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

Jolien De Paepe [a,b,c], Peter Clauwaert [a,c], Maria Celeste Gritti [a], Ramon Ganigué [a,c], Benedikt Sas [d], Siegfried E. Vlaeminck [c,e,#] and Korneel Rabaey [a,c,#*]

[a] Center for Microbial Ecology and Technology (CMET), Department of Biotechnology, Faculty of Bioscience Engineering – Ghent University, Coupure Links 653, 9000 Gent, Belgium

[b] Departament d'Enginyeria Química, Biològica I Ambiental, Escola d'Enginyeria – Universitat Autònoma de Barcelona, Bellaterra 08193 Barcelona, Spain

[c] Center for Advanced Process Technology and Urban Resource Efficiency (CAPTURE, www.capture-resources.be)

[d] Department of Food Quality and Food Safety, Faculty of Bioscience Engineering – Ghent University, Coupure links 653, 9000 Gent, Belgium

[e] Research Group of Sustainable Energy, Air and Water Technology, Department of Bioscience Engineering – University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium

# equally contributed as senior authors

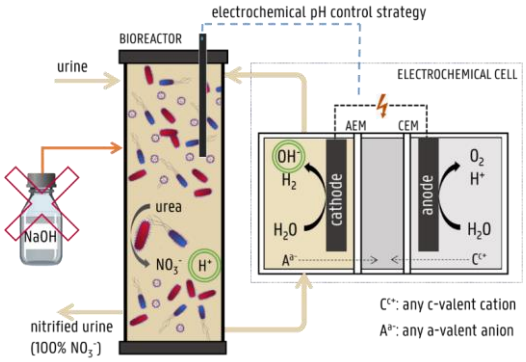
KEYWORDS

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

ABSTRACT

Urine presents a valuable nutrient source to reduce the need for unsustainable synthetic fertilizers. Stabilization is, however, required to prevent ammonia volatilization, uncontrolled precipitation and malodor. This was achieved by alkalinization and subsequent biological conversion of urea and total ammonia nitrogen (TAN) into nitrate (nitrification) and organics into CO_2. Yet, without pH control, the extent of nitrification was limited to 60% as a result of insufficient alkalinity. Simply increasing the alkalinity of the influent with NaOH increased the nitrate/TAN ratio in the effluent, but proved difficult to control due to the N variability in urine. Therefore, this study explored the feasibility of an integrated dynamically-controlled electrochemical cell to obtain on-demand hydroxide production through water reduction at the cathode, compensating for the acidification caused by nitritation. 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 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.

35 **TABLE OF CONTENTS (TOC)/ABSTRACT ART**



36

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 due to microbial activity, thereby 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, 4] This leads to nitrogen and phosphorus loss (consequently lowering the recovery potential), malodor and uncontrolled precipitation of calcium and magnesium salts.[4, 5] The biofouling potential, caused by the presence of organics in urine which fuel microbial growth, further challenges urine treatment and reuse.

Urea hydrolysis can be inhibited by increasing the pH immediately after collection, as demonstrated by Randall, et al. (2016)[6], Senecal, et al. (2017)[7] and De Paepe, et al. (2020)[5]. 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.[5] 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[5]) and organics. Treatment in a nitrification-based bioreactor has been reported as a suitable method to biologically stabilize urine[8-12], 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 excreted 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 into CO_2.

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.[10, 13, 14] 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).[8, 9, 13] 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).[15, 16]

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 nitrification. 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 (1)

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

Water oxidation: $H_2O \rightarrow 0.5\ O_2 + 2\ H^+ + 2\ e^-$ $E^0(SHE) = 1.229$ V (3)

To deal with the inherent variability of the urine influent 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[17, 18], in a bioreactor for continuous culture of yeast cells[19], and in fermentation reactors[20], 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[21] (e.g., Aurin, commercial fertilizer produced by VUNA)[14, 21] or as a culture medium for microalgae (e.g., cyanobacteria)[8, 11, 22], but the nutrient concentrations are relatively low compared to synthetic fertilizers. Hence, for terrestrial applications, a concentration step is preferred in order to reduce the storage and transportation volumes.[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 into a clean, concentrated VFA (volatile fatty acids) stream in the latter study.[20] Similarly, in a nitrification reactor, nitrate migration through an anion exchange membrane can yield a clean 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. Results were compared with an MBBR with a conventional pH control strategy (i.e., NaOH addition). A third MBBR was operated without pH control, resulting in partial nitrification. This set-up was used to study the effect of an increased influent alkalinity on the effluent TAN/NO_3^--N ratio.

2. MATERIALS AND METHODS

2.1 Experimental setup

Three identical MBBRs (SI, Figure S1) were used for (i) partial nitrification without pH control (PN), (ii) full nitrification with NaOH addition (FN-NaOH), or (iii) full nitrification with electrochemical hydroxide addition (FN-EC). An MBBR with biofilm carriers was chosen in order to minimize the amount of suspended biomass which could clog the cathodic compartment in the FN-EC setup. Each MBBR consisted of a plastic cylinder with an active volume of 3.2 L, of which 24% was filled with polyvinyl alcohol (PVA) 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. 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). The reactors were aerated with humidified air using an aquarium pump (~2L d^{-1}, Air pump 400, Eheim, Germany) connected to diffuser stones that were 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 partial nitrification reactor without pH control (PN) consisted of the basic MBBR design.

In the full nitrification reactor with NaOH addition (FN-NaOH), a pH probe (Consort, Belgium) was installed in the reactor which was connected to a pH controller (VP-PRO-PH/RX, Verder, Belgium) to control the pH at 6.7 or 7.5 by adding 1 or 1.5 M NaOH at the top of the MBBR. This reactor only served as a 'control' to compare the OH^- consumption and reactor salinity with the full nitrification reactor with electrochemical hydroxide addition. Therefore, all information regarding the operation and the results of this MBBR are given in SI Section C.

An electrochemical cell was installed in the recirculation loop of the MBBR used for full nitrification with electrochemical hydroxide addition (FN-EC). The bioreactor liquid was recirculated over the cathodic compartment of the electrochemical cell and the pH was measured at the outlet. 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), pH probe (Consort SP10B, Belgium) and 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 electrode coated with iridium oxide (Magneto Special Anodes, The Netherlands) was used as anode. The cathodic and middle compartment were separated by a monovalent anion exchange membrane (AEM, 100 cm^2, PC MVA PCA GmbH, Germany), whereas a cation exchange membrane (CEM, 100 cm^2, Ultrex CMI-7000s, Membranes International Inc., NJ, USA) was installed between the middle compartment and the anodic compartment. Peristaltic pumps were used to recirculate the anolyte and middle compartment solution between the recirculation vessels and electrochemical cell.

In configuration 1 (Figure 1A), the effluent of the bioreactor was pumped from the effluent vessel into the recirculation vessel of the middle compartment using a peristaltic pump (~500 mL d^{-1}). Effluent left the middle compartment recirculation vessel via overpressure. The anodic compartment (initially filled with 0.2 M KH_2PO_4) was operated in a closed loop (i.e., without influent or effluent).

