Electrochemical in situ pH control enables chemical-free full urine nitrification with concomitant nitrate extraction

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Moving bed biofilm reactor; resource recovery; nitrogen recovery; regenerative life support system; MELiSSA

ABSTRACT

Urine is a valuable resource for nutrient recovery. Stabilization is, however, recommended to prevent urea hydrolysis and the associated risk for ammonia volatilization, uncontrolled precipitation and malodor. This can be achieved by alkalinization and subsequent biological conversion of urea and ammonia into nitrate (nitrification) and organics into CO_2. Yet, without pH control, the extent of nitrification is limited as a result of insufficient alkalinity. This study explored the feasibility of an integrated electrochemical cell to obtain on-demand hydroxide production through water reduction at the cathode, compensating for the acidification caused by nitritation, thereby enabling full nitrification. To deal with the inherent variability of the urine influent composition and bioprocess, the electrochemical cell was steered via a controller, modulating the current based on the pH in the bioreactor. This provided a reliable and innovative alternative to base addition, enabling full nitrification while avoiding the use of chemicals, the logistics associated with base storage and dosing, and the associated increase in salinity. Moreover, the electrochemical cell could be used as an in situ extraction and concentration technology, yielding an acidic concentrated nitrate-rich stream. The make-up of the end product could be tailored by tweaking the process configuration, offering versatility for applications on Earth and in Space.

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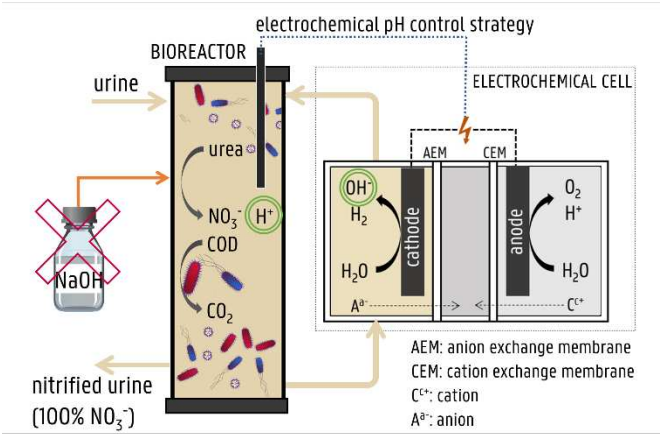


SYNOPSIS

This novel, resource-efficient method to transform fresh human urine into a stable and concentrated nutrient solution can contribute to a more sustainable circular nutrient management.

1. INTRODUCTION

Human urine is a widely available, relatively concentrated source of nitrogen and phosphorus, two essential nutrients in agriculture. Urine source separation at the toilet/urinal allows urine to be collected separately and used as a nutrient resource.[1] The use of urine as a fertilizer or as a raw material for fertilizer production is, however, impeded by the difficulty of collecting the urine and keeping it stable. Urea, the main nitrogen compound in urine, easily and rapidly hydrolyzes to ammonia, ammonium and bicarbonate due to microbial activity, thereby increasing the pH and releasing volatile ammonia, which can be harmful for humans (negative impact on respiratory tract[2]) and the environment (e.g., toxic, and causing eutrophication and acidification).[3-5] This leads to malodor and uncontrolled precipitation of calcium and magnesium salts and lowers the recovery potential due to nitrogen loss (by ammonia volatilization) and phosphorus loss (by precipitation).[4-6] The biofouling potential, caused by the presence of organics in urine which fuel microbial growth, further challenges certain urine treatment technologies (e.g., membrane processes).[7]

Urea hydrolysis can be inhibited by increasing the pH immediately after collection, as demonstrated by Randall, et al. (2016)[8] using $Ca(OH)_2$, Senecal, et al. (2017)[9] using a bed of wood ash and De Paepe, et al. (2020)[6] using a membrane electrolysis cell. The pH increase furthermore triggers precipitation of calcium and magnesium salts, thereby minimizing the risk for downstream scaling and capturing part of the phosphate in precipitates.[6] Yet, urine is only temporarily stabilized by increasing the pH and still contains urea (which can hydrolyze when the pH is lowered or urease is added[6]) and organics (which can cause biofouling). Treatment in a nitrification-based bioreactor has been reported as a suitable method to biologically stabilize urine[7, 10-13], yielding a stable nitrate-rich urine solution low in organics. Urine nitrification requires a well-tuned interplay between heterotrophic and autotrophic bacteria. First, urea is hydrolyzed to total ammonia nitrogen (TAN, i.e., sum of ammonia-N and ammonium-N) by

urease, an enzyme produced by urease positive (mainly heterotrophic) bacteria (a process commonly referred to as 'ureolysis'). Subsequently, TAN is oxidized by ammonium oxidizing bacteria (AOB) with oxygen into nitrite ('nitritation'), which is further oxidized with oxygen into nitrate by nitrite oxidizing bacteria (NOB) ('nitratation'). Concomitantly, heterotrophic bacteria convert the biologically degradable organics (COD, chemical oxygen demand) into CO_2.

Ureolysis: $CO(NH_2)_2 + 2\ H_2O \rightarrow NH_3 + NH_4^+ + HCO_3^-$ (1)

Nitritation: $NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O$ (2)

Nitratation: $NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$ (3)

COD oxidation: $COD + O_2 \rightarrow CO_2 + H_2O$ (4)

Due to the release of protons by nitritation (2 mol H^+ mol^{-1} N nitrified) and the limited alkalinity in urine, only about half of the TAN in urine can be converted into nitrate, while the remaining TAN is protonated as non-volatile ammonium due to the acidification, yielding a slightly acidic ammonium nitrate solution.[11, 14, 15] Full conversion of TAN into nitrate can be achieved by hydroxide addition, typically using a base (e.g., NaOH), and is usually preferred because of the higher process stability (optimal pH and no TAN accumulation) and safety (ammonium nitrate is thermally instable and can be misused as an explosive).[7, 10, 14] On the other hand, it requires supply, storage of and dealing with hazardous chemicals. Moreover, the increase in salinity resulting from the cation addition (e.g., sodium originating from the use of NaOH) can negatively affect the fertilizer potential of the nitrified urine because many plants are sensitive to high salinities (i.e., the ratio of Na^+ per N should be limited).[16, 17]

In order to obtain full nitrification while avoiding base addition and the undesirable associated increase in salinity, the bioreactor content is recirculated over the cathodic compartment of an electrochemical cell in this study. Water and/or oxygen reduction at the cathode produces

hydroxide ions, which can compensate for the acidification caused by nitritation. At the anode, water is oxidized, producing an acidic stream.

Water reduction: $H_2O + e^- \rightarrow 0.5\ H_2 + OH^-$ $E^0(SHE) = -0.8277$ V (6)

Oxygen reduction: $O_2 + 2\ H_2O + 4\ e^- \rightarrow 4\ OH^-$ $E^0(SHE) = 0.401$ V (7)

Water oxidation: $H_2O \rightarrow 0.5\ O_2 + 2\ H^+ + 2\ e^-$ $E^0(SHE) = -1.229$ V (8)

To deal with the inherent variability of the urine influent composition and bioprocess, on-demand and automated OH^- production is essential. This can be implemented by controlling the current flow through the electrochemical cell based on the pH in the bioreactor. In-situ electrochemical pH control has already been applied in hydroponic systems[18, 19], in a bioreactor for continuous culture of yeast cells[20], and in fermentation reactors[21], but has, to the best of our knowledge, not yet been used in combination with nitrification.

