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Graphic for manuscript



1 Nitrite production from urine for sulfide control in sewers

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14 ABSTRACT

15 Most commonly used methods for sewer sulfide control involves dosing chemical agents to 16 wastewater, which incurs high operational costs. Here, we propose and demonstrate a cost-17 effective and environmentally attractive approach to sewer sulfide control through urine 18 separation and its subsequent conversion to nitrite prior to intermittent dosage to sewers. Urine 19 collected from a male toilet urinal was fed to laboratory-scale sequencing batch reactors. The reactors stably converted roughly 50% of the nitrogen in urine to nitrite, with high abundance 20 21 (at 17.46%) of known ammonia-oxidizing bacteria (AOB) of the genus Nitrosomonas, and absence (below detection level) of typical nitrite-oxidizing bacteria of the genus Nitrospira, 22 according to 454 pyrosequencing analysis. The stable nitrite production was achieved at both 23 relatively high (1.0-2.0 mg/L) and low (0.2-0.3 mg/L) dissolved oxygen concentrations. 24 25 Dosing tests in laboratory-scale sewer systems confirmed the sulfide control effectiveness of free nitrous acid generated from urine. Life cycle assessment indicated that, compared with 26 27 commodity chemicals, nitrite/free nitrous acid (FNA) production from urine for sulfide control 28 in sewers would lower the operational costs by approximately 2/3 and greenhouse gas (GHG) emissions by 1/3 in 20 years. 29 Keywords: Urine separation; Free nitrous acid (FNA); Sulfide control; Life cycle assessment; 30

31 Economic analysis

33 **1. Introduction**

34 In urban water management, all pollutants, including ammonium, phosphorus, sulfate, and 35 organics, are collected from households and industries, and then delivered through sewer networks to a treatment plant for removal. Sulfate is easily reduced anaerobically in sewers, 36 resulting in production of sulfide (Liu et al., 2014a). The generation of sulfide in sewer systems 37 38 is a serious problem because it induces odor, concrete and metal corrosion and health hazards (Pikaar et al., 2014). Subsequent rehabilitation and replacement of the damaged sewer pipes 39 are rather expensive. It has been reported that national sewer rehabilitation in the US alone 40 41 could cost up to 3.2 billion dollars annually (US Environmental Protection Agency, 2010). To date, one of the main approaches adopted to mitigate these issues has been the dosage of 42 chemical agents, such as oxygen, iron salts, nitrate and nitrite salts, and alkalis (Ganigue et al., 43 2011; Liu et al., 2014b). However, continuous dosing of these commodity chemicals incurs 44 45 large operational costs.

Recently, the concept of integrated urban water management is receiving more attention 46 47 because it can deliver system-wide optimization with tremendous economic and environmental benefits (Behzadian and Kapelan, 2015). For example, researchers proposed using iron 48 49 chloride to replace commonly used alum as a coagulant in drinking water treatment and then 50 using the same iron for corrosion and odor control in sewers and phosphorus removal in 51 wastewater treatment plants, thus substantially reducing the use of chemicals in the entire urban 52 water system (Gutierrez et al., 2010; Ge et al., 2012; Sun et al., 2015a). In this work, we investigate the feasibility of sulfide control in sewers using urine. This would involve urine 53

54 separation, its conversion to nitrite and the subsequent, intermittent addition of the nitrite to 55 sewers. The nitrite addition would normally need to be accompanied by acid addition to 56 produce free nitrous acid (FNA) at elevated (sub-ppm) levels. FNA was previously discovered 57 to be a cost-effective agent for sulfide control in sewers (Jiang *et al.*, 2013). It has a biocidal 58 effect in sewer biofilms and can also lead to biofilm removal (Jiang *et al.*, 2010; Jiang *et al.*,

2011a; Jiang *et al.*, 2011b; Jiang *et al.*, 2013; Jiang and Yuan, 2014; Sun *et al.*, 2015b). The
source separation approach proposed here is essentially to produce nitrite required from
wastewater itself.

62 Source separation of urine from the remaining household wastewater is a promising approach toward sustainable urban water management (Maurer et al., 2006; Lienert and Larsen, 63 2010; Udert and Wächter, 2012). Urine contains up to 80% of the total nitrogen in domestic 64 wastewater but makes up only less than 1% of the total volume (Larsen and Gujer, 1996; Udert 65 et al., 2006); therefore, source separation of urine can substantially reduce the nitrogen load to 66 67 the downstream wastewater treatment plants. Many methods had been proposed for nutrient 68 recovery from urine by using physical, chemical and biological methods (Maurer et al., 2006; 69 Zhang et al., 2014; Xu et al., 2016). However, to the best of our knowledge, no information 70 has yet been reported on sulfide control in sewers by using source-separated urine.