In configuration 2 (Figure 1B), demineralised water (~100-225 mL d^{-1}) was fed into the middle compartment recirculation vessel using a peristaltic pump and effluent left the recirculation vessel by overpressure. The anodic compartment (initially filled with 0.1 M Na_2SO_4) was again operated in a closed loop.

In configuration 3 (Figure 1C), the effluent of the bioreactor was pumped from the effluent vessel into the recirculation vessel of the anodic compartment using a peristaltic pump (~500 mL d^{-1}). The middle compartment was fed with demineralized water (~90 or 180 mL d^{-1}). Effluent left the recirculation vessels via overpressure.

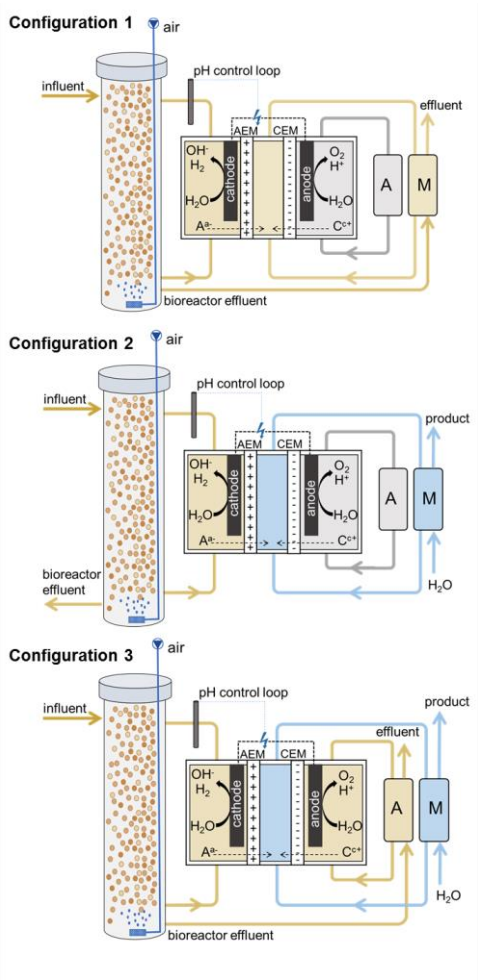


Figure 1. **Schematic overview of the three configurations of the full nitrification reactor with electrochemical hydroxide addition (FN-EC).** Configuration 1: bioreactor effluent is fed into the middle compartment. Configuration 2: demineralized water is fed into the middle compartment. Configuration 3: bioreactor effluent is fed into the anodic compartment and demineralized water is fed into the middle compartment. A: anodic compartment recirculation vessel, M: middle compartment recirculation vessel.

2.2. Urine collection and alkalinization

Fresh male urine was collected using a nonwater urinal with approval from the Ethical committee of Ghent University hospital (registration number B670201731862). Immediately after collection, the urine was diluted with water (33.3% urine-66.6% water) simulating the diluting effect of flush water in urine diverting toilets [23] and the urine was stabilized by increasing the pH to 12 in order to prevent urea hydrolysis during storage. The influent of FN-NaOH was stabilized using 2M NaOH whereas the influent of FN-EC and PN was stabilized with an electrochemical cell according to De Paepe et al. (2020)[5] (SI Section A3). The influent was stored in large vessels (10-25 L) at room temperature prior to feeding into the MBBRs. The average influent composition is displayed in SI Tables S1 (PN), S4 (FN-NaOH) and S6 (FN-EC).

2.3 Reactor operation

2.3.1. Reactor inoculation and start-up

PVA beads, previously used for urine nitrification[9], were reactivated in aerated vessels receiving spikes of a buffered ammonium sulfate solution after storage for more than one year at 4°C. Subsequently, the beads were transferred into the MBBRs and an additional 250 mL of nitrifying inoculum (ABIL, Avecom, Belgium) was added (0.19 g VSS L^{-1} with an activity of 0.4 g TAN g^{-1} VSS d^{-1}, as indicated by the provider). After inoculation, the MBBRs were operated for 7 days in fed-batch mode (to allow for additional biofilm formation on the beads) and for 33 days in continuous mode on a buffered synthetic ammonium sulfate solution with a stepwise increasing N concentration (50-360 mg N L^{-1}). After 40 days, alkalinized urine (1.75 g N L^{-1}) was dosed to the MBBRs and the loading was gradually increased over a period of 30 days by increasing the influent flow rate from 0.2 L d^{-1} to 0.5 L d^{-1}.

2.3.2 Operation of the partial nitrification reactor without pH control (PN)

After a start-up of 70 days (Section 2.3.1), the target loading rate was reached (corresponds to 'day 1') and MBBR PN was operated for 52 days on electrochemically alkalinized urine (at a pH of 11.9 ± 0.2, containing ~29 mmol OH^- L^{-1}) (PN-I) (Table 1). In order to investigate if the NO_3^--N/TAN ratio in the effluent could be increased by feeding more alkaline influent, the MBBR was fed with NaOH-alkalinized urine containing 66 mmol NaOH L^{-1} between day 73 and 126 (PN-III). Prior to PN-III, between day 52 and 73, the bioreactor was conditioned with NaOH-alkalinized influent (22 mmol NaOH L^{-1} to obtain the same influent pH of 11.8 ± 0.3 as in PN-I) (PN-II).

205 **Table 1. Overview of the different operational phases of the partial nitrification reactor without pH control (PN).** Average influent and

206 effluent compositions are reported in SI Section B1. HRT: hydraulic residence time.

		PN-I	PN-II	PN-III	
				PN-IIIa	PN-IIIb
Influent alkalinization method		electrochemical OH^- addition	NaOH addition	NaOH addition	NaOH addition
Hydroxide dosage		~29 mmol OH^- L^{-1}	22 mmol OH^- L^{-1}	66 mmol OH^- L^{-1} (batch 1+2)	66 mmol OH^- L^{-1} (batch 3) [a]
Day		1 - 52	52 - 73	73 - 115	115 - 126
HRT	[d]	7.0 ± 0.3	7.0 ± 0.1	7.0 ± 0.2	6.9 ± 0.3
Duration	[number of HRT]	6.4	3.1	6.1	1.6
Influent flow rate Q_{in}	[L d^{-1}]	460 ± 17	458 ± 4	457 ± 15	479 ± 8
N load	[mg N d^{-1}]	807 ± 59	884 ± 54	790 ± 48	331 ± 43
N loading rate [b]	[mg N L^{-1} d^{-1}]	251 ± 18	274 ± 17	245 ± 15	103 ± 13
COD load	[mg COD d^{-1}]	677 ± 29	1057 ± 28	912 ± 71	426 ± 7
COD loading rate [b]	[mg COD L^{-1} d^{-1}]	210 ± 9	328 ± 9	283 ± 22	132 ± 2

[a] Batch 3 is represented separately since this batch was less concentrated which resulted in a substantially lower N and COD load

[b] Reactor volume = 3.2 L

207

2.3.3 Operation of the full nitrification reactor with electrochemical hydroxide addition (FN-EC)

After reaching the target loading rate, the MBBR with electrochemical hydroxide addition was first operated on electrochemically alkalinized urine with regular pH control (i.e., NaOH addition) at a pH set point of 7.5 (Table 2). On day 13, the electrochemical cell was installed in the recirculation loop of the bioreactor and the reactor was operated in configuration 1 with a pH set point of 7.5 for 43 days and with a pH set point of 6.7 for 27 days. Subsequently, configuration 2 was tested with a pH set point of 7.5 at two different concentration factors: factor 2 (~Q_{in} bioreactor/Q_{in} middle compartment, i.e., influent flow rate to the middle compartment of ~225 mL d^{-1}) for 35 days and factor 5 (~95 mL d^{-1}) for 23 days. Afterwards, the concentration factor was again decreased to 2 for 26 days before changing to configuration 3. Configuration 3 was also tested with a concentration factor of 2 (between day 168 and day 182) and 5 (between day 182 and day 195).