Nitrified urine can be used as a fertilizer in agriculture (e.g., Aurin, commercial fertilizer produced by VUNA)[15, 22] or as a culture medium for microalgae (e.g., cyanobacteria)[10, 12, 23]. In order to reduce the storage and transportation volumes, a concentration step is preferred for terrestrial applications due to the low nutrient concentrations in urine compared to synthetic fertilizers.[1] Interestingly, the electrochemical cell can be used as an in-situ extraction and concentration technology, as demonstrated by Andersen et al. (2015) for a fermentation reactor. Besides countering acidogenic fermentation, the electric field in the electrolysis cell drove carboxylate ions over the anion exchange membrane (AEM) into a cleaner, aqueous concentrated VFA (volatile fatty acids) stream in the latter study.[21] Similarly, in a nitrification reactor, nitrate migration through an AEM can yield a cleaner, aqueous and concentrated nitrate rich stream.

This study aimed to explore the feasibility of an integrated electrochemical cell to dose hydroxide on-demand in a urine nitrification reactor, and to concentrate/refine the produced

nitrate. Alkalinized urine (pH 12, to prevent urea hydrolysis in the influent during storage) was fed into a moving bed biofilm reactor (MBBR) which was coupled to an electrochemical cell. Three different configurations were tested at different pH set points and/or concentration factors. To compare the novel electrochemical pH control strategy with base addition and partial nitrification, an identical MBBR with NaOH addition and an MBBR without pH control were operated. All information regarding the operation and the results of these two MBBR are given in the supporting information (SI) Section C and D.

2. MATERIALS AND METHODS

2.1 Experimental setup

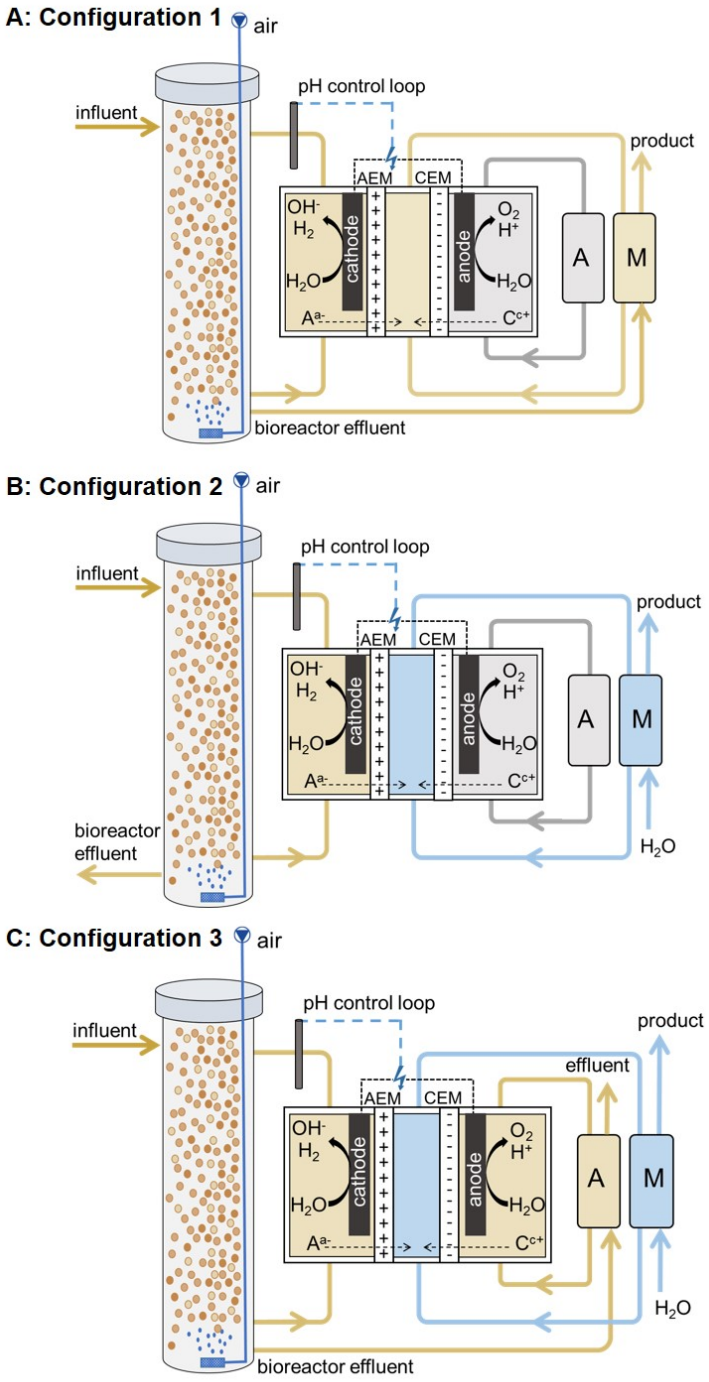
An electrochemical cell was installed in the recirculation loop of an MBBR. An MBBR with biofilm carriers was chosen in order to minimize the amount of suspended biomass which could clog the electrochemical cell. The MBBR consisted of a plastic cylinder with an active volume of 3.2 L, of which 24% was filled with polyvinyl alcohol beads (Kuraray Aqua Co, Ltd., Tokyo, Japan), confined in bags made from fine fishnet material and kept in suspension by the aeration and liquid recirculation (SI Figure S1-S2). The reactor was aerated with humidified air using an aquarium pump (~2-3 L d^{-1}, Air pump 400, Eheim, Germany) connected to diffuser stones installed at the bottom of the reactor. Influent (~500 mL d^{-1}) was dosed using a peristaltic pump and timer, and effluent (and suspended biomass) left the reactor via an overflow at the top of the reactor. The temperature was not controlled and ranged between 20-24°C.

The reactor liquid was recirculated from the top to the bottom of the reactor via an external recirculation loop using a peristaltic pump at a flow rate of 18.7 L h^{-1} (5.8 reactor volumes per hour) and passed through the cathodic compartment of the electrochemical cell. The cell was galvanostatically controlled at a current density of 0-20 A m^{-2} (membrane projected surface) depending on the pH using a programmable power supply (Z+ series, TDK lambda, Japan), a pH probe installed at the outlet of the cathodic compartment (Consort SP10B, Belgium) and a control system programmed in LabVIEW (National Instruments) (SI Section A2). The pH, current and voltage were recorded every 15 seconds.

The electrochemical cell consisted of three compartments, made from Perspex® plates and frames with an internal volume of 200 mL (20 x 5 x 2 cm^3, anodic and cathodic compartment) or 100 mL (20 x 5 x 1 cm^3, middle compartment). A stainless steel wire mesh (564 µm mesh width, 20 x 5 cm^2, Solana, Belgium) functioned as a cathode and a dimensionally stable titanium

145 electrode coated with iridium MMO (Magneto Special Anodes, The Netherlands) was used as
146 anode. The cathodic and middle compartment were separated by a monovalent AEM (100 cm^2,
147 PC MVA PCA GmbH, Germany), whereas a cation exchange membrane (CEM, 100 cm^2,
148 Ultrex CMI-7000s, Membranes International Inc., NJ, USA) was installed between the middle
149 compartment and the anodic compartment. Peristaltic pumps were used to recirculate the
150 anolyte and middle compartment solution between the recirculation vessels and electrochemical
151 cell.
152 In configuration 1 (Figure 1A), the aim was to obtain full nitrification and to recover all nitrate.
153 Therefore, the effluent of the bioreactor was pumped from the effluent vessel into the
154 recirculation vessel of the middle compartment using a peristaltic pump (~500 mL d^{-1}) to
155 capture all nitrate that migrated through the AEM. Effluent left the middle compartment
156 recirculation vessel via overpressure. The anodic compartment (initially filled with 0.2 M
157 KH_2PO_4 to minimize the Ohmic resistance) was operated in a closed loop (i.e., without influent
158 or effluent).