In this study, we experimentally demonstrate nitrite production from urine and its effectiveness in sulfide control in sewers. Urine was collected from a male toilet urinal in a public building. Nitrite was then produced using laboratory-scale sequencing batch reactors with urine as the feed. The effectiveness of the urine-generated nitrite in suppressing sulfide

production in sewers was then demonstrated using a laboratory-scale sewer reactor.
Additionally, life cycle assessment (LCA) was conducted to assess the economic and
environmental performance of the proposed approach.

78 **2. Materials and methods**

79 2.1 Urine collection and characteristics

Urine was collected from a male toilet urinal in the Sino-Italian Environment and Energyefficient Building located in Tsinghua University, Beijing. The collected urine, which was stored in a large container for over three days, was then used as the feed to a nitrifying reactor. The feed contained approximately 400–900 mg N/L ammonia nitrogen, 450–950 mg N/L total nitrogen, 300–700 mg/L COD, and 1500–2800 mg/L alkalinity as CaCO₃ with a pH of 8.8±0.1. The nitrogen concentrations were much lower compared with fresh urine because of the dilution with flushing water during collection.

87 2.2 Reactor setup and operation

Experiments on the production of FNA were performed in laboratory-scale sequencing batch reactor. The reactors were made from Plexiglas cylinders with an effective volume of 2.7 L (30 cm in height and 12 cm in inner diameter). Each 8 h cycle consisted of period of feeding (5 min), mixing and aeration (7 h), settling (50 min), and decanting (5 min). The hydraulic retention time (HRT) and sludge retention time (SRT) of the reactor were 24 h and 40 d, respectively.

94 The experiments included three phases: I, start-up of a reactor and long-term operation with
95 ammonia nitrogen concentration of ~550 mg/L as the influent for achievement of stable nitrite

96 production from urine; II, operation of two reactors in parallel under different dissolved oxygen (DO) conditions with influent ammonia nitrogen concentration fluctuation to investigate the 97 98 potential impact of DO on the ammonium to nitrite conversion; and III, operation of a low DO 99 reactor to investigate the effect of alkalinity supplement in the urine on the reactor performance. 100 The DO concentration was monitored continuously using a DO probe (WTW, Oxi340i), and 101 controlled at set-points of 1.0-2.0 mg/L and 0.2-0.3 mg/L in the high and low DO reactors, respectively. Temperature and pH were automatically recorded using a pH meter (WTW, 102 pH340i). Both reactor was inoculated with nitrification sludge from a full-scale municipal 103 104 wastewater treatment plant on the campus of Tsinghua University (Tsinghua Water Reuse, Beijing) in both Phase I and Phase II. The seed sludge in the Phase III was from a laboratory 105 nitritation reactor. The ammonium, nitrite and nitrate concentrations in the effluent were 106 107 measured 1-4 times every week. The nitrite accumulation ratio in the effluent was calculated 108 as the percentage of nitrite nitrogen in the total nitrate and nitrite nitrogen, as described previously (Zheng et al., 2013a). Cycle studies were conducted by measuring the ammonium, 109 110 nitrite and nitrate concentrations throughout a cycle. In Phase I, microbial communities in the inoculated sludge and in the reactor sludge were analyzed using 454 pyrosequencing, as 111 described in 2.4. 112

113 2.3 Batch tests to determine sulfide control effectiveness

Batch tests were carried out in laboratory reactors to determine the effectiveness of the effluent from the above nitrifying reactors in controlling sulfide production by sewer sediments. Nine reactors were used in the study. Among them, one was used as control. Each

117 reactor had an effective volume of 1 L and was made from a Plexiglas cylinder with a diameter of 80 mm and a height of 200 mm. Sediments were collected from a mature gravity sanitary 118 119 sewer receiving domestic wastewater in Tsinghua University, Beijing, using a shovel. The bulk 120 density of the sewer sediment was 1.46 g/cm³. The total and volatile solids contents were 0.19 121 g/mL and 0.14 g/mL, respectively. The collected sediments were exposed for 12 h to different nitrite concentrations (50, 100, 150 and 200 mg N/L) at a pH of 6.5 (equal to FNA 122 concentrations of 0.036, 0.072, 0.108, and 1.144 mg HNO₂-N/L, respectively). Nitrite was 123 prepared from the treated urine in four groups and NaNO₂ in other four groups. Then, 100 mL 124 125 of the exposed sediments and 900 mL of deoxygenated water with organics and sulfate were added into the reactors with no headspace. A shaker with a rotating speed at 30 r/min was used 126 to keep a slight disturbance on the sediment. After 12 h, the produced sulfide concentration in 127 128 the reactors was measured, and used for calculating the sediment sulfide production rate. The control test was repeated six times to obtain the baseline sulfide production rate, as 1.02±0.07 129 mg S/($d \cdot m^2$). 130

