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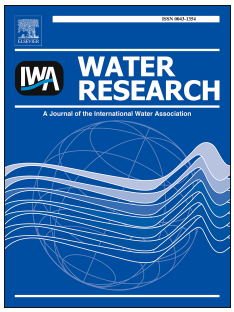

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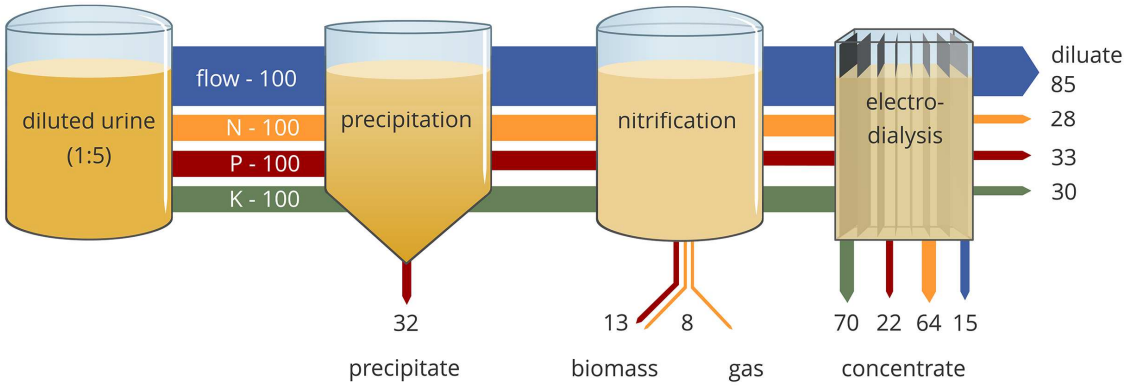

Refinery and concentration of nutrients from urine with electrodialysis enabled by upstream precipitation and nitrification

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ABSTRACT

Human urine is a valuable resource for nutrient recovery, given its high levels of nitrogen, phosphorus and potassium, but the compositional complexity of urine presents a challenge for an energy-efficient concentration and refinery of nutrients. In this study, a pilot installation combining precipitation, nitrification and electrodialysis (ED), designed for one person equivalent ($1.2L_{urine}$ d^{-1}), was continuously operated for ~7 months. First, NaOH addition yielded calcium and magnesium precipitation, preventing scaling in ED. Second, a moving bed biofilm reactor oxidized organics, preventing downstream biofouling, and yielded complete nitrification on diluted urine (20-40%, i.e. dilution factors 5 and 2.5) at an average loading rate of 215 mg N L^{-1} d^{-1}. Batch tests demonstrated the halotolerance of the nitrifying community, with nitrification rates not affected up to an electrical conductivity of 40 mS cm^{-1} and gradually decreasing, yet ongoing, activity up to 96 mS cm^{-1} at 18% of the maximum rate. Next-generation 16S rRNA gene amplicon sequencing revealed that switching from a synthetic influent to real urine induced a profound shift in microbial community and that the AOB community was dominated by halophilic species closely related to *Nitrosomonas aestuarii* and *Nitrosomonas marina*. Third, nitrate, phosphate and potassium in the filtered (0.1 µm) bioreactor effluent were concentrated by factors 4.3, 2.6 and 4.6, respectively, with ED. Doubling the urine concentration from 20% to 40% further increased the ED recovery efficiency by ~10%. Batch experiments at pH 6, 7 and 8 indicated a more efficient phosphate transport to the concentrate at pH 7. The newly proposed three-stage strategy opens up opportunities for energy- and chemical-efficient nutrient recovery from urine. Precipitation and nitrification enabled the long-term continuous operation of ED on fresh urine requiring minimal maintenance, which has, to the best of our knowledge, never been achieved before.

Keywords:

source separation, resource recovery, MBBR, MBR, electrodialysis

1. INTRODUCTION

Fertilizers are pivotal in meeting the global food demand. At present, fertilizer production mainly relies on the use of non-renewable energy (to produce ammonia) and finite natural resources, such as phosphate rock and potassium minerals (Ledezma et al. 2015). The growing world population, the limited resources and the environmental burden of eutrophication demand a paradigm shift towards recovery and reuse of nutrients (Verstraete et al. 2016). Recently, source-separated urine has gained great interest as a valuable resource of nutrients, given its relatively high concentration in macronutrients (~9 g N L^{-1}, 0.7 g P L^{-1} and 2 g K L^{-1}) (Udert et al. 2006). Efficient nutrient recovery from source-separated urine could provide an estimated 20% of the nitrogen, phosphorus and potassium for the current fertilizer production in the EU (Kuntke 2013, Ledezma et al. 2015). Since urine is often diluted with flushing water, it is important to concentrate the nutrients in order to reduce transport and storage volumes and to facilitate the recovery of nutrients (Maurer et al. 2006). Numerous technologies have been proposed to separate and/or concentrate nutrients from urine. However, none of these technologies are capable of capturing the broad urine nutrient spectrum in a micropollutant-free fertilizer in a scalable, energy- and chemical-efficient manner. Evaporation, reverse osmosis, freeze-thawing and nitrification-distillation are energy-intensive, microbial fuel cells and electrolysis cells are difficult to scale up, whereas struvite precipitation, NH_3 stripping and ion exchange only target specific nutrients (i.e., N or P) (Ledezma et al. 2015, Maurer et al. 2006, Tice and Kim 2014, Udert and Wachter 2012). In order to fill this gap, electrodialysis (ED) was selected in the present study.

ED is a membrane separation process with an electric potential gradient as the driving force to separate charged ions from an aqueous solution. Anion and cation exchange membranes compartimentalise the system, producing two effluent streams, an ion-depleted diluate and ion-accumulating concentrate stream. ED has been implemented at large scale (> 20 000 m^3 d^{-1}), mostly for desalination of brackish water and demineralization of industrial process water (Strathmann 2010). A few studies report on urine treatment with ED for the purpose of i) water recovery (Brown et al. 1963) or urine desalination (Aponte and Colon 2001) in Space life support systems, ii) micropollutant removal (Escher et al. 2006) and iii) nutrient recovery

(Pronk et al. 2006a, Pronk et al. 2006b, Pronk et al. 2007). Operating an ED stack on urine is challenging due to the high calcium and magnesium concentration and the high organic load, which can cause scaling and biofouling on the membranes, and clogging of spacers and tubing. As a consequence, the reduced flux and increasing resistance in the stack are limiting factors in achieving high efficiencies (Mikhaylin and Bazinet 2016). Thus far, autoclaved, filtered urine (Aponte and Colon 2001) or urine pre-treated with charcoal adsorption (Brown et al. 1963) or microfiltration (Pronk et al. 2006a, Pronk et al. 2006b) has been used in short-term ED experiments. Only one study addressed the long-term (>3 months), interrupted operation (short operation times of 1-2 weeks) of an ED stack on real, hydrolysed urine (Pronk et al. 2007). The desalination rate, however, decreased with approximately 50% over a period of 195 days due to fouling (Pronk et al. 2007).

In the present study, fresh urine was used and scaling and biofouling were prevented by a combination of precipitation and an aerobic bioreactor. Disadvantages of using hydrolysed urine include uncontrolled precipitation of phosphorus and evaporation of ammonia during storage, leading to phosphorus and nitrogen losses (Maurer et al. 2006 Udert et al. 2003b, Udert et al.2003c). Enzymatic urea hydrolysis can be prevented during storage through addition of acids, metals, caustics …, but it is considered challenging to stop the process for 100% (Randall et al. 2016, Ray et al. 2018). Calcium and magnesium were removed in a precipitation reactor in which NaOH was dosed to increase the pH to 11, shifting the speciation of phosphate and carbonate ions, causing supersaturation and thus triggering precipitation. Subsequently, the urine was treated in an aerobic bioreactor. The purpose of the bioreactor was to i) convert biodegradable organics into CO_2 and sludge by heterotrophic bacteria, thereby reducing the biofouling potential in ED, and ii) to convert urea (~85% of the nitrogen in fresh urine) into nitrate through ammonification followed by nitrification. In addition, nitrification decreases the pH, leading to undersaturation, thereby preventing further precipitation in ED.

Ammonification, or the hydrolysis of urea to bicarbonate and ammonium, is initiated by the urease enzyme, produced by many heterotrophic bacteria and some nitrifiers (Defoirdt et al. 2017, Koops et al. 1991, Mobley and Hausinger 1989). The nitrification process consists of ammonium oxidizing bacteria

(AOB) converting ammonium into nitrite ('nitritation') followed by the oxidation of the nitrite into nitrate ('nitratation') by nitrite oxidizing bacteria (NOB). Nitrification is a well-documented stabilisation method of urine as nitrate is a stable molecule, in contrast to ammonia, which upon volatilisation causes significant malodour and nitrogen losses (Coppens et al. 2016, Feng et al. 2008, Udert et al. 2003, Udert and Wachter 2012). Additionally, nitrate is the preferred nitrogen species for recovery as it is a charged molecule which can be captured in the ED concentrate, and, in a lot of cases, the preferred nitrogen fertilizer for plants (Marschner 1995).

