td>
<td>0.29</td>
<td>0.37</td>
<td>0.46</td>
<td>0.22</td>
</tr>
<tr>
<td>Potassium conversion rate (mg/mg)</td>
<td>0.02</td>
<td>0.33</td>
<td>0.40</td>
<td>0.51</td>
<td>0.27</td>
</tr>
<tr>
<td>COD removal rate (%)</td>
<td>66.3</td>
<td>65.0</td>
<td>63.1</td>
<td>58.1</td>
<td>N.A.</td>
</tr>
<tr>
<td>TSS removal rate (%)</td>
<td>46.9</td>
<td>31.3</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>TN removal rate (%)</td>
<td>42.3</td>
<td>40.6</td>
<td>45.9</td>
<td>49.4</td>
<td>33.1</td>
</tr>
</tbody>
</table>

Notes:
N.A. means negligible changes of removal rate due to low initial concentrations.

The content of other macronutrients such as Ca showed a significant difference in plants at different dilution ratios. Ca mass increased as the dilution ratio went up, which is possibly caused by salt-induced mineral perturbations (similar to K). With reference to Mg, it is an essential macronutrient and its deficiency is a detrimental plant disorder (Hopkins and Hüner, 2008). It shows comparable results in all urine experiments. Na content of plants in urine was much higher than that in nutrient solution due to the high salinity of urine.

3.3.2 Micronutrient elements
Micronutrient elements play a strengthening role in plant growth regardless of their low quantity. A remarkable enhancement of plant growth could be achieved from the joint addition of B, Cu, Mn and Zn (Arnon, 1938). B deficiency affects vegetative and
reproductive growth resulting from inhibition of cell expansion, death of meristem and reduced fertility (Marschner, 1995). Accumulated B in SDM was twice compared with the corresponding values in RDM. Higher levels of Cu, Mn, Zn, Fe and Mo in RDM compared with those in SDM were related with the formation of iron plaques on the root surface, which was also mentioned by other researchers (Tanner, 1996).

3.4. Urine purification

3.4.1. Nitrogen source transformation

Nitrogen transformation plays an important role at different stages during the 21 days experiment as shown in Fig. 5. In the pretreated urine, the major form of nitrogen was NH$_4^+$-N (about 70%), while organic nitrogen and NO$_x$-N (NO$_3^-$-N + NO$_2^-$-N) accounted 30% and less than 1%, respectively. In the nutrient solution, more than 80% of nitrogen was in the form of NO$_3^-$-N. After 12 days treatment, almost all organic nitrogen was removed and total amount of inorganic nitrogen almost remained constant. It might result from plant associated microbes which capture organic nitrogen more easily than inorganic nitrogen (Dunn et al., 2006). Afterwards, NH$_4^+$-N concentration started declining due to several possible chemical and biological reactions including plant uptake (Rogers et al., 1991), volatilization (Sánchez-Monedero et al., 2001), nitrification-denitrification (Flite et al., 2001) and anoxic ammonia oxidation (Mulder et al., 1995).
Fig. 5. Nitrogen forms during 21 days cultivation in urine at different dilution ratios. (A) Unplanted Channel, (B) 1:10, (C) 1:20, (D) 1:30, (E) 1:50, (F) Nutrient Solution. (NH₄⁺-N, NO₃⁻-N, NO₂⁻-N, Organic-N, Removed-N).
Both NO₃⁻-N and NO₂⁻-N had very low initial concentrations and demonstrated significant increase after 12 days, indicating that nitrification by nitrosation and nitrifying bacteria was undergone in a two-step process. Nitrification is the predominant process of NH₄⁺-N reduction. Dissolved oxygen was utilized in nitrification process concurrently with COD degradation, since they are on aerobic basis. Competition for oxygen utilization took place between nitrobacteria for nitrification and microbes for COD degradation as shown in Fig. 6. The observed variation shows that at high COD concentration, COD degradation was favored compared to nitrification. Oxygen transfer through internal pressurization and
convection was mostly utilized by COD microbes. This inhibited the establishment of large population of nitrobacteria (Gray et al., 1996). Nitrobacteria began to use the existing oxygen and noticeable nitrification process took place when COD degradation reached around 60%. It was remarkable that pH was decreased by 2 units from day 12 to 15 because of the nitrification process (data not shown).