Table 2. Overview of the different operational phases of the full nitrification reactor with electrochemical pH control (FN-EC). Q_{in}=influent flow rate, Q_{out}=effluent flow rate. Average influent and effluent compositions are reported in SI Section D1.

		NaOH	Configuration 1		Configuration 2			Configuration 3	
			pH 7.5	pH 6.5	Factor 2	Factor 5	Factor 2	Factor 2	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
Duration [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]	484 ± 21	483 ± 11	489 ± 16	511 ± 20	507 ± 4	401 ± 43	423 ± 9
Q_{in} middle compartment	[L d^{-1}]				223 ± 16	94 ± 4		180 ± 11	90 ± 5
Q_{out} middle compartment	[L d^{-1}]				223 ± 9	119 ± 6		200 ± 10	125 ± 16
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

223 2.3.4 Sampling

224 Samples were taken every 2-3 days, filtered over a 0.22 µm Chromafil® Xtra filter (Macherey-
225 Nagel, PA, USA) and stored in a fridge (4°C) prior to analysis. The bulk liquid dissolved
226 oxygen (DO) concentration and pH were measured during sampling using a luminescent DO
227 probe (LDO10103, Hach, Belgium) connected to a HQ40d meter (Hach, Belgium) and a
228 portable pH meter (C5010, Consort, Belgium).

229 **2.4 Analytical methods**

230 Ion chromatography was used to determine the concentration of anions (Metrohm 930 equipped
231 with a Metrosep A supp 5-150/4.0 column and conductivity detector) and cations (Metrohm
232 761 equipped with a Metrosep C6-250/4.0 column and conductivity detector). The total
233 nitrogen (TN) and COD concentrations were measured with Nanocolor tube test kits
234 (Nanocolor® TN220 and Nanocolor® COD160/1500, Macherey-Nagel, PA, USA). The
235 electrical conductivity (EC) was measured using a conductivity meter (Consort C6010 with a
236 Metrohm 6.0912.110 conductivity electrode).

3. RESULTS AND DISCUSSION

3.1. Two-step urine stabilization: alkalinization and subsequent nitrification

This study aimed to stabilize urine by biological conversion of urea and organics into nitrate, CO_2 and biomass. In order to prevent urea hydrolysis during storage, which could reduce the nutrient content and create odor nuisance and scaling, the pH of the urine was increased to ~12 immediately after collection using a chemical-free electrochemical method developed by De Paepe et al. (2020).[5] Batches of 2-4 L of urine were alkalinized with an electrochemical cell, pooled together into large batches of alkalinized urine (10-25 L) to average out fluctuations in urine composition in order to provide a stable influent (with a constant composition) to the nitrification bioreactor. The influent batches were stored for up to three months at room temperature. The TAN/TN ratio remained below 5% in the influent of the bioreactor (SI Figures S5 and S9, Table S6), confirming that urea hydrolysis was inhibited by the high pH. Also the COD concentration in the influent did not decrease. Only one batch (used in configuration 2, between day 132 and 136) got contaminated, resulting in a lower influent pH (~9.2) and COD concentration and an increased TAN concentration in the influent (SI Figure S9). This batch was replaced after 5 days.

The electrochemical pre-treatment also reduced the scaling potential by precipitation of divalent cations with phosphate and sulfate. Additionaly, the salinity of the urine decreased through the migration of anions (mainly chloride) to the middle compartment. Combined, these two processes resulted in a removal of 88 ± 7 % of calcium, 91 ± 6 % of magnesium, 78 ± 16 % of phosphate, 64 ± 14 % of chloride and 58 ± 18 % of sulfate (SI Section A3).

Yet, urine is only temporarily stabilized by increasing the pH and still contains urea (which can easily hydrolyze when the pH is lowered or urease is added [5]) and organics (which can cause biofouling). Therefore, the urine was further processed in an aerated nitrification bioreactor.

3.2. The extent of nitrification (NO_3^--N/TAN ratio in the effluent) increases as a function of the influent alkalinity

The extent of nitrification on alkalinized urine (pH influent ≥ 11.9) was studied in an MBBR without pH control. In a first phase (PN-I), 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 (Figure 2). This NO_3^--N/TAN ratio (60:40) is higher than reported in literature (typically ~50:50 [10, 24, 25]), because of the hydroxide addition in the electrochemical pre-treatment (~29 mmol OH^- L^{-1}, assuming a coulombic efficiency of 100%). Notably, the observed 60% of nitrification corresponds well with the interpolated value of 62%, calculated by comparing the added OH^- equivalents to the OH^- demand for full nitrification, which was estimated based on the TN concentration in the influent (SI Section B2). At the end of PN-I, after interrupting the influent flow for two days, the nitrate and TAN concentration remained unaffected (SI Section B3), confirming that the extent of nitrification was solely limited by the lack of alkalinity and not by a too slow nitrification rate (i.e., TAN accumulates when the nitrification rate is lower than the N loading rate).

In an attempt to increase the NO_3^--N/TAN ratio, influent with a higher alkalinity was fed to the reactor in phase PN-III. The influent was alkalinized with NaOH instead of an electrochemical cell, in order to define the exact amount of OH^- added to the urine. The latter is difficult to determine in electrochemically alkalinized urine, since the coulombic efficiency might drop at high pH (>12) due to hydroxide migration from the cathode to the middle compartment. Prior to PN-III, the bioreactor was conditioned with NaOH-alkalinized influent (22 mmol OH^- L^{-1} to obtain the same influent pH of 11.8 ± 0.3 as in PN-I), resulting in a similar NO_3^--N/TAN ratio (60:40) as in phase PN-I on electrochemically alkalinized urine (Figure 2). After increasing the OH^- content of the influent to 66 mmol OH^- L^{-1} (pH of 12.8 ± 0.2), the NO_3^--N/TAN ratio increased to 73:27 in phase PN-IIIa. In phase PN-IIIb, the NO_3^--N/TAN ratio further increased

to 93:7, because of the lower N concentration in the influent (other batch of urine with only 668 ± 90 mg N L^{-1} compared to 1706 ± 126 mg N L^{-1} in PN-IIIa) and, hence, a lower OH^- demand. Similar to PN-I, the increased extent of nitrification in response to the increased influent alkalinity throughout operation on NaOH-alkalinized urine was in line with the interpolated values (SI Section B2).