159 In configuration 2 (Figure 1B), the goal was to extract and concentrate nitrate in the middle
160 compartment. Demineralised water (~100-225 mL d^{-1}) was fed into the middle compartment
161 recirculation vessel using a peristaltic pump and effluent left the recirculation vessel by
162 overpressure. The anodic compartment (initially filled with 0.1 M Na_2SO_4 to minimize the
163 Ohmic resistance) was again operated in a closed loop.
164 In configuration 3 (Figure 1C), the effluent from the bioreactor was pumped from the effluent
165 vessel into the recirculation vessel of the anodic compartment using a peristaltic pump (~500
166 mL d^{-1}) to enable, besides nitrate recovery, potassium recovery from the urine. The middle
167 compartment was fed with demineralized water (~90 or 180 mL d^{-1}). Effluent left the
168 recirculation vessels via overpressure.
169 **FIGURE 1**



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171 Figure 1. **Schematic overview of the three configurations.** A: Configuration 1: bioreactor
172 effluent is fed into the middle compartment. B: Configuration 2: demineralized water is fed into
173 the middle compartment. C: Configuration 3: bioreactor effluent is fed into the anodic
174 compartment and demineralized water is fed into the middle compartment. A: anodic
175 compartment recirculation vessel, M: middle compartment recirculation vessel.

176 **2.2. Urine collection and alkalinization**

177 Fresh male urine was collected using a nonwater urinal with approval from the Ethical
178 committee of Ghent University hospital (registration number B670201731862). Immediately
179 after collection, the urine was diluted with water (33.3% urine-66.6% water) simulating the
180 diluting effect of flush water in urine diverting toilets[24] and the pH was increased to 12 using a
181 chemical-free electrochemical method developed by De Paepe et al. (2020)[6] (SI Section A4) in
182 order to prevent urea hydrolysis during storage. Batches of 2-4 L of urine were alkalinized with
183 an electrochemical cell, pooled together into large batches of alkalinized urine (10-25 L) to
184 average out fluctuations in urine composition in order to provide a stable influent (with a
185 constant composition) to the nitrification bioreactor. The influent batches were stored for up to
186 three months at room temperature. The average influent composition is displayed in SI Table
187 S1. The TAN/TN ratio remained below 5% in the influent of the bioreactor (SI Figure S5),
188 confirming that urea hydrolysis was inhibited by the high pH. Only one batch (used in
189 configuration 2, between day 132 and 136) got contaminated, resulting in a lower influent pH
190 (~9.2) and COD concentration and an increased TAN concentration in the influent (SI Figure
191 S5). This batch was replaced after 5 days. The electrochemical alkalinization pre-treatment also
192 reduced the scaling potential by precipitation of divalent cations with phosphate and sulfate.
193 Additionaly, the salinity of the urine decreased through the migration of anions (mainly
194 chloride) to the middle compartment. Combined, these two processes resulted in a removal of
195 88 ± 7 % of calcium, 91 ± 6 % of magnesium, 78 ± 16 % of phosphate, 64 ± 14 % of chloride
196 and 58 ± 18 % of sulfate (SI Section A4). The TAN concentration after electrochemical pre-
197 treatment was about 18% lower than before treatment, indicating some ammonia volatilization
198 or ammonia diffusion to the middle compartment. The N loss was however limited as less than
199 5% of the total influent nitrogen was present as TAN.

200

2.3 Reactor operation

Reactor inoculation and the start-up of the MBBR are described in SI, Section A3. After a start-up of 70 days, the target loading rate was reached (corresponds to 'day 1'). During the first 13 days, the MBBR was operated with regular pH control (i.e., NaOH addition) at a pH set point of 7.5 (Table 1). On day 13, the electrochemical cell was installed in the recirculation loop of the bioreactor and the reactor was operated in configuration 1. Two different pH set points were compared in terms of nitrification efficiency, hydroxide demand and electrode energy consumption. The setup was operated with a pH set point of 7.5 for 43 days and with a pH set point of 6.5 for 27 days. Subsequently, the setup was adapted with the goal to extract and concentrate nitrate in the middle compartment. Configuration 2 was tested with a pH set point of 7.5 at two different influent flow rates to the middle compartment: ~225 mL d^{-1} (corresponding to a Q_{in} bioreactor/Q_{in} middle compartment of ~2) for 35 days and ~95 mL d^{-1} (concentration factor ~5) for 23 days to compare the extraction efficiency, concentration factor and electrode energy consumption. Afterwards, the influent flow rate to the middle compartment was again increased to ~225 mL d^{-1} (concentration factor ~2) for 26 days before changing to configuration 3. Configuration 3 was also tested with two different influent flow rates to the middle compartment: ~180 mL d^{-1} (between day 168 and day 182) and ~90 mL d^{-1} (between day 182 and day 195).

TABLE 1

Table 1. Overview of the different operational phases. Q_{in}=influent flow rate, Q_{out}=effluent flow rate. Average influent and effluent compositions are reported in SI Section B1.

223

		NaOH	Configuration 1		Configuration 2			Configuration 3	
			pH 7.5	pH 6.5	concentration factor 2	concentration factor 5	concentration factor 2	concentration factor 2	concentration factor 5
Configuration		NaOH control	1	1	2	2	2	3	3
pH set point		7.5	7.5	6.5	7.5	7.5	7.5	7.5	7.5
Concentration factor		1	1	1	2	5	2	2	5
Day		1 - 13	13 - 56	56 - 84	84 - 118	118 - 141	141 - 168	168 - 182	182 - 195
HRT in reactor	[d]	6.8 ± 0.2	7.1 ± 0.3	7.1 ± 0.2	6.9 ± 0.3	6.5 ± 0.3	6.8 ± 0.7	7.8 ± 0.7	7.3 ± 0.4
Number of reactor HRT		1.9	6.2	4.1	5.1	3.7	2.6	1.8	1.9
HRT in middle compartment					2.0 ± 0.1	2.6 ± 0.1	2.0 ± 0.3	1.6 ± 0.1	2.5 ± 0.3
Number of HRT in middle compartment					17.2	9.2	17.5	9	5.6
HRT in anodic compartment								1.3 ± 0.1	1.2 ± 0.1
Number of HRT in anodic compartment								11.2	11.8
Q_{in} bioreactor	[L d^{-1}]	488 ± 21	488 ± 22	495 ± 9	501 ± 22	531 ± 24	493 ± 21	447 ± 741	472 ± 24
Q_{out} bioreactor	[L d^{-1}]	502 ± 4 [b]			489 ± 16	511 ± 20	507 ± 4		
Q_{in} middle compartment	[L d^{-1}]				223 ± 16	94 ± 4		180 ± 11	90 ± 5
Q_{out} middle compartment	[L d^{-1}]		484 ± 21	483 ± 11	223 ± 9	119 ± 6		200 ± 10	125 ± 16
Q_{out} anodic compartment	[L d^{-1}]							401 ± 43	423 ± 9
N load	[mg N d^{-1}]	791 ± 42	783 ± 87	716 ± 35	773 ± 23	802 ± 28	796 ± 42	748 ± 74	760 ± 47
N loading rate [a]	[mg N L^{-1} d^{-1}]	247 ± 13	277 ± 25	208 ± 10	224 ± 6.5	233 ± 8	252 ± 13	217 ± 21	220 ± 14
COD load	[mg COD d^{-1}]	697 ± 30	676 ± 44	650 ± 36	679 ± 27	538 ± 188	702 ± 30	810 ± 100	753 ± 42
COD loading rate [a]	[mg COD L^{-1} d^{-1}]	218 ± 9	196 ± 13	188 ± 10	197 ± 8	156 ± 54	223 ± 9	235 ± 29	218 ± 12