131 2.4 Analytical methods

Measurements of COD, NH₄⁺–N, NO₂⁻–N, NO₃⁻–N and sulfide concentrations in the reactor liquid phase were performed in accordance with standard methods (Ministry of Environmental Protection, 2006). Free ammonia (FA) and FNA concentrations were calculated as described by Anthonisen *et al.* (1976), based on the measured ammonium and nitrite concentrations, respectively, along with the pH and temperature levels.

137 For DNA extraction and 454 pyrosequencing, the sludge samples were collected and

138 concentrated by centrifugation at 5000 rpm for 4 min. Then, the total DNA was extracted from each prepared sample using a Fast DNA Spin Kit for Soil (MP Biomedicals, LLC, Solon, OH, 139 140 USA). The OD260/OD280 and OD260/OD230 values were determined on a NanoDrop 2000 141 to evaluate the purity and quantity of each DNA sample. For each DNA sample, the V1-V3 region of the 16S ribosomal RNA gene was amplified using the primers 27F and 533R. The 142 143 amplified amplicons were detected by 454 pyrosequencing, and the sequenced data were processed using Quantitative Insights Into Microbial Ecology (QIIME) pipeline with default 144 settings. The operational taxonomic units (OTUs) with a 97% sequence density threshold were 145 146 clustered using UCLUST software. Sequences that were associated with one genus divided by total sequences were defined as the relative abundance of that genus in the sludge sample. 147

148 *2.5 LCA and economic analysis*

To determine the practical feasibility of the proposed approach for nitrite production from urine, the capital cost, operational cost, and environmental impacts such as greenhouse gas (GHG) emissions and energy inputs must be assessed and compared with the industrial production of the chemical. In this study, we used the experimental results and then employed LCA methodology to design and compare life cycle energy inputs, GHG emissions and cost between the following two sulfide control strategies: 1) a commercial operation of nitrite production from urine and 2) a conventional chemical dosing method.

Sulfide control in the sewer using nitrite produced from urine consists of three steps: urine
collection, on-site treatment and dosing. A hypothetical commercial building with a male
population of approximately 1000 was assumed to have a built-in urine diverting system for

159 urine collection from the toilet urinals in men's lavatories, which, compared with typical urinal units, only requires additional pipes. The on-site treatment units are assumed to be located in 160 161 the basement of the building and to include a urine collection tank, a treatment reactor, and a 162 storage tank for dosing. The collected fresh urine was thoroughly decomposed in the collection tank with simultaneous conversion of urea to ammonia in 2 days, driving the pH to increase 163 164 from approximately 6 to 9 (Mobley and Hausinger, 1989). Nitrite was then produced in the treatment reactor, and the nitrite-containing effluent was stored in the third unit and 165 166 intermittently dosed into the sewer for sulfide control to nearby branch pipes with a gravity system. Assuming that 15 liters of diluted urine was produced per male person per day, and 167 that people worked 5 days a week, a total of 7.14 m^3/day diluted urine was estimated to be 168 produced in the building during working hours. The volume of the collection tank, reactor tank 169 170 and storage tank was designed as 16 m³, 8 m³ and 8 m³, respectively. The life-time of the system was assumed to be 20 years. 171

The infrastructure inputs included pipes, steel, concrete, and excavation. The operation 172 inputs included electricity for mixing, aeration, and pumping. The infrastructure input data 173 were taken from Cashman et al. (2014); electricity consumption data for operation were 174 calculated based on data from Jiang et al. (2014); and life cycle inventory data for chemicals, 175 176 electricity and construction materials were from the GaBi database (GaBi Software, 2012). 177 GHG emissions were expressed as CO₂ equivalents (CO₂-e) using the 100-year global warming potentials (GWPs) of CO₂, CH₄ and N₂O from the IPCC's fifth report (Edenhofer et al., 2014). 178 179 The conversion ratio of nitrous oxide (N₂O) nitrogen to consumed ammonia nitrogen under

180	aeration conditions was assumed to be 1.7%, as evaluated in a full-scale nitritation reactor for
181	reject water treatment (Kampschreur et al., 2008). Energy inputs, GHGs and cost were reported
182	for 1 kg N of nitrite produced from urine (kg nitrite-N). The capital cost of the reactor was
183	estimated using a unit price per volume of approximately \$60/m ³ (Hao and Hao, 2009). The
184	price of electricity was set at \$0.12/kWh from the literature (Law et al., 2015). The dosing rate
185	requirement of NaNO ₂ was calculated to achieve an equivalent sulfide control effect as realized
186	with the FNA application approach. Acid dosage is likely required to lower the sewage pH in
187	order to achieve an effective FNA level. However, this was neglected in the analysis as the
188	amount of acid needed in the two cases would be the same or similar. The life cycle inventory
189	energy and GHG emissions were obtained from the GaBi database (GaBi Software, 2012). The
190	price of NaNO ₂ was assumed to be \$460/tonne (Alibaba website).