Over the course of 130 days, the partial nitrification reactor was stably operated at a N and COD loading rate of 103 mg N L^{-1} d^{-1} (PN-IIIb) - 250 mg N L^{-1} d^{-1} (PN-I, PN-II, PN-IIIa) and 132 mg COD L^{-1} d^{-1} (PN-IIIb) - 328 mg COD L^{-1} d^{-1} (Table 1). The pH was low enough to prevent free ammonia inhibition, even with a large fraction of TAN in the reactor liquid (up to 621 mg N L^{-1}). The nitrite concentration stayed below 15 mg N L^{-1}. The TN concentration in the effluent equalled the sum of the TAN and NO_3^--N concentration in the effluent and corresponded to the TN concentration in the influent, indicating that all urea was hydrolyzed and no nitrogen was lost through denitrification or ammonia stripping (Figure 2). After switching to NaOH-alkalinized urine, the sodium concentration increased threefold, increasing the electrical conductivity from ~10 mS cm^{-1} (PN-I) to ~15 mS cm^{-1} (PN-IIIa) (Table S2).

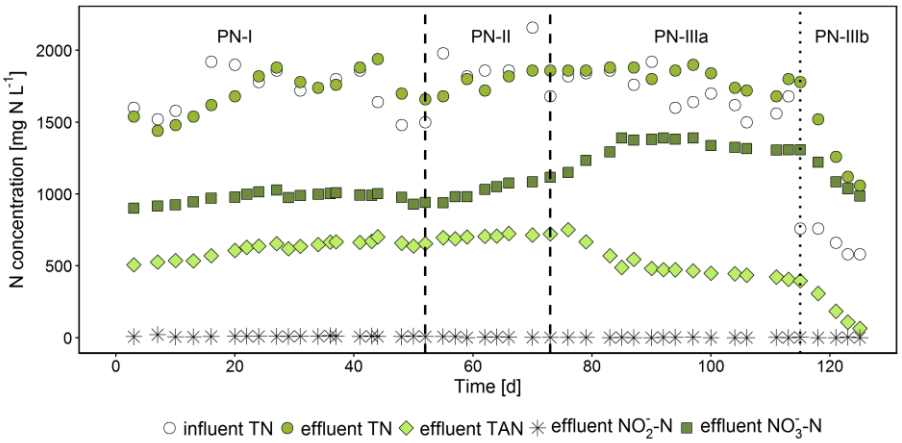


Figure 2. **Partial nitrification reactor without pH control (PN): total nitrogen (TN) concentration in the influent and TN, total ammonia nitrogen (TAN), nitrite and nitrate concentration in the effluent.** The influent and effluent composition is given in SI Figure S5 and Tables S1-S2.

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

The OH^- addition to increase the pH to 12 in the electrochemical pre-treatment (~29 mmol L^{-1}) was not sufficient to obtain fully nitrified urine in the MBBR without pH control (Section 3.2, PN-I). Although the NO_3^--N/TAN could be steered via the influent alkalinity, it was difficult to control since the OH^- demand depends on the N concentration in the urine, which highly fluctuates (Section 3.2, PN-IIIb). In order to control the pH in the reactor at a given set point, an electrochemical cell producing hydroxide ions 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 in order 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 2 weeks (day 13-26), the pH could be controlled in a narrow range (7.6 ± 0.1) between day 26 and 56 (Figure 3A), resulting in full nitrification in the MBBR. The TAN and nitrite concentration in the bioreactor remained below 2 mg N L^{-1} (Figure 4A). The nitrate concentration in the effluent equalled the TN concentration in the effluent, indicating that all urea was hydrolyzed and nitrified, but was lower than the TN concentration in the influent, due to nitrate migration over the AEM (Section 3.4). On average 0.055 ± 0.023 A was applied, supplying 101 mmol OH^- L^{-1} to the reactor (assuming a coulombic efficiency of 100%).

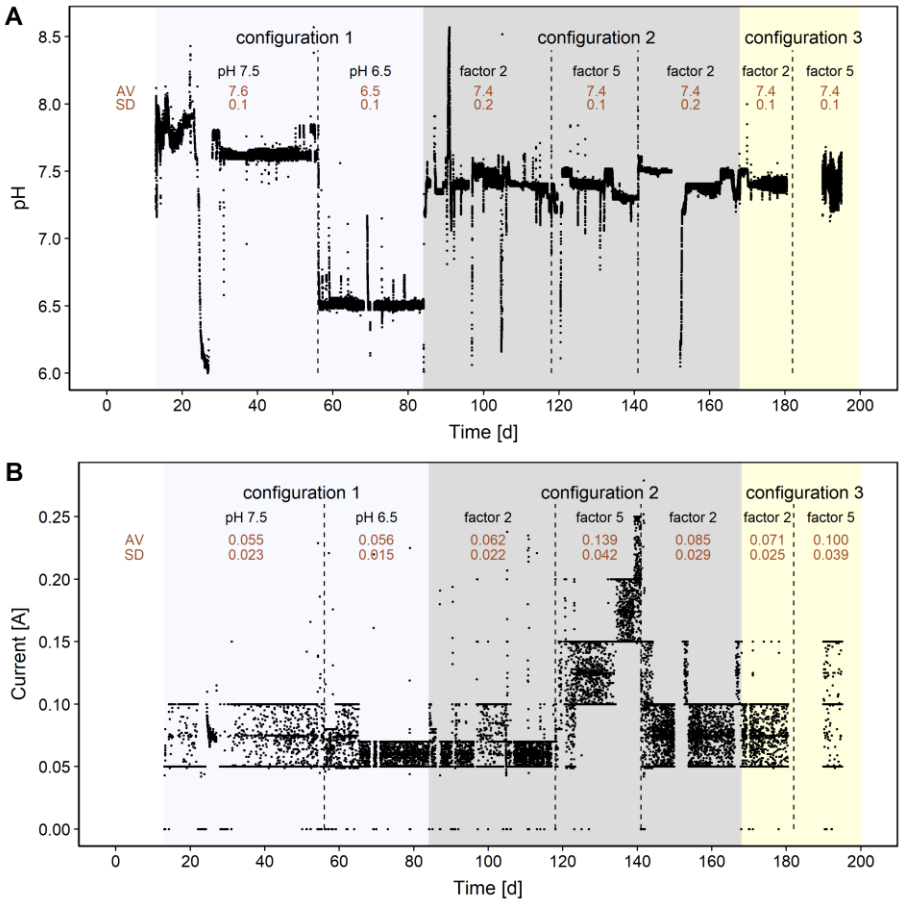
Together with the hydroxide added during the electrochemical pre-treatment (~29 mmol OH^- L^{-1}), a total of 130 mmol OH^- L^{-1} was added which was slightly higher than the theoretical OH^- demand for full nitrification (SI Table S10).