[a] volume of 3.2 L in NaOH control or 3.45 L (volume of MBBR and cathodic compartment) in all other phases

[b] Effluent flow rate is higher than influent flow rate due to NaOH addition

2.4 Sampling and analytical methods

Samples (10 mL) were taken every 2-3 days, filtered over a 0.22 μm Chromafil® Xtra filter (Macherey-Nagel, PA, USA) and stored in a fridge (4°C) prior to analysis. The samples were brought to room temperature before analysing. The bulk liquid dissolved oxygen (DO) concentration and pH were measured during sampling using a luminescent DO probe (LDO10103, Hach, Belgium) connected to a HQ40d meter (Hach, Belgium) and a portable pH meter (C5010, Consort, Belgium). Ion chromatography was used to determine the concentration of anions (Metrohm 930 equipped with a Metrosep A supp 5-150/4.0 column and conductivity detector, eluent: 1.0 mM $NaHCO_3$, 3.2 mM Na_2CO_3) and cations (Metrohm 761 equipped with a Metrosep C6-250/4.0 column and conductivity detector, eluent: 1.7 mM HNO_3, 1.7 mM dipicolinic acid). The total nitrogen (TN) and COD concentrations were measured with Nanocolor tube test kits (Nanocolor® TN220 and Nanocolor® COD160/1500, Macherey-Nagel, PA, USA). The electrical conductivity (EC) was measured using a conductivity meter (Consort C6010 with a Metrohm 6.0912.110 conductivity electrode).

3. RESULTS AND DISCUSSION

3.1. Electrochemical hydroxide production enables full urine nitrification by dynamically compensating for the associated acidification

This study aimed to stabilize urine by alkalinization and biological conversion of urea and organics into nitrate, CO_2 and biomass. The urine was first alkalinized with an electrochemical cell immediately after collection (Section 2.2) to prevent urea hydrolysis during storage, which could reduce the nutrient content and create odor nuisance and scaling. The alkalinized urine was then fed into a nitrification MBBR. To enable full nitrification, an electrochemical cell producing OH^- through water reduction at the cathode was coupled to the MBBR. The current applied by the power supply was controlled based on the pH of the reactor. At a low pH, a relatively high current was applied to increase the OH^- production and, hence, the pH, whereas a high pH in the bioreactor resulted in a lower applied current. After optimizing the settings (i.e., pH set points and corresponding current, SI Section A2) during the first two weeks (day 13-26), the pH could be controlled in a narrow range (7.6 ± 0.1) between day 26 and 56 (Figure 2A), resulting in full nitrification in the MBBR. The TAN and nitrite concentration in the bioreactor remained below 2 mg N L^{-1} (Figure 3A). The nitrate concentration in the effluent equalled the total nitrogen concentration in the effluent, indicating that all urea was hydrolyzed and nitrified, but was lower than the total nitrogen concentration in the influent, due to nitrate migration over the AEM (Section 3.2). On average 0.055 ± 0.023 A was applied, supplying 101 mmol OH^- L^{-1} to the reactor (assuming a coulombic efficiency of 100%). Together with the hydroxide added during the electrochemical alkalinization pre-treatment (~29 mmol OH^- L^{-1}), a total of 130 mmol OH^- L^{-1} was added which was slightly higher than the theoretical hydroxide demand for full nitrification (SI Table S6).

To investigate whether a lower pH set point would result in a lower current and thus reduce the energy consumption, the MBBR was controlled at a pH of 6.5 ± 0.1 between day 56 and 84. All urea and TAN were converted into nitrate (Figure 3A), but the average current (0.056 ± 0.015) was indifferent from the current at a pH set point of 7.5 (Figure 2B). Titration curves of the middle compartment effluent (SI Figure S8) revealed that only a small increment of 3-5 mmol OH^- L^{-1} was required to increase the pH from 6.5 to 7.5, which is low compared to the total of ~130 mmol OH^- L^{-1} added. This explains the similar current at both pH set points. Hence, lowering the pH set point did not have a significant impact on the energy consumption, but it might affect the nitrification rate. This was observed in the control MBBR with NaOH addition, where the nitrification rate decreased with 35% by changing the set point from 7.5 to 6.7, causing TAN accumulation (since the loading rate was higher than the nitrification rate) (SI Section D3 and D4).

To obtain a concentrated nitrate rich stream in the middle compartment, the configuration was adapted as further outlined in Section 3.3 and 3.4. Apart from some peaks (e.g., on day 97, 106, 152) caused by malfunctioning of the software control system, the pH was stably controlled at a pH of 7.4, resulting in full nitrification in configuration 2 and 3 (effluent TAN and NO_2^--N < 10 mg N L^{-1}, except on day 141 after a shift to a more concentrated influent batch) (Figure 3A). The OH^- demand for full nitrification was similar to configuration 1 (SI Table S6), since the nitrogen loading rate was stable over time (Table 1). The higher proton concentration in the middle compartment (due to the concentration obtained by the lower influent flow rate to the middle compartment), probably resulted in more proton diffusion over the AEM from the middle compartment to the cathodic compartment in configuration 2 and 3. To compensate for this acidification, more OH^- had to be produced by the electrochemical cell. As a result, a higher current was required to keep the pH at 7.5 compared to configuration 1 (Figure 2B). A higher concentration factor (5 instead of 2) in the middle compartment required a higher OH^-

286 production and current (235 mmol OH^- L^{-1} ~0.139 A in configuration 2, concentration factor 5
287 compared to 111 mmol OH^- L^{-1} ~0.062 A in configuration 2, concentration factor 2). Similar
288 findings were obtained in configuration 3, aiming at both anion and cation extraction and
289 concentration.

290 Throughout the course of 182 days, the voltage fluctuated between 2.8 and 3.5 V, depending
291 on the current (i.e., a higher current resulted in a higher voltage) (SI Figure S7). The voltage
292 did not display an increasing trend over time, indicating that no or very limited membrane
293 scaling and fouling occurred. The average electrode energy consumption ranged between 7.8
294 kWh m^{-3} or 4.3 kWh kg^{-1} NO_3^--N recovered (without concentration in the middle compartment,
295 configuration 1) and 20.9 kWh m^{-3} or 20.5 kWh kg^{-1} NO_3^--N recovered (with a concentration
296 factor of 5, configuration 2) (Table S5).