191 **3. Results**

192 *3.1 Reactor operation to achieve stable nitrite production from urine*

Experiments were performed in three phases to investigate the effects of influent characteristics and operational conditions on nitrite production from urine, as described in section 2.2. The reactor performance is shown in Figure 1-3, and summarized in Table 1.

196 3.1.1 Phase I: start-up and stable operation with influent ammonia concentration at ~550

197 *mg N/L*

Initially, the influent ammonia nitrogen concentration was 424.3 ± 81.7 mg N/L, and the average FA concentration reached up to 140 mg NH₃–N/L. The high FA concentration significantly inhibited the activities of both AOB and nitrite oxidizing bacteria (NOB)

201 (Anthonisen et al., 1976). However, the activity of AOB recovered after four days (Figure 1). The effluent nitrite nitrogen concentration increased sustainably and stabilized at above 250 202 203 mg/L on day 10. The effluent nitrate nitrogen concentration slightly increased to 46.1 mg/L in 204 the initial 6 days, before gradually decreasing to below 10 mg/L on day 10, and stayed at this low level. On day 10, the effluent pH became stable at approximately 5.7, and stayed at 5.6-205 206 5.8 for the remaining period of the study. The typical reactor operational cycle studies showed that the pH gradually decreased as ammonia was converted to nitrite because of the nitritation 207 process (data not shown). On day 15, the influent ammonia nitrogen concentration was 208 209 increased and maintained at 550 mg/L. Stable nitrite production with average nitrogen concentration at 280.3±16.6 mg/L was achieved during 100 days' operation (Figure 1). The 210 average nitrite accumulation ratio in the effluent was high as 99.9±0.3%. The average ammonia 211 212 removal efficiency was 48.4±2.0%, which indicates that nearly half of the ammonium in the 213 feed was converted to nitrite.

3.1.2 Phase II: Reactor operation with influent ammonia nitrogen concentration fluctuation
under low and high DO conditions

Two reactors were operated in parallel in Phase II. The reactor operated at a lower DO level (0.2–0.3 mg/L) performed similarly to the reactor at a higher DO level (1.0–2.0 mg/L) in the initial 40 days (Figure 2). The nitrite accumulation ratios in the effluent were both very high, and reached $89.4\pm6.2\%$ and $87.9\pm6.3\%$ in the low and high DO reactors, respectively, with a high influent ammonia concentration at 850 mg/L. When the influent ammonia concentration decreased to a lower level down to 375 mg/L, the effluent nitrite accumulation ratio

222 considerably decreased approximately 50% in the high DO reactor, while the ratio remained 223 basically unchanged in the low DO reactor. The results indicate that DO indeed played an 224 important role for the achievement of nitrite production with relatively low influent ammonia 225 concentration but did not have a significant impact on the reactor performance fed with 226 wastewater containing a high-level ammonia like urine collected in most cases. A lower DO is 227 also preferred due to lower energy consumption.

228 3.1.3 Phase III: Reactor operation with alkalinity supplement in the urine

229 Figure 3 shows the reactor performance with and without alkalinity supplement in the urine. The operational DO was set as 0.2-0.3 mg/L. Initially, the reactor was operated to stably 230 produce nitrite as described above. On day 31, additional NaHCO₃ was added to the influent 231 as an alkalinity supplement, which facilitated higher ammonia removal efficiencies of up to 232 233 94.8±13.5%. The average nitrite accumulation ratio was as high as 90.3±13.3%. The ammonia removal efficiency returned to approximately 50% on day 84, when alkalinity supply was 234 235 terminated. These results indicate that the technology is flexible and can produce nitrite at appropriate levels when properly controlled. 236

237 3.1.4 Community analysis

The microbial communities in the inoculated sludge (sample T0) and reactor sludge when stable nitrite production was achieved in Phase I (sample T1 take on Day 85, see Figure 1) are compared in Figure 4. The 454-pyrosequencing analysis generated 19352 DNA gene sequences from the two samples and separated them into 373 OTUs. The OTUs calculated from each sample using Good's coverage estimator showed that this test captured 99% of the species in

the samples. The relative abundances of the *Nitrosomonas* and *Nitrospira* genera in the inoculated sludge were 3.08% and 4.79%, respectively. The relative abundance of the *Nitrosomonas* genus reached up to 17.46%, while that of the *Nitrospira* genus was as low as 0.02% during the stable production of FNA. In the biomass, the AOB in the *Nitrosomonas* genus became predominant, whereas the NOB in the *Nitrospira* genus were selectively eliminated.