The integration of precipitation, nitrification and ED to refine and concentrate nutrients from fresh urine of one person equivalent was, for the first time, evaluated in an automated pilot-scale installation during four months on a 20% urine solution (i.e., dilution factor of 5). As upstream precipitation and nitrification enable ED to act as a key recovery stage, all units are considered equally important. The installation was operated for another 40 days on a 40% urine solution (i.e., dilution factor of 2.5) to study the effect of a lower urine dilution and thus a higher salinity on the nitrification and ED. The microbial community on the biofilm carriers of the nitrification reactor was followed up over time through next-generation amplicon sequencing of the 16S rRNA gene. To study the impact of salinity on the maximum ammonium and nitrite oxidation rates of the biofilm, batch experiments were performed in which carriers were exposed to salinities between 20 and 96 mS cm^{-1}. Furthermore, a batch experiment was carried out on ED to investigate whether the phosphate recovery in ED was influenced by the pH of the ED feed stream. Three different pH values (in the pH range of nitrification) were tested: pH 6, 7 and 8.

2. MATERIALS AND METHODS

2.1 Automated treatment train

Details and pictures of the equipment are provided in Figure S1-S3.

2.1.1 Precipitation reactor

Diluted human urine was dosed to the precipitation reactor based on a level control PLC (programmable logic controller, Siemens Simatic HMI) feedback loop, in which detection of low level initiated pumping and high level stopped pumping. The pH was controlled at 11 by dosing 2M NaOH and temperature was controlled at 40°C by means of a heating and stirring plate. The content of the reactor was continuously recycled (3 L h^{-1}) by a peristaltic pump to enhance the formation and growth of crystals. To prevent precipitate from entering the bioreactor, urine was passed through a dead-end filter basket with glass fiber cloth and two filters (10 µm and 0.2 µm).

2.1.2 Nitrification bioreactor

Nitrification and COD oxidation occurred in a moving bed biofilm reactor (MBBR) filled for 20% (v/v) with polyvinyl alcohol beads and integrated in a membrane bioreactor (MBR) with an external ultrafiltration (UF) module. The reactor received effluent from the precipitation reactor when the level dropped below 30.7 L. The pH was controlled between 6.7 and 6.8 by dosing 2M NaOH or 1M HCl. Aeration (dissolved oxygen (DO) level >2 mg O_2 L^{-1}) and mixing were provided by injection of pressurized ambient air. The UF module consisted of three 0.1 µm sidestream tubular α-Al_2O_3 membranes with a total surface area of 0.057 m^2 placed inside a stainless steel module and was operated at a crossflow velocity of 0.5 m s^{-1}. The UF filtrate was dosed to the diluate tank of the ED unit, the UF retentate was recirculated to the bioreactor. When the pressure over the UF membrane increased (>1 bar), the filtration module was disconnected and cleaned by recirculating a 1% P110 (alkaline) and a 1% P73 (acid) ultrasil solution (Ecolab®, MN, USA).

2.1.3 Electrodialysis unit

The ED unit contained electrolyte, diluate and concentrate tanks next to a PCCell ED 64004 stack (PCA GmbH, Heusweiler, Germany). The stack comprised 10 cell pairs with standard PC SA anion and PC SK cation exchange membranes with an active membrane area of 64 cm^2 and 0.45 mm thick silicone/polyethylene spacers (PCA GmbH, Heusweiler, Germany). The electrodes were stretched titanium,

coated with a mixed metal oxide platinum/iridium coating for the cathode/anode, respectively (PCA GmbH, Heusweiler, Germany). A 1M $NaNO_3$ electrode rinsing solution was circulated in the electrode compartments over the electrolyte tank. The diluate and concentrate were circulated at a constant flow rate (1 L min^{-1}) between the tank and stack. The concentrate was drained when the level or the conductivity in the concentrate tank exceeded the threshold (2 L or 70 mS cm^{-1}, respectively). The diluate tank was continuously filled with the filtrate of the bioreactor and partially drained when the level in the tank exceeded 2.1 L. The current of the power supply was controlled at 80-90% of the limiting current density (LCD). The LCD was determined according to the method described by Mulder (1996) using the electrical conductivity (EC) of the ED diluate. When the potential difference surmounted 10 V (about three times the nominal value), the stack was cleaned in place by recirculating a 0.1% oxonia solution and a 0.01 M HCl solution through the diluate and concentrate compartments.