297 **FIGURE 2 - 3**

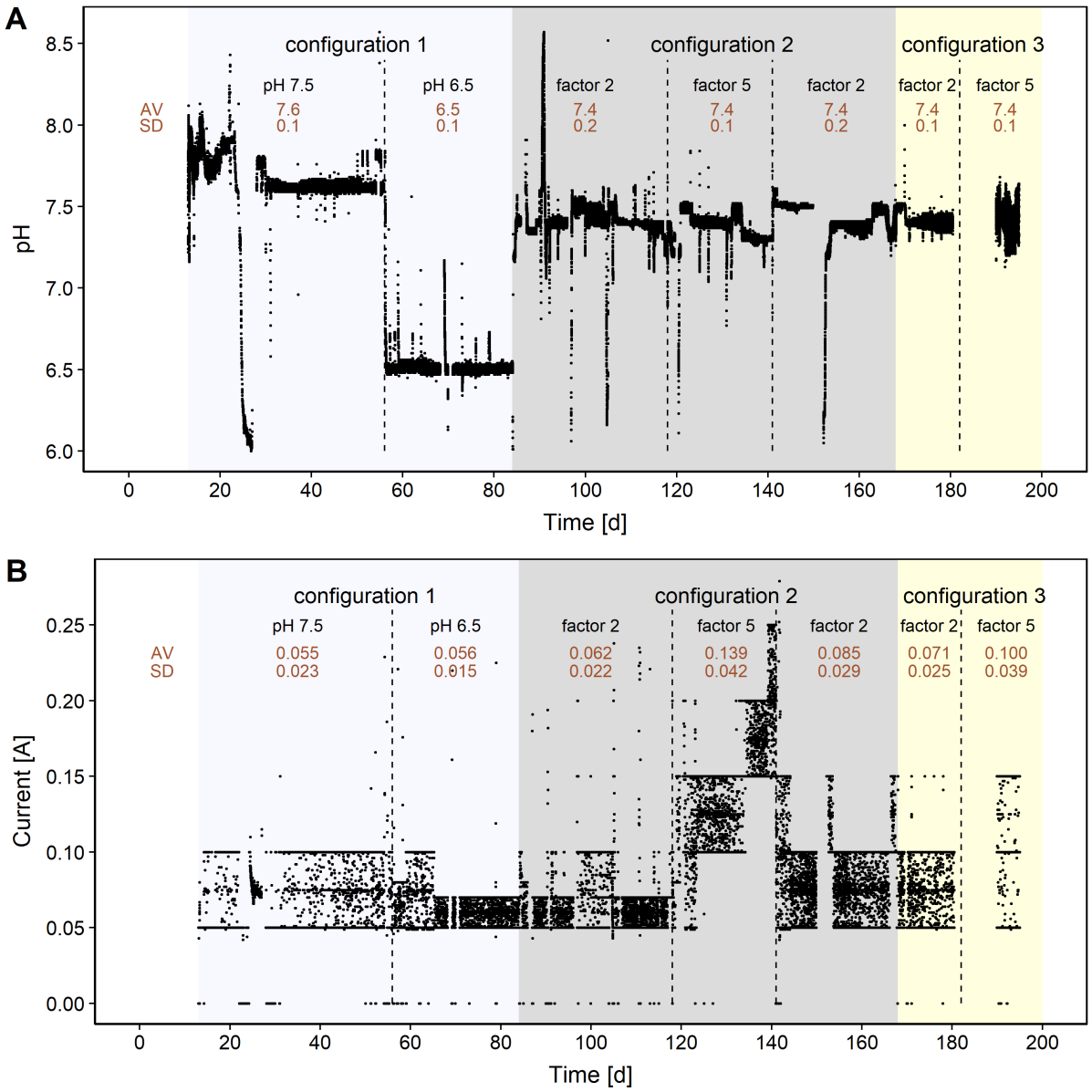
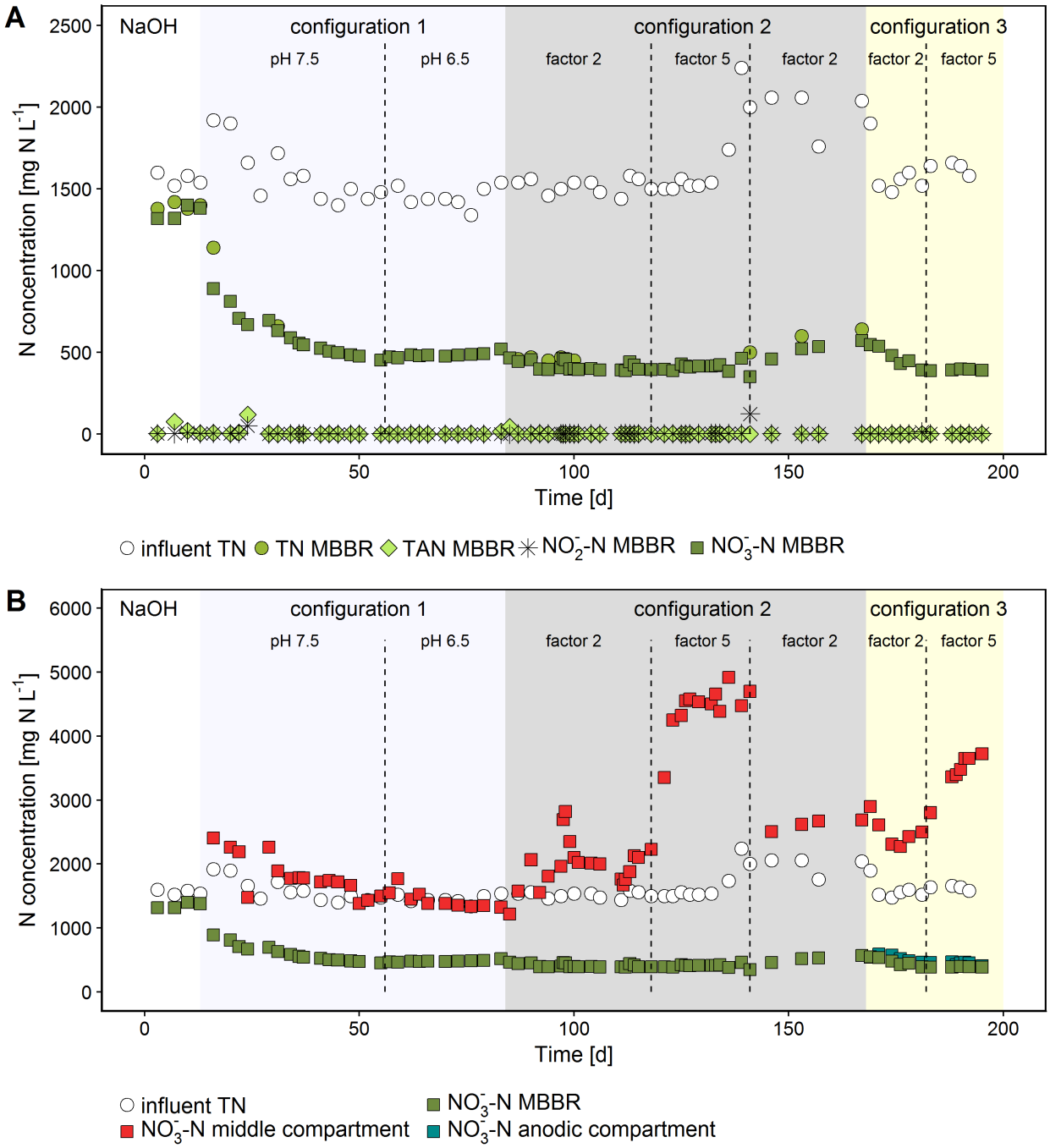


Figure 2. A) pH profile, B) current applied by the electrochemical cell. Averages (AV) and standard deviations (SD) for each operational phase are displayed at the top.



302

303 **Figure 3. A) total nitrogen (TN) concentration in the influent and TN, TAN, nitrite and**
304 **nitrate concentration in the effluent, B) TN concentration in the influent and nitrate**
305 **concentration in the effluent of the bioreactor, middle compartment and anodic**
306 **compartment.** The average composition of the influent and effluent of the bioreactor, middle
307 compartment and cathodic compartment are given in SI Tables S1-S4.

3.2. Configuration 1 enables full nitrate recovery

Due to the electron flow driven by the power supply, anions migrated from the cathodic compartment through the AEM to the middle compartment to restore the charge balance. Nitrate was the predominant anion in the nitrified urine and accounted for an estimated 68-78% of the migration through the AEM in configuration 1 (SI Section B3). Chloride accounted for ~9% of the migration, whereas the contribution of sulfate and phosphate was negligible (<1.5%). The remaining 12-22% can likely be attributed to bicarbonate (was not measured) or hydroxide migration, or to some leakage proton diffusion from the middle compartment (pH<2) to the cathodic compartment. Due to migration, the nitrogen and chloride concentration in the bioreactor were respectively 67-70% and 54-55% lower than the influent concentration, while the concentration of sodium, potassium, phosphate and sulfate did not decrease (Figure 4A, Figure 3A). In order to recover all nitrogen, the effluent from the bioreactor was sent through the middle compartment in configuration 1. The nitrogen and chloride concentration in the effluent of the middle compartment corresponded to the concentration in the influent (Figure 3B, SI Tables S1 and S3). Hence, all nitrate and chloride that migrated through the membrane could be captured again in the urine. Evidently, this does not enable creating a concentrate of nitrate.