249 3.1.5 Organics consumption in the nitrifying reactor

The aeration process also resulted in a decrease in the organics concentration of the urine. The average COD concentration decreased from 464.6±56.9 mg/L in the influent to 94.5±28.4 mg/L in the effluent, but the loss of total organics in domestic wastewater could be ignored because most of those organics were from feces (Larsen and Gujer, 1996) and other sources.

254 *3.2 Dosing tests for sulfide control in the laboratory-scale sewer system*

Previous studies demonstrated that dosing of FNA prepared from hydrochloric acid and 255 sodium nitrite is effective for sulfide control in sewers (Jiang et al., 2011a; Jiang et al., 2013). 256 Sulfide production was reduced by more than 80% in 10 days following each dosing of FNA 257 at a concentration of 0.26 mg HNO₂–N/L and with a dosing duration of 8 h in real rising main 258 sewers in Australia (Jiang et al., 2013). Thus, dosing tests for sulfide control comparing the 259 260 FNA agent produced from urine with the chemical FNA agent were experimentally conducted 261 in the laboratory-scale sewer systems. The sediment sulfide production rate gradually 262 decreased with the nitrite/FNA concentration increase in the groups of reactors (Figure 5). After a 12 h exposure to FNA at 0.14 mg HNO₂–N/L, achieved through the addition of either 263

NaNO₂ or nitrite produced from urine (a similar amount of HCl was added in both cases to achieve a pH level of 6.5), the sediments sulfide production rate decreased by approximately 50%, and was down to 0.48-0.52 mg S/($d \cdot m^2$) in both cases. This means that nitrite produced from urine is as effective as a commodity nitrite source when applied to sulfide control in sewers.

269 *3.3 Life cycle GHG, energy and cost analysis*

According to the results reported above, nitrite produced from urine has the same sulfide 270 271 control effect as NaNO₂. For the testing case designed in 2.5, the annual nitrite production is estimated to be 781 kg N, equivalent to 3850 kg NaNO₂ as a commodity product for achieving 272 273 the same sulfide control effectiveness. The production cost, energy inputs and GHG emission results of our approach are compared to those of the commercial supply in Table 2. The 274 275 production of FNA from urine showed significant advantages compared with the commercial NO₂⁻ supply in all aspects. The life cycle energy and GHGs for the commercial production of 276 pure NaNO₂ are 3514 MJ/kg-N and 13.75 kg CO_{2e}/kg-N, respectively. In comparison, the 277 energy requirement and GHG emissions for urine conversion to nitrite are 44.74 MJ/kg-N 278 (98.7% reduction) and 9.76 kg CO_{2e}/kg-N (29% reduction), respectively. Additionally, the 279 production cost is also reduced by 69.1%. For the GHG emissions, N₂O emission during the 280 281 biological conversion of ammonia to nitrite represents the most significant source due to the 282 high global warming potential of N₂O. Uncertainty analysis indicated that the savings in GHG 283 emissions would be negative when the N₂O conversion factor is increased to above 2.7% in the 284 urine nitrification reactor. Therefore, efforts are needed to minimize N₂O emissions during

- urine treatment.
- 286 **4. Discussion**

287 4.1 The proposed integrated urban water management strategy

288 This study demonstrates that source-separated urine can be used for sulfide control in sewers. 289 With aerobic treatment, approximately 50% of the ammonia can be converted to nitrite, 290 generating FNA in sewage for sewer biofilm control when discharged to sewers particularly when acid is also dosed. This decentralized treatment also reduces the total nitrogen load to the 291 292 downstream centralized wastewater treatment plants. The experimentally obtained results also 293 showed that the sulfide control effectiveness of nitrite thus produced is essentially the same as NaNO₂ as a commodity product. According to Jiang et al. (2010) and Jiang et al. (2011a), FNA 294 is effective for sewer biofilm control when added to sewage at 100 mg N/L for 12 hours every 295 296 1-2 weeks. As an ammonium to nitrite conversion ratio of 50% in the urine treatment reactor, this means a total nitrogen addition of 200 mg N/L during each dosing period. This means that 297 298 roughly 15-30% of the urine should be collected in a catchment in order to produce an adequate 299 amount of nitrite for sewer biofilm control. The strategy is evaluated as being easy to implement and providing economic and energy savings. 300

Conventionally, ammonium in domestic wastewater is transported through sewers and then removed by nitrification and denitrification processes in downstream treatment plants. Strict effluent standards for total nitrogen incur not only high aeration costs but also loss of carbon sources for denitrification (Zheng *et al.*, 2013b; Zheng *et al.*, 2014). Resources can be recovered more effectively if wastewater streams are not mixed but treated separately.