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 4A), but the average current (0.056 ± 0.015) was indifferent from the current at a pH set point of 7.5 (Figure 3B). Titration curves of the middle compartment effluent (SI Figure S11) 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. 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 C2 and C3).

To obtain a concentrated nitrate rich stream in the middle compartment, the configuration was adapted as further outlined in Section 3.5 and 3.6. 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 (effluent TAN and NO_2^--N < 10 mg N L^{-1}, except on day 141 after a shift to a more concentrated influent batch) in configuration 2 and 3 (Figure 4A). The OH^- demand for full nitrification was similar to configuration 1 (SI Table S10), since the nitrogen loading rate was stable over time (Table 2). Because of the higher proton concentration in the middle compartment (due to the concentration obtained by the lower influent flow rate to the middle compartment), more protons diffused through 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

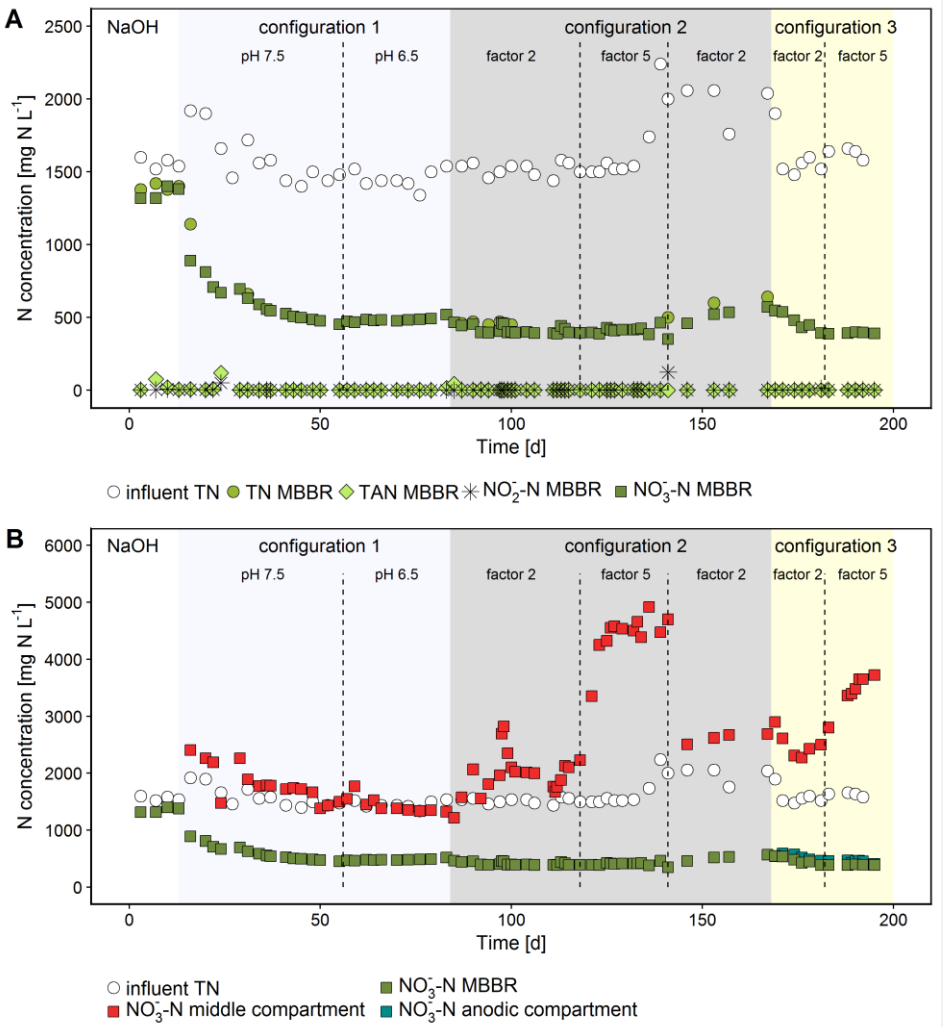
higher current was required to keep the pH at 7.4 compared to configuration 1 (Figure 3B). A higher concentration factor (5 instead of 2) in the middle compartment required a higher OH^- production and current (235 mmol OH^- L^{-1} ~0.139 A in configuration 2, factor 5 instead of 111 mmol OH^- L^{-1} ~0.062 A in configuration 2, factor 2). Similar findings were obtained in configuration 3, aiming at both anion and cation extraction and concentration.

Throughout the course of 182 days, the voltage fluctuated between 2.8 and 3.5 V, depending on the current (i.e., a higher current resulted in a higher voltage) (SI Figure S10). The voltage did not display an increasing trend over time, indicating that no or very limited membrane scaling and fouling occurred. The average energy consumption ranged between 7.9 Wh L^{-1} (without concentration in the middle compartment, configuration 1) and 21.3 Wh L^{-1} (with a concentration factor of 5, configuration 2).



362

363 Figure 3. **Full nitrification reactor with electrochemical hydroxide addition (EC): A) pH**
364 **profile, B) current applied by the electrochemical cell.** Averages (AV) and standard
365 deviations (SD) for each operational phase are displayed at the top.



366

367 Figure 4. **Full nitrification reactor with electrochemical hydroxide addition (FN-EC): A)**
368 **total nitrogen (TN) concentration in the influent and TN, TAN, nitrite and nitrate**
369 **concentration in the effluent, B) TN concentration in the influent and nitrate**
370 **concentration in the effluent of the bioreactor, middle compartment and anodic**
371 **compartment.** The average composition of the influent and effluent of the bioreactor, middle
372 compartment and cathodic compartment are given in SI Tables S6-S9.