Likewise, the electric field resulted in cation migration from the anodic compartment to the middle compartment (Figure 4A). The anolyte initially consisted of 0.2 M KH_2PO_4, resulting in proton and potassium migration through the CEM. However, after a few days, potassium was depleted in the anolyte, yielding only proton migration. Due to proton migration, the urine was acidified in the middle compartment (effluent pH<1.5). Assuming that all protons migrated from the anode to the middle compartment, about 100 mmol H^+ L^{-1} was supplied to the middle compartment (based on the current). This was confirmed by means of titrations of the middle

compartment effluent, where about 90-115 mmol OH^- L^{-1} was required to increase the pH to 6.5-7.5 (SI Figure S8).

FIGURE 4

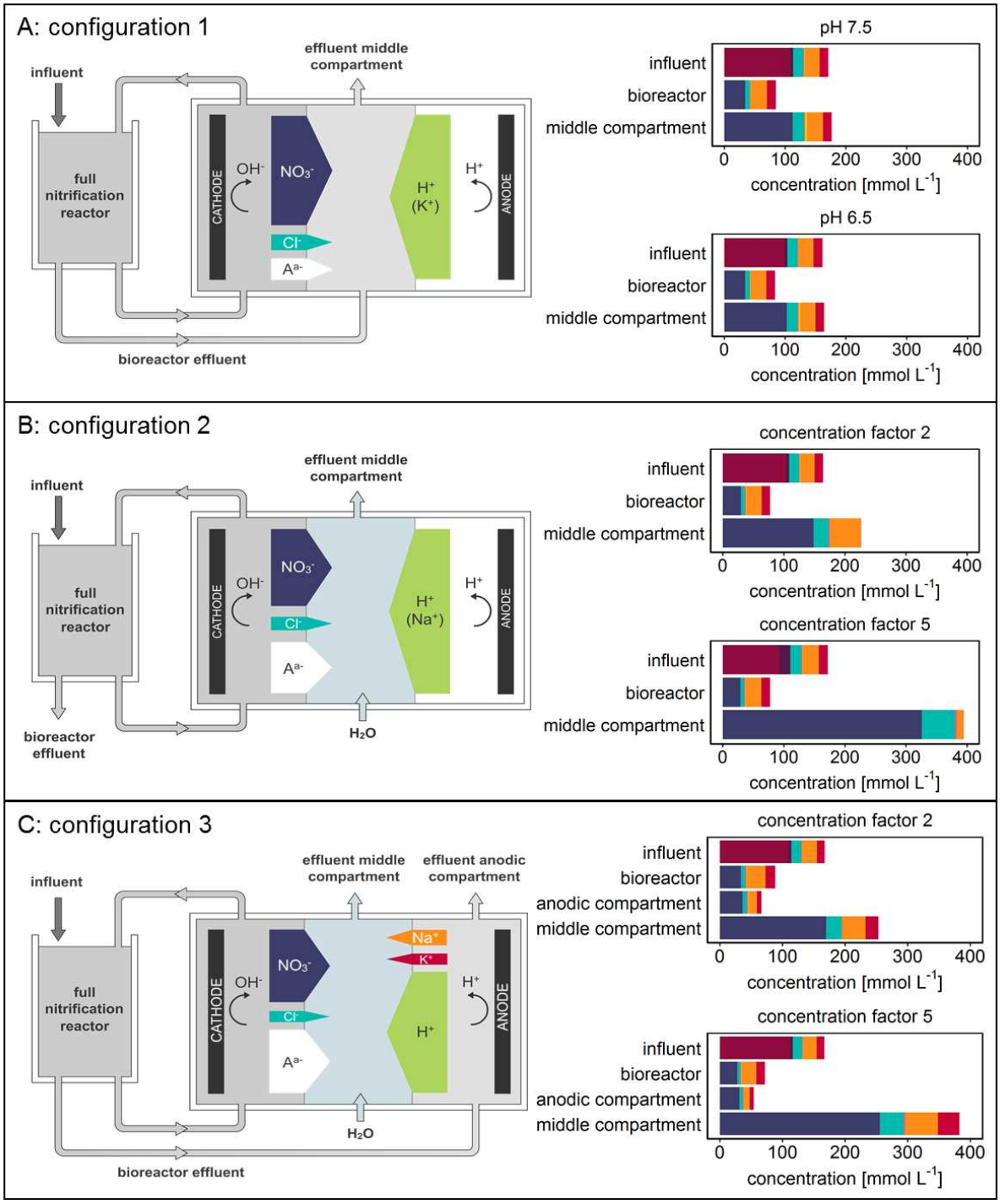


Figure 4. Ion migration through the AEM and CEM and composition of the influent, bioreactor liquid, middle compartment and anodic compartment in configuration 1-3. The size of the arrows represent the relative contribution of each ion to the total migration (estimated based on the electric charge) (calculation in SI Section B3). A mass balance is presented in SI, Figure S6.

3.3. Configuration 2 enables anion extraction and concentration in the middle compartment

In configuration 2, nitrate was extracted from the urine and concentrated in the middle compartment, which was fed not with nitrified urine but with demineralized water, yielding a purified acidic nitrate-rich side stream (effluent of the middle compartment) and a nitrate-depleted urine stream (effluent of the bioreactor). Similar to configuration 1, nitrate and protons were the predominant migrating ions through the AEM and CEM, respectively (Figure 4B). Besides protons, sodium migrated from the anolyte (initially 0.2 M Na_2SO_4) through the CEM at an initially high rate. After all sodium was depleted, the sodium concentration in the middle compartment rapidly decreased, as apparent from the difference between the two tested concentration factors (since the anolyte was not replaced when shifting from a concentration factor of 2 to 5). Cation migration from the urine to the middle compartment was mostly prevented by the AEM, and also sulfate and phosphate migration was negligible due to their low concentration. Hence, the effluent from the middle compartment mainly consisted of nitrate (2.1 ± 0.3 g NO_3^--N L^{-1} at factor 2 and 4.6 ± 0.2 g NO_3^--N L^{-1} at factor 5), chloride (0.9 ± 0.2 g Cl^- L^{-1} at factor 2 and 1.9 ± 0.2 g Cl^- L^{-1} at factor 5), and sodium (originating from the anolyte) in an aqueous matrix with a pH below 1.2 (Figure 4B, SI Table S3). This is in contrast to configuration 1, where only one effluent stream, derived from the middle compartment, with a composition and matrix similar to the influent was obtained (only urea was converted into nitrate and COD was removed). While most pathogens and micropollutants are retained by the ion exchange membrane[25-27], these urine originating contaminants can be re-introduced in the effluent of configuration 1 by redirecting the bioreactor content to the middle compartment. This as opposed to configuration 2, where a contaminant-free aqueous end product is obtained, although in depth analysis would be necessary to fully confirm this.