306 Fundamentally, urine source separation has been reported as a promising innovation in 307 sustainable urban water management (Lienert and Larsen, 2010). In these publications, the 308 main purposes of separate urine treatment were hygienization, volume reduction, stabilization, 309 nutrient recovery, and micropollutant handling (Maurer and Larsen, 2006; Udert and Wächter, 2012; Zhang et al., 2014). However, developing low cost resource recovery technologies is 310 311 still critical. In this study, we converted ammonia to nitrite for sulfide control in sewers, a 'niche' application. As previously reported, after being treated by FNA at ppm levels, only 2-312 3% of microorganisms in an anaerobic sewer biofilm remained viable (Jiang et al., 2011b), a 313 314 result that is a good indication of the feasibility of using the FNA product from urine to control 315 the ubiquitous sulfide problem in sewers.

Commonly used methods for sulfide control in sewers involved dosing chemicals agents 316 317 such as oxygen, iron salts, nitrates, nitrites, sodium hydroxide, alkalis and microbial inhibitors have been reported (Ganigue et al., 2011; Liu et al., 2014b). Among them, FNA dosing had 318 the lowest cost and had been evaluated in field trials by Jiang et al. (2013). Using the urine-319 generated FNA agent should make this approach even more competitive. Differences in the 320 purity of the FNA agent from urine and industrial chemicals should be noted. Before 321 environmental and human health risk assessments, expanding the scope of using the urine-322 generated FNA beyond the sewers is unexpected. 323

In general, dosing urine-generated FNA in nearby upstream pipes should have an inherent advantage because these pipes have a high area-to-volume ratio and are important sources of sulfide leading to corrosion of main pipes (Sharma *et al.*, 2008; Guisasola *et al.*, 2009).

327 Previous studies showed that through the optimization of intermittent and simultaneous dosage in pressure pipe systems, FNA treatment at a concentration of 0.26 mg HNO₂–N/L with a 12 h 328 329 exposure was able to effectively suppress sulfide production (Jiang et al., 2011a; Jiang et al., 330 2013). However, in commonly used gravity pipe systems, maintaining the same exposure time 331 requires substantially more chemicals and thus has additional negative effects on downstream 332 wastewater treatment plants. Therefore, the dosing strategy significantly depends on the successive exposure time of FNA in pipes, and the effect of dilution on the dosage needs to be 333 considered further when using the urine-generated FNA. In addition, the wastewater volume 334 in the sewer system can exceed the capacity of the sewer system during periods of heavy 335 rainfall or snowmelt. In this case, the dosing of the urine-generated FNA should be terminated 336 to avoid nitrite emissions. 337

338 *4.2 Achieving nitrite production and its relationship with the pH limit for AOB metabolism.*

Through urine wastewater treatment, nitrite production was achieved in the reactor. Half of 339 the influent ammonium was converted to nitrite at a pH of approximately 5.7. The results are 340 similar to nitrite production from anaerobic digester liquor in a treatment plant (Law et al., 341 342 2015). Many experimental results presented before showed a strong dependency of the nitrite pathway on DO concentration (Ma et al., 2009; Wang et al., 2016), while this study 343 demonstrated that achieving the nitrite pathway was not significantly affected by DO. 344 345 Community analysis confirmed that stable maintenance of the nitration process was due to 346 AOB predominance and NOB elimination in the biomass, which has been identified in the literature as a key factor for achieving the nitrite pathway (Wang et al., 2014; Zheng et al., 347