3.4.2. Pollutant removal efficiency

COD is an important parameter to assess the effectiveness of wastewater polishing. COD was 4120 mg/L before any dilution and 58-66% was removed at the end of the experiments (Table 2). The removal was almost ceased at day 6. Accumulated bacteria in the root system were the main consumer of COD through degradation, adsorption or infiltration (Vaillant et al., 2003). The COD concentration in the effluents with 1:30 and 1:50 dilution ratios met European and Singaporean discharge level (Directive, 1991; NEA, 2005). This proves that hydroponic system can effectively reduce the COD concentration in the urine.

Total nitrogen (TN) in undiluted urine was around 1200 mg/L. After hydroponic treatment, 41-49% removal rate of TN was achieved and urine with 1:50 dilution ratio had the highest removal efficiency. In the nutrient solution, only 33% of TN was removed due to relatively high initial TN concentration. TSS content in the effluent was quite low according to regulations except dilution 10. Urine effluent with 1:50 dilution ratio as shown in Table 3 meets the discharge standard in Singapore and Europe after minor pH adjustment (Directive, 1991; NEA, 2005). Overall, the source-separated human urine treatment processes, starting from urea hydrolysis, induced-struvite precipitation and ammonia
stripping (Liu et al., 2014, 2013; Zhang et al., 2013) to hydroponic systems, achieved a remarkable purification with total removal of 95.6%, 73.1% and 92.1% for \( \text{PO}_4^{3-} \), COD and TN, respectively.

### Table 3
Parameters of urine effluent after treatment (1:50) and corresponding discharge standards.

<table>
<thead>
<tr>
<th>Parameters*</th>
<th>Treated urine (1:50)</th>
<th>Singapore(^a)</th>
<th>European Union</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.31</td>
<td>6-9</td>
<td>N.A.</td>
</tr>
<tr>
<td>COD</td>
<td>35</td>
<td>60</td>
<td>125</td>
</tr>
<tr>
<td>TSS</td>
<td>10</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>TN</td>
<td>12.6</td>
<td>N.A.</td>
<td>15</td>
</tr>
<tr>
<td>( \text{NO}_3^- )-N</td>
<td>2.1</td>
<td>4.5</td>
<td>N.A.</td>
</tr>
<tr>
<td>TP</td>
<td>0.3</td>
<td>0.67</td>
<td>1</td>
</tr>
<tr>
<td>Fe</td>
<td>0.05</td>
<td>1</td>
<td>N.A.</td>
</tr>
<tr>
<td>B</td>
<td>0.03</td>
<td>0.5</td>
<td>N.A.</td>
</tr>
<tr>
<td>Mn</td>
<td>0.04</td>
<td>0.5</td>
<td>N.A.</td>
</tr>
<tr>
<td>Cu</td>
<td>0.10</td>
<td>0.1</td>
<td>N.A.</td>
</tr>
<tr>
<td>Zn</td>
<td>0.09</td>
<td>0.5</td>
<td>N.A.</td>
</tr>
<tr>
<td>Ca</td>
<td>2.27</td>
<td>150</td>
<td>N.A.</td>
</tr>
<tr>
<td>Mg</td>
<td>1.10</td>
<td>150</td>
<td>N.A.</td>
</tr>
<tr>
<td>( \text{SO}_4^{2-} )</td>
<td>63</td>
<td>200</td>
<td>N.A.</td>
</tr>
<tr>
<td>Cl</td>
<td>172.5</td>
<td>400</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

Notes:
* The unit is in mg/L, except pH.
\(^a\) Discharge to controlled watercourse from which potable water is supplied.
N.A. means data are not available.