About 30% of the nitrate remained in the urine and was not recovered in the middle compartment. As anion migration restores the charge balance by compensating for the production of negatively charged OH^- ions, the extent of nitrate migration is limited by the OH^- demand, which in turn depends on the nitrogen load. Hence, the nitrate recovery could be increased by increasing the OH^- demand in the bioreactor. The latter could be achieved by eliminating the electrochemical alkalinization pre-treatment (which provided 11-22% of the OH^-, SI Table S6). However, given the discontinuous nature of urine supplies, the high variability in composition and the fact that nitrifiers are very susceptible to peak loadings, storage in an equalization tank is important in order to provide a constant influent flow and loading to the bioreactor. Eliminating or minimizing the OH^- addition in the electrochemical pre-treatment would result in a pH<12, thereby increasing the risk for urea hydrolysis during storage. In De Paepe et al. (2020)[6], increasing the pH to 11 was insufficient to prevent urea hydrolysis for longer than one week. Moreover, no or less chloride would be removed by the electrochemical pre-treatment, increasing the competition between nitrate and chloride for migration in the electrochemical cell of the bioreactor. Alternatively, the OH^- demand could be increased by redirecting a part of the anolyte to the bioreactor. This would result in an acidification of the bioreactor, and, a concurrent increase of the OH^- demand, at the expense of a higher energy consumption. A third option to improve the nitrate recovery, is the use of an AEM with a high nitrate to chloride selectivity in order to favor nitrate migration. The chloride/nitrogen ratio in the middle compartment corresponded to the ratio of the influent, indicating that the monovalent AEM used in this study had the same selectivity for nitrate and chloride. Membranes with a nitrate to chloride selectivity of 2 [28] to 4.68 [29] have been developed. A fourth option to improve the nitrate recovery is the use of a cell with multiple AEM and CEM cell pairs, which enables to uncouple the nitrate migration from the OH^- demand.

In addition to extraction, concentration was achieved in the middle compartment by minimizing the influent (water) flow, thereby capturing the nutrients in a smaller volume. In a first phase, with an influent flow rate of 223 mL H_2O d^{-1} to the middle compartment (Q_{in} bioreactor/Q_{in} middle compartment~2), the nitrate concentration was about 1.3 times higher compared to the total nitrogen concentration in the influent of the MBBR. By further decreasing the influent water flow rate to 94 ± 4 mL d^{-1} (Q_{in} bioreactor/Q_{in} middle compartment~5), this value increased to 3. As for nitrate, protons were more concentrated at factor 5 (pH of 0.7 ± 0.1 compared to 1.1 ± 0.1 at factor 2) (SI Figure S9). The discrepancy between the theoretical concentration factor (~2 and ~5) and the actual nitrate concentration factor (1.3 and 3) is due to incomplete nitrate recovery (i.e., 30% remained in urine). Osmotic and electro-osmotic water transport became more substantial with an increasing concentration factor because of the increased concentration gradient and ion migration. In case of factor 5, with an influent water flow rate of 94 mL d^{-1}, the effluent flow rate of the middle compartment amounted to 120 mL d^{-1}, corresponding to an influent ratio (Q_{in} bioreactor/Q_{in} middle compartment) of 5.7 and an effluent ratio (Q_{in} bioreactor/Q_{out} middle compartment) of only 4.5. This presents limits to the maximum achievable concentration in the middle compartment. Furthermore, the energy consumption increases with the concentration factor. Three times more energy was required for a factor 5 concentration compared to configuration 1 without concentration (SI Table S5).

TABLE 2

Table 2. Overview of concentration factors and nitrogen recovery in configuration 2 and 3.

Concentration factor	Configuration 2		Configuration 3	
	2	5	2	5
$\frac{Q_{in}\ \text{bioreactor}}{Q_{in}\ \text{middle compartment}}$	2.2	5.7	2.5	5.3
$\frac{Q_{in}\ \text{bioreactor}}{Q_{out}\ \text{middle compartment}}$	2.2	4.5	2.2	3.8
$\frac{[NO_3^- - N]\ \text{middle compartment}}{[TN]\ \text{influent}}$	1.3	3.0	1.5	2.3
Nitrogen recovery				
$1 - \frac{[NO_3^- - N]\ \text{bioreactor}}{[TN]\ \text{influent}}$	73	74	70-73	76

3.4. Configuration 3 enables anion and cation extraction and concentration in the middle compartment

In configuration 2, all cations were retained in the urine. In order to recover part of the potassium (which is an important plant nutrient), the effluent of the bioreactor was sent through the anodic compartment in configuration 3. As a result, besides protons produced at the anode, also sodium and potassium from the urine migrated through the CEM to the middle compartment, accounting for an estimated 8-11% and 4-6% of the migration, respectively (Figure 4C, SI Section B3). This resulted in a recovery of on average 40% of the potassium and 44% of the sodium from urine.

Anion migration through the AEM was identical to configuration 2, meaning that ~30% of the nitrate and ~40% of the chloride remained in the urine. As a consequence, by diverting the effluent of the bioreactor to the anodic compartment, chloride entered the anodic compartment, where it could be oxidized at the anode to chlorine, which could diffuse to the atmosphere (chlorine gas is toxic) or further react to HOCl, a known disinfectant, or undesired disinfection

by-products such as organochlorides and chlorate (ClO_3^-) [30]. If HOCl were to diffuse to the bioreactor, it could inactivate or kill the microbial community, hampering nitrification and COD oxidation. The latter was not observed, indicating that configuration 3 is a promising alternative to configuration 2, offering the advantage of both anion and cation recovery. As HOCl decreases the lifespan of most membranes, a HOCl resistant CEM might be required for long-term operation.

No major differences were observed with respect to the concentration effects. In line with configuration 2, a 5 times concentration factor showed a higher concentration of nitrate, a lower pH in the middle compartment, more osmotic and electro-osmotic water transport, and a higher energy consumption compared to a concentration factor of 2.

3.5 COD removal eliminates the risk for downstream biofouling

Besides nitrification, COD was converted into CO_2 and biomass by heterotrophic bacteria in the bioreactor. In all reactors, throughout all operational phases, 82-95% of the COD was removed, which is in line with typical COD removal percentages by open communities reported in literature (SI Tables S1, S2, S10, S11 and S13).[7, 10, 11, 14, 31-33] The COD removal percentage was not affected by the pH set point in configuration 1 or by the concentration factor in configuration 2 and 3. Since all rapidly biodegradable COD was removed, the risk for downstream biofouling was likely eliminated.

3.6. Electrochemical pH control offers competitive benefits compared to partial nitrification and full nitrification with base addition

To compare the novel electrochemical pH control strategy with more common approaches such as base addition or partial nitrification, an identical MBBR with NaOH addition and an MBBR without pH control were operated (SI Section C and D).

Due to a lack of alkalinity, partial nitrification was obtained in the MBBR without pH control. In a first phase, with an influent pH of 11.9 ± 0.2, about 40% of the nitrogen in the effluent consisted of TAN, whereas ~60% was nitrified to nitrate (SI Section C3). This NO_3^--N/TAN ratio (60:40) is higher than reported in literature (typically ~50:50[11, 32, 34]), because of the OH^- addition for alkalinization (SI Table S12). In an attempt to increase the NO_3^--N/TAN ratio, influent with a higher alkalinity was fed to the reactor. Although the NO_3^--N/TAN could be increased via the influent alkalinity (SI Section C3), it proved difficult to regulate since the OH^- demand depends on the nitrogen concentration in urine, which highly fluctuates.