348 2016). However, to achieve the nitrite pathway, this study further revealed that in addition to the above community response, obtaining a final pH as low as 5.7 is essential. Normally, pH 349 350 drops with proton production and with alkalinity consumption coupled with ammonia 351 oxidation. The rate of the ammonia oxidation declines as pH decreases and very often stops at a pH slightly below 6. Therefore, in the case of nitritation, cessation of ammonia oxidation is 352 353 very important to obtain a low final pH and to achieve the nitrite pathway. Conventional kinetic studies assumed that FNA inactivates NOB more significantly than it does on AOB (Wang et 354 355 al., 2014) and relatively high concentrations of FNA would completely inhibit the activity of 356 AOB (Park et al., 2010). These assumptions indicate that the oxidation of ammonia to nitrite, together with a decrease in pH by AOB, would increase FNA inhibition and result in a cessation 357 of ammonia oxidation. However, the pH could also drop from 9 to approximately 6 in the case 358 359 of nitrification without FNA inhibition using a moving bed biofilm reactor (MBBR) with biofilm carriers (Udert et al., 2003). Fumasoli et al. (2015) proposed a mathematical model to 360 simulate the pH limit of a known AOB, *Nitrosomonas eutropha*, and indicated that energy 361 limitation would be responsible for the cessation of ammonia oxidation. Thus, cessation of 362 ammonia oxidation might be mainly influenced by the FA substrate rather than by external 363 FNA inhibition. In addition, the lowest possible ammonium concentration for achieving the 364 FNA pathway requires further research. 365

366 5. Conclusions

367 This study proposed a promising approach to treating urine to produce nitrite for sulfide 368 control in sewers. The methods for achieving the production of nitrite and then using the urine-

369	generated nitrite (forming FNA along with acid dosage) for sulfide control we	ere experimentally
370	investigated. Economic and environmental analysis was also conducted. The	main conclusions
371	are as follows:	
272		

- Nitrite produced from urine is effective for sulfide control in sewers.
- Based on urine collection from a public building, the proposed strategy of dosing urine-
- 374 generated nitrite/FNA for sulfide control in sewers would significantly reduce the cost and
- 375 GHG emissions compared with conventional chemical agent approach.
- A nitrifying reactor can easily convert 50% of ammonium in urine to nitrite suitable for
- 377 sewer discharge for sulfide control.

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Experimente	Time (days)	DO	Influent	Effluent				Ammonia removal
		control	Ammonia (mg N/L)	Ammonia (mg N/L)	Nitrite (mg N/L)	Nitrate (mg N/L)	Nitrite accumulation ratio (%)	efficiency (%)
Ι	1-14		424.3±81.7	239.6±53.3	140.4±116.3	16.3±12.3	73.0±29.6	41.9±17.0
	15-98	-	546.5±8.8	282.0±9.6	280.3±16.6	0.2±0.7	99.9±0.3	48.4±2.0
Π	1-25	Low*	Low*	323.5±130.8	200.7±144.0	20.1±22.4	80.9±31.4	47.9±19.7
		High**	013.9±34.8	352.4±130.9	139.4±162.5	23.6±21.8	71.7±30.2	42.9±20.6
	25.27	Low	850.0±0.0	394.2±58.9	389.8±49.3	47.1±26.1	89.4±6.2	53.6±6.9
	23-37	High		440.8±44.8	442.2±46.1	61.9±35.4	87.9±6.3	52.8±5.3
	Lov 38-55 Hig	Low	.0W	185.4±29.4	251.5±26.1	35.9±9.8	87.6±2.5	67.2±5.2
		High	505.0±0.0	204.0±34.1	155.1±25.3	166.2±53.2	49.5±13.2	63.9±6.0
	56 70	Low	375.0±0.0	156.8±47.3	211.1±42.6	0.0±0.0	100.0±0.0	72.2±8.4
	- - / 0 Н	High		153.0±45.4	90.8±9.9	87.8±31.9	52.1±10.9	72.9±8.0
III	1-30		803.7±115.4	353.0±63.6	330.7±55.9	0.0±0.0	100.0±0.0	56.1±3.9
	31-83***	Low	457.6±6.4	23.9±62.7	358.2±40.3	21.1±14.8	90.3±13.3	94.8±13.5
	84-126		559.3±72.4	288.7±14.1	217.0±91.8	16.4±17.4	87.6±19.4	58.6±5.0

495 **Table 1**. Reactor performance for the production of nitrite.

496 *Reactor was performed with low DO of 0.2–0.3 mg/L.

497 **Reactor was performed with high DO in the range of 1.0–2.0 mg/L.

498 ***An alkalinity supplement of NaHCO₃ was added in the influent to increase ammonia removal efficiency.