3.4. Configuration 1 enables full nitrate recovery

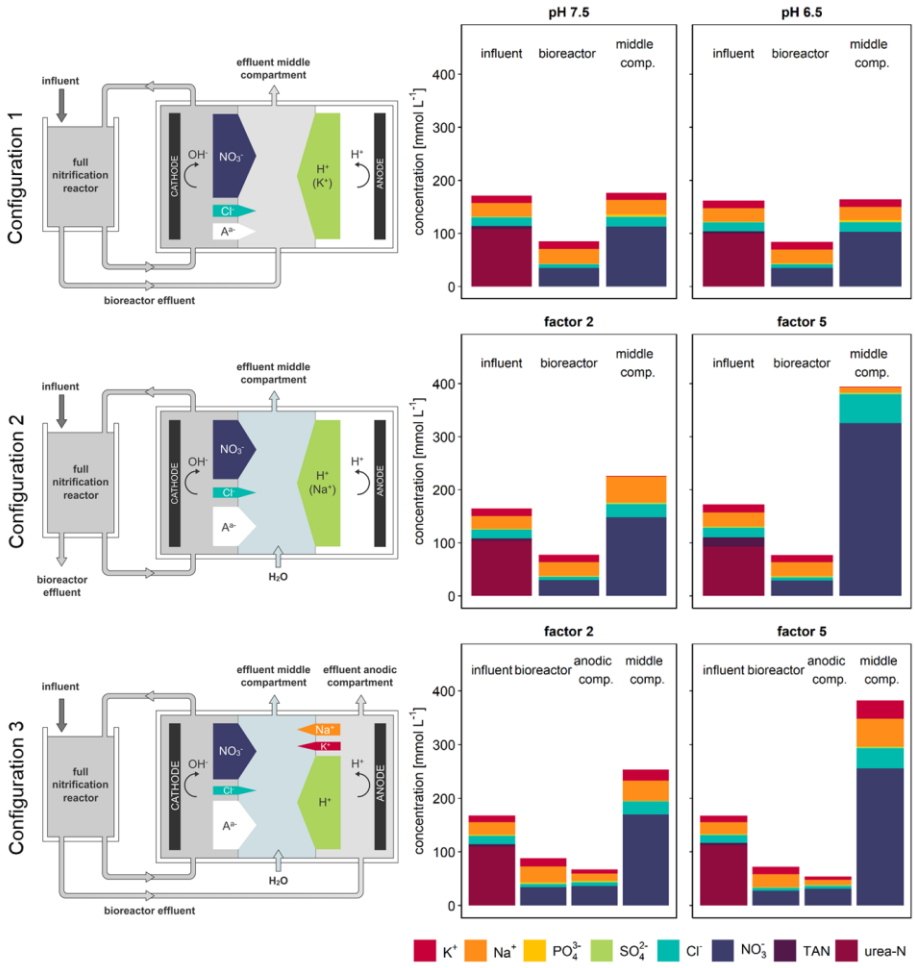
Due to the electron flow driven by the power supply, anions need to migrate from the cathodic compartment through the AEM to the middle compartment to restore the charge balance. Nitrate was the predominant anion in the nitrified urine and accounted for an estimated 68-78% of the migration through the AEM in configuration 1 (SI Section D3). 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 5A, Figure 4A). In order to recover all nitrogen, the effluent from the bioreactor was sent through the middle compartment in configuration 1. The nitrate and chloride concentration in the effluent of the middle compartment corresponded to the TN and chloride concentration in the influent (Figure 4B, SI Tables S6 and S8). 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, proton production at the anode resulted in cation migration from the anodic compartment to the middle compartment (Figure 5A). 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

397 titrations of the middle compartment effluent, where about 90-115 mmol OH^- L^{-1} was required
398 to increase the pH to 6.5-7.5 (SI Figure S11).

399 The salinity of the effluent of the middle compartment was markedly lower compared to the
400 salinity of the control reactor with NaOH addition (FN-NaOH) (SI Section E). This is on the
401 one hand due to the upstream electrochemical alkalinization (to pH 12), which reduced the
402 chloride concentration in the influent of FN-EC with more than 60% compared to the NaOH-
403 alkalinized influent used to feed FN-NaOH (Section 3.1). On the other hand, sodium addition
404 (originating from the use of NaOH for alkalinization and full nitrification) increased the sodium
405 concentration fivefold in FN-NaOH compared to the original concentration in urine (before pH
406 increase), whereas no sodium was dosed to FN-EC.

407 An additional advantage of the electrochemical pH control is that the nitrifiers in the bioreactor
408 are exposed to an even lower salinity (electrical conductivity of ~5 mS cm^{-1}), because of anion
409 migration to the middle compartment. This is particularly relevant when working with salt-
410 sensitive synthetic communities in high strength urine.[12]



411

412 Figure 5. **Full nitrification reactor with electrochemical hydroxide addition (FN-EC): ion**
413 **migration through the AEM and CEM and composition of the influent, bioreactor liquid,**
414 **middle compartment and anodic compartment in configuration 1-3.** The size of the arrows
415 represent the relative contribution of each ion to the total migration (estimated based on the
416 electric charge) (calculation in SI Section D3). (Comp: compartment.)

3.5. 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 5B). 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 factor 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 (2083 ± 320 mg NO_3^--N L^{-1} at factor 2 and 4561 ± 167 mg NO_3^--N L^{-1} at factor 5), chloride (861 ± 163 mg Cl^- L^{-1} at factor 2 and 1909 ± 186 mg Cl^- L^{-1} at factor 5), and sodium (originating from the anolyte) in an aqueous matrix with a pH below 1.2 (Figure 5B, SI Table S8). 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 [26-28], 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 N 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 pre-treatment (which provided 11-22% of the OH^-, SI Table S10). 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)[5], 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 in the recirculation loop 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 [29] to 4.68 [30] have been developed.

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 water flow rate of 223 mL d^{-1} ($Q_{in\ bioreactor}/Q_{in\ middle\ compartment}$~2), the nitrate

concentration was about 1.3 times higher compared to the TN 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 S12). 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 6.7 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.

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

	configuration 2		configuration 3	
	factor 2	factor 5	factor 2	factor 5
Concentration factor				
$\frac{Q_{in}\ bioreactor}{Q_{in}\ middle\ compartment}$	2.24	5.67	2.48	5.26
$\frac{Q_{in}\ bioreactor}{Q_{out}\ middle\ compartment}$	2.24	4.47	2.24	3.78
$\frac{[NO_3^- - N]\ middle\ compartment}{[TN]\ influent}$	1.3	3.0	1.5	2.3
Nitrogen recovery				
1 - $\frac{[NO_3^- - N]\ bioreactor}{[TN]\ influent}$	73	74	70-73	76

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

In configuration 2, all cations, including potassium, 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 5C, SI Section D3). This resulted in a recovery of on average 40% of the potassium and 44% of the sodium from urine. Due to the increased competition for migration through the CEM, less protons migrated, resulting in a slightly higher pH in the middle compartment in configuration 3 (pH of 1.5 ± 0.1) compared to configuration 2 (pH of 0.7 ± 0.1) (SI Figure S13). A lower proton concentration in the middle compartment limited the proton diffusion to the cathode, giving way to a lower current and energy consumption by the electrochemical cell.

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 to chlorine, and further react to HOCl, a known disinfectant. If this 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 life span 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.

3.7 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 reported in literature by open communties (SI, Tables S1, S2, S4, S6 and S7).[8-10, 13, 24, 31, 32] 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.

3.8 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. 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[9, 33, 34], while the electrode power consumption is higher (25-60 kWh electrical energy or 80-194 kWh primary energy m^{-3} urine compared to 4.4 kWh electrical energy m^{-3} urine[9]). Distillation reaches higher concentration factors but at the expense of a higher energy investment (~700 kWh primary energy m^{-3} urine).[13]

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.[35, 36] 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.