### 3.5. Kinetic study
Zero-, first- and second-order kinetic models (Eq.1, 2, 3) were engaged in order to understand the kinetics of pollutant removal efficiency in the hydroponic system. According to the following equations, the removal rate constant for a targeted pollutant was determined as a function of treatment time, and initial and final concentrations of the
1 pollutant.

\[ Ct = Co - k_i t \quad (1) \]

\[ \frac{Ct}{Co} = \exp(-k_i t) \quad (2) \]

\[ \frac{1}{Ct} - \frac{1}{Co} = k_2 t \quad (3) \]

where \( Ct \) is the pollutant concentration at \( t^{th} \) day (mg/L); \( Co \) is the initial pollutant concentration (mg/L); \( k_i=0,1,2 \) is the \( i^{th} \)-order removal rate constant (mg/L·d\(^{-1}\), d\(^{-1}\), L/mg·d\(^{-1}\)) and \( t \) is the treatment time (d).

Table 4
Kinetic reaction rate constants of COD, TN and NH\(_4^+\)-N in the hydroponic system with different dilution conditions.

<table>
<thead>
<tr>
<th>Dilution ratios</th>
<th>Pollutants</th>
<th>Zero-order ( k_0 ) (mg/L·d(^{-1}))</th>
<th>First-order ( k_1 ) (d(^{-1}))</th>
<th>Second-order ( k_2 ) (L/mg·d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10*</td>
<td>COD</td>
<td>100.5</td>
<td>0.9993</td>
<td>0.0006</td>
</tr>
<tr>
<td>1:10</td>
<td>COD</td>
<td>120.3</td>
<td>0.9977</td>
<td>0.0009</td>
</tr>
<tr>
<td>1:20</td>
<td>COD</td>
<td>65.0</td>
<td>0.9987</td>
<td>0.0024</td>
</tr>
<tr>
<td>1:30</td>
<td>COD</td>
<td>44.5</td>
<td>0.9993</td>
<td>0.0036</td>
</tr>
<tr>
<td>1:50</td>
<td>COD</td>
<td>22.3</td>
<td>0.9966</td>
<td>0.0033</td>
</tr>
<tr>
<td>1:10*</td>
<td>TN</td>
<td>7.5771</td>
<td>0.9304</td>
<td>0.0008</td>
</tr>
<tr>
<td>1:10</td>
<td>TN</td>
<td>7.0158</td>
<td>0.8864</td>
<td>0.0008</td>
</tr>
<tr>
<td>1:20</td>
<td>TN</td>
<td>3.8086</td>
<td>0.8472</td>
<td>0.0014</td>
</tr>
<tr>
<td>1:30</td>
<td>TN</td>
<td>2.5314</td>
<td>0.8715</td>
<td>0.0027</td>
</tr>
<tr>
<td>1:50</td>
<td>TN</td>
<td>1.4976</td>
<td>0.9222</td>
<td>0.0049</td>
</tr>
<tr>
<td>1:10*</td>
<td>NH(_4^+)-N</td>
<td>7.0462</td>
<td>0.9143</td>
<td>0.0018</td>
</tr>
<tr>
<td>1:10</td>
<td>NH(_4^+)-N</td>
<td>6.6724</td>
<td>0.8494</td>
<td>0.0010</td>
</tr>
<tr>
<td>1:20</td>
<td>NH(_4^+)-N</td>
<td>3.3547</td>
<td>0.8542</td>
<td>0.0021</td>
</tr>
<tr>
<td>1:30</td>
<td>NH(_4^+)-N</td>
<td>2.3191</td>
<td>0.9205</td>
<td>0.0046</td>
</tr>
<tr>
<td>1:50</td>
<td>NH(_4^+)-N</td>
<td>1.4868</td>
<td>0.8956</td>
<td>0.0078</td>
</tr>
</tbody>
</table>

Notes:
* indicates unplanted channel.
The bold characters indicate the best description of the models.

The \( k_i \) of TN and NH\(_4^+\)-N were calculated using the data during the whole period, while the COD removal rate constant was derived from the first 6 days because the
concentration was relatively stable after that period. As shown in Table 4, zero-order model fits best for the pollutant removal kinetics of COD and NH₄⁺-N with good correlation coefficient $R^2$ ranging between 0.9966-0.9997 and 0.8494-0.9205, respectively. TN correlated best with second-order kinetic model with $R^2$ ranging between 0.8961-0.9457.