Similar to the reactor with electrochemical pH control, full nitrification was obtained in the MBBR with NaOH addition (SI Section D). The salinity of the reactor with NaOH addition was however markedly higher compared to the salinity of the middle compartment effluent in configuration 1 (SI Section E). This is on the one hand due to the upstream electrochemical alkalinization (to pH 12), which reduced the concentration of chloride (and other anions) in the influent of the MBBR with electrochemical OH^- addition with more than 60% compared to the NaOH-alkalinized influent used to feed the MBBR with NaOH addition (Section 2.2). On the other hand, sodium addition (originating from the use of NaOH for alkalinization and full nitrification) increased the sodium concentration fivefold compared to the original concentration in urine (before alkalinization) in the MBBR with NaOH addition, whereas no sodium was dosed to the MBBR with electrochemical OH^- addition. An additional advantage of the electrochemical pH control is that the nitrifiers in the bioreactor are exposed to an even lower salinity because of anion migration to the middle compartment (which decreased the chloride and nitrate concentration in the bioreactor with 55-70%). This is particularly relevant when working with salt-sensitive synthetic communities in high strength urine.[13]

A preliminary cost comparison (SI Table S16) showed that the energy cost for electrochemical pH control (€3.8-9.0 m^{-3} urine) is in the same range as the cost of NaOH addition (€3.2 m^{-3} urine).

3.7 Application of electrochemical in-situ pH control and extraction

In this study, we showed that coupling of a urine nitrification bioreactor with an electrochemical system can provide a convenient and innovative alternative to base addition, enabling full nitrification while avoiding the use of chemicals (bases), the logistics associated with base storage and dosing, and the associated increase in salinity. Furthermore, the electrochemical cell can flexibly be integrated with the nitrification reactor, and each resulting configuration has its own benefits and application potential. When full nitrogen recovery is sought and further nitrogen concentration/refinery is not important, configuration 1 proved to be the better option. All urine compounds, including macronutrients and trace elements (except organics and the phosphorus, calcium and magnesium precipitated in the alkalinization step) are then recovered in the nitrified urine. The urine precipitates could be redissolved in the acidic nitrified urine. In contrast, configurations 2 and 3 each yielded two streams, i.e., a purified concentrated acidic nitrate-rich product stream and a nitrate-depleted treated urine effluent (SI Figure S16). These configurations result in a lower nitrate recovery but are particularly valuable when interested in a refined and concentrated end product, which facilitates storage and transport. Only a limited number of compounds is recovered in the concentrated stream, which can be an advantage (if only nitrate recovery is targeted) or a disadvantage (since other macronutrients and trace elements are lost). Important in this respect is that configuration 3 allows to recover more compounds, including potassium, an important fertilizer constituent. The concentration factors fall within the same range as those obtained by electrodialysis[7, 35, 36], while the electrode energy consumption is higher (25-60 kWh electrical energy or 75-200 kWh primary energy m^{-3} urine compared to only 4.4 kWh electrical energy m^{-3} urine with electrodialysis[7]) (SI Table S5).

Additionally, both methods consume energy for pumping. Distillation reaches higher concentration factors but at the expense of a higher energy investment (~700 kWh primary energy m^{-3} urine).[14]

Besides hydroxide ions or protons, hydrogen gas (25-62 mmol H_2 d^{-1}) and oxygen gas (12-31 mmol O_2 d^{-1}) were produced by water reduction and water oxidation at the cathode and anode, respectively. Redirecting the oxygen gas to the bioreactor could cover 10-25% of the theoretical oxygen demand for nitrification and COD oxidation (assuming an oxygen demand of 4.33 g O_2 g^{-1} N nitrified and 0.8 g O_2 g^{-1} COD removed). Alternatively, recycling of the cathodically generated hydrogen gas to the anode could shift the anode reaction from water oxidation to hydrogen gas oxidation, thereby decreasing the anode potential and thus the energy consumption by the electrochemical cell, as demonstrated by Kuntke et al. in a TAN recovery electrochemical system.[37, 38] This implies that a gas stream (containing hydrogen gas) is recirculated over the anodic compartment (containing a gas diffusion electrode) instead of a liquid stream (anolyte solution). This is not compatible with configuration 3, since the bioreactor effluent is redirected over the anodic compartment. In configuration 1 and 2 on the contrary, it can be implemented, but requires additional stripping of the hydrogen gas from the bioreactor liquid at the exit of the cathodic compartment.

Water oxidation: $H_2O \rightarrow 0.5\ O_2 + 2\ H^+ + 2\ e^-$ $E^0(SHE) = -1.229$ V (9)

Hydrogen gas oxidation: $0.5\ H_2 + OH^- \rightarrow H_2O + e^-$ $E^0(SHE) = 0.8277$ V (10)

The technology is mainly suited for small decentralized urine treatment systems. The electrochemical cell connected to the bioreactor (3.5 L) had a size of 100 cm^2 and was operated at a current density of 0-20 A m^{-2}, which is low (current density can be increased up to ~100 A m^{-2} or higher). Hence, for a bioreactor of 35 L, the same electrochemical cell could have been used. For larger reactors, the size of the electrochemical cell can be increased or multiple units

520 can be operated in parallel. The main limitation is however the fact that the bioreactor liquid
521 has to be recirculated over the cathode. Recirculation is rather uncommon in large scale
522 bioreactors in practice.

523 Urine treatment and recycling are not only relevant on Earth, but are of major importance in
524 regenerative life support systems (RLSS) as urine is the main resource of water and nutrients.
525 Even when recovery is not envisaged, urine stabilization is essential since ammonia, originating
526 from urea hydrolysis, can pose a hazard to the crew upon volatilization. Currently, on board of
527 the International Space Station, sulfuric/phosphoric acid and toxic chromium trioxide are added
528 to urine in order to inhibit urea hydrolysis.[39] Subsequently, water is recovered from the urine
529 using vapor compression distillation and filtration, while the nutrients are concentrated in a
530 toxic brine.[40] Alternatively, urine could be stabilized by the two-step approach presented in
531 this study. Immediately after collection, the pH should be increased to 12 in order to prevent
532 urea hydrolysis during storage, which is essential to provide a constant flow and composition
533 to the bioreactor, where all urea is nitrified to nitrate. The nitrified urine can be valorized as
534 substrate for plants and microalgae. Nitrification combined with the production of microalgae
535 and plants is being explored in the framework of the Micro-Ecological Life Support System
536 Alternative (MELiSSA), the RLSS programme from the European Space Agency.[13, 41] To take
537 this one step further, this study addressed the issue of payload limitations to Space by
538 implementation of electrochemical cells enabling in-situ production of acids and bases,
539 obviating the need for transportation and storage of these hazardous consumables.
540 Configuration 1 is most appropriate for Space application as maximum recovery is pivotal while
541 concentration does not present an added value in Space. Furthermore, the low salinity and
542 acidicity (~1 mol H^+ mol^{-1} NO_3^--N) of the end product of configuration 1 are compatible with
543 hydroponic plant production, as 0-1 mol H^+ mol^{-1} NO_3^--N is required to compensate for the
544 release of OH^- ions (0-1 mol OH^- mol^{-1} NO_3^--N) that accompanies nitrate uptake by most

plants.[18, 42, 43] The low pH is furthermore well suited for dissolving the urine precipitates formed in the alkalinization step, which would increase the phosphate content and the pH of the end-product. For other applications (e.g., microalgae cultivation), further neutralization might be required. This could be achieved by adapting the configuration (e.g., by sending another waste stream through the anodic compartment) or by using another electrochemical cell to avoid the use of chemicals.

ASSOCIATED CONTENT

Supporting Information. The Supporting Information is available free of charge at: ...

Experimental setup and operation; additional tables and figures of the full nitrification reactor with electrochemical hydroxide addition (composition of influent, bioreactor effluent, middle compartment effluent and anodic compartment effluent, electrochemical hydroxide production and energy consumption, electromigration), material and methods and results of the partial nitrification reactor without pH control and of the full nitrification reactor with NaOH addition; Comparison of the three pH control strategies (salinity and cost).

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Notes

The authors declare no competing financial interest

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