555 Water oxidation: $H_2O \rightarrow 0.5\ O_2 + 2\ H^+ + 2\ e^-$ $E_0(SHE) = 1.229$ V (9)

556 Hydrogen gas oxidation: $0.5\ H_2 + OH^- \rightarrow H_2O + e^-$ $E_0(SHE) = 0.8277$ V (10)

557 Urine treatment and recycling is not only relevant on Earth, but is of major importance in
558 regenerative life support systems (RLSS) as urine is the main resource of water and nutrients.
559 Even when recovery is not envisaged, urine stabilization is essential since ammonia, originating
560 from urea hydrolysis, can pose a hazard to the crew upon volatilization. Currently, on board of
561 the International Space Station, sulfuric/phosphoric acid and toxic chromium trioxide are added
562 to urine in order to inhibit urea hydrolysis.[37] Subsequently, water is recovered from the urine
563 using vapor compression distillation and filtration, while the nutrients are concentrated in a
564 toxic brine.[38] Alternatively, urine could be stabilized by our two-step approach. Immediately
565 after collection, the pH should be increased to 12 in order to prevent urea hydrolysis during
566 storage, which is essential to provide a constant flow and composition (i.e., no peak loading) to
567 the bioreactor, where all urea is nitrified to nitrate. The nitrified urine can be valorized as
568 substrate for plants and microalgae. Nitrification combined with the production of microalgae
569 and plants is being explored in the framework of the Micro-Ecological Life Support System
570 Alternative (MELiSSA), the RLSS programme from the European Space Agency.[12, 39] To take
571 this one step further, this study addressed the issue of payload limitations to Space by
572 implementation of electrochemical cells enabling in-situ production of acids and bases,
573 obviating the need for transportation and storage of these hazardous consumables.
574 Configuration 1 is most appropriate for Space application as maximum recovery is pivotal while
575 concentration does not present an added value in Space. Furthermore, the low salinity and
576 acidicity (~1 mol H^+ mol^{-1} NO_3^--N) of the end product of configuration 1 is compatible with
577 hydroponic plant production, as 0-1 mol H^+ mol^{-1} NO_3^--N is required to compensate for the
578 release of OH^- ions (0-1 mol OH^- mol^{-1} NO_3^--N) that accompanies nitrate uptake by most
579 plants.[17, 40, 41]

ASSOCIATED CONTENT

Supporting Information. The Supporting Information is available free of charge on the ACS Publications website at DOI: ...

Additional figures, tables and calculations (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: Korneel.Rabaey@UGent.be

Notes

The authors declare no competing financial interest

ACKNOWLEDGMENTS

This article has been made possible through the authors' involvement in the MELiSSA project, the life support system research program from the European Space Agency (ESA) (https://www.melissafoundation.org/).

The authors would like to acknowledge

i) the MELiSSA foundation to support JDP via the POMP1 (Pool Of MELiSSA PhD) program,

ii) KR …

iii) the Belgian Science Policy (BELSPO) to support RG via the URINIS-A phase 2 project,

iv) Avecom for providing the ABIL sludge,

v) Mike Taghon and Annick Beyaert for making the Labview control system,

Met opmerkingen [KR1]: FWO microelectronics associates as theme in part is controllers:

vi) Kim De Paepe for critically reviewing the manuscript.

602 REFERENCES

603 1. Maurer, M.; Pronk, W.; Larsen, T. A., Treatment processes for source-separated urine.
604 *Water Res.* **2006,** *40*, (17), 3151-3166.
605 2. National Research Council (US), *Committee on Acute Exposure Guideline Levels.*
606 *Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 6. Washington*
607 *(DC): National Academies Press (US); 2008. 2, Ammonia Acute Exposure Guideline Levels.*
608 *Available from: https://www.ncbi.nlm.nih.gov/books/NBK207883/*. 2008.
609 3. Udert, K. M.; Larsen, T. A.; Biebow, M.; Gujer, W., Urea hydrolysis and precipitation
610 dynamics in a urine-collecting system. *Water Res.* **2003,** *37*, (11), 2571-2582.
611 4. Udert, K. M.; Larsen, T. A.; Gujer, W., Fate of major compounds in source-separated
612 urine. *Water Sci. Technol.* **2006,** *54*, (11-12), 413-420.
613 5. De Paepe, J.; De Pryck, L.; Verliefde, A. R. D.; Rabaey, K.; Clauwaert, P.,
614 Electrochemically Induced Precipitation Enables Fresh Urine Stabilization and Facilitates
615 Source Separation. *Environ Sci Technol* **2020**.
616 6. Randall, D. G.; Krahenbuhl, M.; Kopping, I.; Larsen, T. A.; Udert, K. M., A novel
617 approach for stabilizing fresh urine by calcium hydroxide addition. *Water Res.* **2016,** *95*, 361-
618 369.
619 7. Senecal, J.; Vinneras, B., Urea stabilisation and concentration for urine-diverting dry
620 toilets: Urine dehydration in ash. *Sci Total Environ* **2017,** *586*, 650-657.
621 8. Coppens, J.; Lindeboom, R.; Muys, M.; Coessens, W.; Alloul, A.; Meerbergen, K.;
622 Lievens, B.; Clauwaert, P.; Boon, N.; Vlaeminck, S. E., Nitrification and microalgae
623 cultivation for two-stage biological nutrient valorization from source separated urine.
624 *Bioresour. Technol.* **2016,** *211*, 41-50.
625 9. De Paepe, J.; Lindeboom, R. E. F.; Vanoppen, M.; De Paepe, K.; Demey, D.;
626 Coessens, W.; Lamaze, B.; Verliefde, A. R. D.; Clauwaert, P.; Vlaeminck, S. E., Refinery and
627 concentration of nutrients from urine with electrodialysis enabled by upstream precipitation
628 and nitrification. *Water Res.* **2018,** *144*, 76-86.
629 10. Udert, K. M.; Fux, C.; Munster, M.; Larsen, T. A.; Siegrist, H.; Gujer, W.,
630 Nitrification and autotrophic denitrification of source-separated urine. *Water Sci. Technol.*
631 **2003,** *48*, (1), 119-130.
632 11. Feng, D. L.; Wu, Z. C.; Xu, S. H., Nitrification of human urine for its stabilization and
633 nutrient recycling. *Bioresour. Technol.* **2008,** *99*, (14), 6299-6304.
634 12. Christiaens, M. E. R.; De Paepe, J.; Ilgrande, C.; De Vrieze, J.; Barys, J.; Teirlinck, P.;
635 Meerbergen, K.; Lievens, B.; Boon, N.; Clauwaert, P.; Vlaeminck, S. E., Urine nitrification
636 with a synthetic microbial community. *Systematic and Applied Microbiology* **2019**, 126021.
637 13. Udert, K. M.; Wachter, M., Complete nutrient recovery from source-separated urine
638 by nitrification and distillation. *Water Res.* **2012,** *46*, (2), 453-464.
639 14. Fumasoli, A.; Etter, B.; Sterkele, B.; Morgenroth, E.; Udert, K. M., Operating a pilot-
640 scale nitrification/distillation plant for complete nutrient recovery from urine. *Water Sci.*
641 *Technol.* **2016,** *73*, (1), 215-222.
642 15. Parida, A. K.; Das, A. B., Salt tolerance and salinity effects on plants: a review.
643 *Ecotoxicology and Environmental Safety* **2005,** *60*, (3), 324-349.
644 16. Munns, R.; Tester, M., Mechanisms of salinity tolerance. *Annual review of plant*
645 *biology* **2008,** *59*, 651-81.
646 17. Spinu, V. C.; Langhans, R. W.; Albright, L. D. In *ELECTROCHEMICAL PH*
647 *CONTROL IN HYDROPONIC SYSTEMS*, 1998; International Society for Horticultural
648 Science (ISHS), Leuven, Belgium: 1998; pp 275-282.