The effect of dilution ratios ($DRs$) on removal rate constant was further examined by linear regression with exponential functions using the $k$-$DRs$ data. Good correlation coefficients $R^2$ for COD, TN and NH₄⁺-N were obtained as shown in Eq. 4, 5, 6.

$$k_0 = 205 \exp(-0.5DRs), \quad R^2 = 0.9890$$

$$k_2 = 0.0004 \exp(0.6DRs), \quad R^2 = 0.9992$$

$$k_0 = 10 \exp(-0.5DRs), \quad R^2 = 0.9824$$

### 3.6. Engineering aspects

The experimental data indicates that large amount of dilution water is required for practical implementation because of the high dilution ratio (1:50). Assuming all the urine discharged in Singapore is source-separated and diluted with DI water for hydroponic cultivation, $\sim 6.6 \times 10^7$ S$/year are needed to supply the DI water alone, based on the water tariff of $\sim 2.31$ S$/m^3$ and population of 5,469,700 (2014), without considering the treatment cost from tap water to DI water. The high cost highlights the need to explore other water sources to fulfill the demand for urine dilution. Since Singapore is a typical rainwater-abundant country, rainwater might be a good alternative. The average annual rainfall in Singapore is 2357.8 mm/y (NEA, 2007) and urine excretion volume per person per day is 1~1.5 L/p/d (Kujawa-Roeleveld et al., 2003). Assuming a high-rise residential apartment of
250 occupants with a land area 600 m², the total volumes of rainwater provided and required can be computed according to Eq. 7, 8.

\[
V_1 = H \times A / 1000
\]  

(7)

\[
V_2 = U \times N \times (D - 1) / 1000 \times 365
\]  

(8)

where \( V_1 \) is the total volume of rainwater provided per year (m³/y); \( V_2 \) is the total volume of rainwater required per year (m³/y); \( H \) is the average annual rainfall (mm/y); \( A \) is the building area (m²); \( U \) is the urine excretion volume per person per day (L/p/d), define \( U \) as 1.5; \( N \) is the total number of persons in this building (p); and \( D \) is the dilution ratio. Since the supply (\( V_1 \approx 1400 \text{ m}^3 \)) is larger than the demand (\( V_2 \approx 1300 \text{ m}^3 \)), it is theoretically feasible to use rainwater as a dilution water source for this hydroponic system. There are some proposals of using domestic gray water for urine dilution as well.

As described in section 2.3, 5 L diluted urine (1:50) can support the growth of 45 plantlets with total dry mass 1.65 g. The amount of urine generated by the above-mentioned apartment can yield 452 kg/y dry mass. The space needed for the hydroponic system might require further consideration. Currently, there are several on-going projects exploring the plant growth on vertical walls and/or building façades for landscape purpose, which might be able to solve the space constraint. In addition, roof gardens and other landscape applications attract more attentions nowadays. Direct consumption of cultivated plants as edible food is also feasible but this requires further investigation. Cultivated plants should be tested for food safety, such as pathogens (e.g. escherichia coli, cyclospora, listera monocytogenes and salmonella enteritis), heavy metal and micropollutant accumulations, etc.
4. Conclusions

This study demonstrated the feasibility of applying hydroponic systems for both urine treatment and water spinach cultivation. Plants cultivated in urine with 1:50 dilution ratio achieved the comparable growth characteristics (e.g. growth rate, leaf number, etc.) to those in the nutrient solution. This system also removed remarkable amount of COD, TN and TSS and the effluent can fulfill the local sewer discharge standard. Nitrogen transformation and removal mechanism attributes mainly to microbial nitrification and plant utilization. The amount of rainwater should be sufficient to supply the dilution requirement. However, the actual engagement might require further study.

Acknowledgements

This study was supported by the National Research Foundation, Singapore; program number NRF-CRP5-2009-02. Authors are also grateful to Interdisciplinary Graduate School (IGS), Residues and Resource Reclamation Centre (R3C), Nanyang Environment and Water Research Institute (NEWRI), Nanyang Technological University (NTU), Singapore. We appreciate Mr. Bernard Ng for his assistance in the experimental work as well.

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