<u>`</u>			
	System Assumptions	Urine nitrite	NaNO ₂
	Lifetime (Period over which capital costs are annualized)	20	
	Male population in the building	1,000	
	Operation time (days/year)	365	
Nitrite	Flow rate of the urine (m ³ /day)	7.14	
Draduction from	NH ₄ ⁺ -N concentration in urine (mg N/L)	600	
	NH ₄ ⁺ -N flow (kg N/year)	1564	~
Urine	Volume of the urine collection tank (m ³)	16	
	Volume of the FNA production reactor (m ³)	8	
	Volume of the FNA storage tank (m ³)	8	
	NO ₂ -N production (kg N/year)	781	781
	PVC pipe length (m)	500	
	PVC pipe mass (kg)	1,520	
	Steel (kg/m ³)	0.15	
Construction	Steal (kg)	4.80	
Materials	Concrete (m^3/m^3)	0.0009	
	Concrete (kg)	69	
	Excavation (m ³)	32	
	$\Omega_{2}/NH_{4}+N(\sigma/\sigma)$	3 43	
	$O_2/COD(\sigma/\sigma)$	1	
	Reactive NH. ⁺ -N ($k \sigma N/v$)	781	
	COD(kg COD/y)	781	
Operation Inputs	$O_{\text{requirement}}(kg/y)$	3460	
	O_2 requirement (kg/y) Electricity for NH \pm N ovidation (kWh/kg O_2)	0.66	
	Electricity for numping $(l_W h/(m^3 d))$	0.00	
	Electricity for pointing (k wil/(IP.d))	0.078	
	Lie 1 (MU(20)	0.12	
Life Cycle	Life cycle energy for construction (MJ/ 20 years)	110,894	
Energy	Tradeling and a series (MI/ he NO N)	587,970	2.514
Consumption	1 otal life cycle energy (MJ/ kg NO ₂ -N)	44./4	3, 514
	Life cycle energy saving	98.7	/%
	On-site N ₂ O emission (kg/year) CHCs from NO emission (kg/year)	20.88	
	GHGs from N ₂ O emission (kg $CO_2e/20$ years)	110695	
Life Cycle GWP	GHGs from construction (kg $CO_2e/20$ years)	5,074	
	GHGs from operation electricity (kg $CO_2e/20$ years)	36829	
	Total GHGs (kg CO ₂ e/ kg NO ₂ -N)	9.76	13.75
	GHG Emissions Saving	29.0)%
	Cost of tanks (\$/m ³)	60	
	Capital cost of three tanks and major equipment (\$)	2160	
	Interest applied for initial capital expenditure	8.50%	
	Annualized capital cost (\$/y)	228	
Life Cycle Cost	Power price (\$/kWh)	0.12	
	Cost of O_2 supply (y)	247	
	Cost of mixing (\$/y)	61	
	Total cost (\$/y)	536	
	Cost (\$/ kg NO ₂ -N)	0.69	2.22
	Cost saving	69. 1	1%

499 Table 2. LCA and cost analysis of the production of nitrite from urine and the commodity nitrite500 (functional unit in 1 kg nitrite-N produced).

501 Figure Captures

- 502 Figure 1. Phase I results. Reactor start-up (left) and stable (right) performance with the
- 503 treatment of urine from a male toilet urinal with influent ammonia concentration around 550
- 504 mg/L. \blacktriangle : Sludge sample collection for community analysis.
- 505 Figure 2. Phase II results. Reactor performance with fluctuation in the influent ammonia
- 506 nitrogen concentration under low DO of 0.2-0.3 mg/L (A) and high DO in the range of 1.0-2.0
- 507 mg/L (B) operational conditions.
- 508 Figure 3. Phase III results. Reactor performance with and without alkalinity supplement in the
- 509 influent (DO = 0.2-0.3 mg/L).
- 510 Figure 4. The relative abundance of the predominant groups at genus level according 454
- 511 pyrosequencing. T0: The inoculated nitrification sludge. T1: The sludge collected from the
- 512 reactor stably producing nitrite.
- 513 Figure 5. Measured sulfide production rates under different nitrite/FNA concentrations (pH =
- 514 6.5).



Figure 1. Phase I results. Reactor start-up (left) and stable (right) performance with the
treatment of urine from a male toilet urinal. The influent ammonia concentration was around
550 mg/L during stable operation. ▲: Sludge sample collection for community analysis.

519



521 Figure 2. Phase II results. Reactor performance with fluctuation in the influent ammonia

- 522 nitrogen concentration under low DO of 0.2-0.3 mg/L (A) and high DO in the range of 1.0-2.0
- 523 mg/L (B) conditions.
- 524



526 **Figure 3**. Phase III results. Reactor performance with and without alkalinity supplement in the

527 influent (DO = 0.2-0.3 mg/L).

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Figure 4. The relative abundance of the predominant groups at genus level according to 454

- 531 pyrosequencing. T0: The inoculated nitrification sludge. T1: The sludge collected from the
- 532 reactor stably producing nitrite.
- 533



534

535 Figure 5. Measured sulfide production rates after 12 h exposure to different nitrite/FNA

536 concentrations (pH = 6.5).

Highlights

- A green chemical produced from urine for sulfide control in sewer
- Urine can be partially (-50%) converted to nitrite
- The approach is cost-effective with a lower carbon footprint