18. Schwartzkopf, S. H. *Electrochemical Control of pH in a Hydroponic Nutrient Solution. Available at:* https://ntrs.nasa.gov/archive/nasa/casi.ntrs.nasa.gov/19860010447.pdf; 1986.
19. Walther, I.; van der Schoot, B. H.; Jeanneret, S.; Arquint, P.; de Rooij, N. F.; Gass, V.; Bechler, B.; Lorenzi, G.; Cogoli, A., Development of a miniature bioreactor for continuous culture in a space laboratory. *Journal of Biotechnology* **1994,** *38*, (1), 21-32.
20. Andersen, S. J.; Candry, P.; Basadre, T.; Khor, W. C.; Roume, H.; Hernandez-Sanabria, E.; Coma, M.; Rabaey, K., Electrolytic extraction drives volatile fatty acid chain elongation through lactic acid and replaces chemical pH control in thin stillage fermentation. *Biotechnology for Biofuels* **2015,** *8*, (1), 221.
21. Bonvin, C.; Etter, B.; Udert, K. M.; Frossard, E.; Nanzer, S.; Tamburini, F.; Oberson, A., Plant uptake of phosphorus and nitrogen recycled from synthetic source-separated urine. *Ambio* **2015,** *44*, S217-S227.
22. Muys, M.; Coppens, J.; Boon, N.; Vlaeminck, S. E., Photosynthetic oxygenation for urine nitrification. *Water Sci. Technol.* **2018,** *78*, (1), 183-194.
23. Wohlsager, S.; Clemens, J.; Nguyet, P. T.; Rechenburg, A.; Arnold, U., Urine--a valuable fertilizer with low risk after storage in the tropics. *Water environment research : a research publication of the Water Environment Federation* **2010,** *82*, (9), 840-7.
24. Bischel, H. N.; Schertenleib, A.; Fumasoli, A.; Udert, K. M.; Kohn, T., Inactivation kinetics and mechanisms of viral and bacterial pathogen surrogates during urine nitrification. *Environ. Sci.-Wat. Res. Technol.* **2015,** *1*, (1), 65-76.
25. Feng, D. L.; Wu, Z. C.; Wang, D. H., Effects of N source and nitrification pretreatment on growth of Arthrospira platensis in human urine. *J. Zhejiang Univ.-SCI A* **2007,** *8*, (11), 1846-1852.
26. Escher, B. I.; Pronk, W.; Suter, M. J. F.; Maurer, M., Monitoring the removal efficiency of pharmaceuticals and hormones in different treatment processes of source-separated urine with bioassays. *Environ. Sci. Technol.* **2006,** *40*, (16), 5095-5101.
27. Pronk, W.; Kone, D., Options for urine treatment in developing countries. *Desalination* **2009,** *248*, (1-3), 360-368.
28. Pronk, W.; Biebow, M.; Boller, P., Treatment of source-separated urine by a combination of bipolar electrodialysis and a gas transfer membrane. *Water Sci. Technol.* **2006,** *53*, (3), 139-146.
29. Eyal, A.; Kedem, O., Nitrate-selective anion-exchange membranes. *J. Membr. Sci.* **1988,** *38*, (2), 101-111.
30. Kikhavani, T.; Ashrafizadeh, S. N.; Van der Bruggen, B., Nitrate Selectivity and Transport Properties of a Novel Anion Exchange Membrane in Electrodialysis. *Electrochimica Acta* **2014,** *144*, 341-351.
31. Jiang, F.; Chen, Y.; Mackey, H. R.; Chen, G. H.; van Loosdrecht, M. C. M., Urine nitrification and sewer discharge to realize in-sewer denitrification to simplify sewage treatment in Hong Kong. *Water Sci. Technol.* **2011,** *64*, (3), 618-626.
32. Chen, L. P.; Yang, X. X.; Tian, X. J.; Yao, S.; Li, J. Y.; Wang, A. M.; Yao, Q. A.; Peng, D. C., Partial nitritation of stored source-separated urine by granular activated sludge in a sequencing batch reactor. *AMB Express* **2017,** *7*, 1-10.
33. Pronk, W.; Zuleeg, S.; Lienert, J.; Escher, B.; Koller, M.; Berner, A.; Koch, G.; Boller, M., Pilot experiments with electrodialysis and ozonation for the production of a fertiliser from urine. *Water Sci. Technol.* **2007,** *56*, (5), 219-227.
34. Pronk, W.; Biebow, M.; Boller, M., Electrodialysis for recovering salts from a urine solution containing micropollutants. *Environ. Sci. Technol.* **2006,** *40*, (7), 2414-2420.
35. Kuntke, P.; Rodríguez Arredondo, M.; Widyakristi, L.; ter Heijne, A.; Sleutels, T. H. J. A.; Hamelers, H. V. M.; Buisman, C. J. N., Hydrogen Gas Recycling for Energy Efficient

Ammonia Recovery in Electrochemical Systems. *Environ. Sci. Technol.* **2017,** *51*, (5), 3110-3116.
36. Kuntke, P.; Rodrigues, M.; Sleutels, T.; Saakes, M.; Hamelers, H. V. M.; Buisman, C. J. N., Energy-Efficient Ammonia Recovery in an Up-Scaled Hydrogen Gas Recycling Electrochemical System. *ACS Sustainable Chemistry & Engineering* **2018,** *6*, (6), 7638-7644.
37. Muirhead, D.; Carter, D. L.; Williamson, J., Preventing Precipitation in the ISS Urine Processor In *48th International Conference on Environmental Systems*, Albuquerque, New Mexico, 2018.
38. Carter, D. L.; Williamson, J.; Brown, C. A.; Bazley, J.; Gazda, D.; Schaezler, R.; THomas, F., Status of ISS Water Management and Recovery. In *48th International Conference on Environmental Systems*, Albuquerque, New Mexico, 2017.
39. Gòdia, F.; Albiol, J.; Montesinos, J. L.; Pérez, J.; Creus, N.; Cabello, F.; Mengual, X.; Montras, A.; Lasseur, C., MELISSA: a loop of interconnected bioreactors to develop life support in Space. *Journal of Biotechnology* **2002,** *99*, (3), 319-330.
40. FAN, X. H.; TANG, C.; RENGEL, Z., Nitrate Uptake, Nitrate Reductase Distribution and their Relation to Proton Release in Five Nodulated Grain Legumes. *Annals of Botany* **2002,** *90*, (3), 315-323.
41. Bolan, N. S.; Hedley, M. J.; White, R. E., Processes of soil acidification during nitrogen cycling with emphasis on legume based pastures. *Plant and Soil* **1991,** *134*, (1), 53-63.