2.2 Operation

2.2.1 Urine collection

Urine from male donors not taking medication, was collected in a water-less urinal and stored in a freezer (-20°C). Urine collection was approved by the Ethical committee of Ghent University hospital under registration number B670201523246. Before feeding to the precipitation reactor, the urine was thawed and preserved at 4°C. Demineralised water was added to simulate flush water. The installation was operated during four months on a 20% urine solution. Daily, 1.2 L of urine and 4.6 L of demineralised water were seperately fed into the system and subsequently mixed in the precipitation reactor. To study the effect of a higher salinity on the nitrification and the recovery efficiencies and concentration factors of the ED, the installation was operated using a 40% urine solution (1.3 L d^{-1} of urine mixed with 2.2 L d^{-1} of demineralised water) for two months.

2.2.2 Reactor inoculation

Based on the inoculum screening described by Coppens et al. (2016), three inoculum sources (urine nitrification, OLAND and commercial aquaculture inoculum) were selected to inoculate a cultivation reactor (CR), used to establish a nitrifying biofilm on the beads. The CR (22L) was operated as a moving bed biofilm

reactor (MBBR) and received a synthetic influent with a salinity around 10 mS cm^{-1} consisting of 0.7 g N L^{-1} (as $(NH_4)_2SO_4$), 7.6 g L^{-1} $NaHCO_3$, 25 mg P L^{-1} (as KH_2PO_4) , 27 mg N L^{-1} (as $NaNO_2$), 104 mg COD L^{-1} (as sodium acetate) and 2.5 g NaCl L^{-1}, dissolved in tap water. Prior to operating the CR as MBBR, it was operated for several months as a SBR. The initial influent composition was based on the information provided by the suppliers of the inoculum and gradually tuned, also using batch assays, towards maximizing nitritation and nitratation rates, while maintaining the cultures' functionality for ureolysis and COD removal at slightly saline conditions. The additional nitrite was used to create a slight overcapacity in nitratation compared to nitritation to minimize nitrite toxicity risks during start up. The volumetric nitrogen loading rate amounted to 165 mg N L^{-1} d^{-1}. The pH was between pH 6.5-8.0 ($NaHCO_3$ buffer) and oxygen was continuously supplied with an aquarium pump (DO > 2 mg O_2 L^{-1}). An average hydraulic retention time (HRT) of 4.4 days was applied to ensure sufficient selective pressure for biofilm growth by washing out the suspended biomass. The bioreactor of the pilot installation was inoculated with beads from the cultivation reactor.

2.2.3 Automation, operation and sampling

The pilot installation was equipped with pH, DO and EC electrodes connected to an SC1000 controller (Hach, CO, USA). An online ammonium analyser (AMTAX sc, Hach, CO, USA) continuously monitored the ammonium concentration in the UF filtrate of the bioreactor. The process parameters were automatically logged and controlled by a PLC (Siemens Simatic HMI), allowing an automated operation of the installation. Additionally, samples were taken daily at different positions in the installation through sampling ports, filtered over a 0.20 µm Chromafil® Xtra filter (Macherey-Nagel, PA, USA) and stored in the fridge (4°C) prior to analysis. The operational conditions during the tests on a 20% and 40% urine solution are presented in Table 1.

TABLE 1 (double column, full width)

Table 1. **Operational conditions for the precipitation reactor, nitrification bioreactor and electrodialysis (ED) unit during the tests on 20% and 40% urine solutions.** Average values and standard deviations are based on a period of 100 days operating using a 20% urine solution and 40 days operating using a 40% urine solution. HRT: hydraulic retention time; EC: electrical conductivity; DO: dissolved oxygen.

	Precipitation reactor		**Nitrification bioreactor**		**ED diluate**		**ED concentrate**	
Urine solution [vol%]	**20%**	**40%**	**20%**	**40%**	**20%**	**40%**	**20%**	**40%**
Volume [L]	0.8 - 1		30		1.8 - 2.1		0.5 - 2	
Flow rate [L d^{-1}]	5.8	3.5	5.8	3.5	5.1	2.8	0.7	0.7
HRT [h or d]	4 h	6.8 h	5 d	8.6 d	8 h	14 h	2 – 3 d	2 – 3 d
pH [-]	10.8 ± 0.6	10.9 ± 0.2	6.7 ± 0.4	6.8 ± 0.1	6.2 ± 0.3	6.9 ± 0.2		
EC [mS cm^{-1}]	7.6 ± 2.4	14.6 ± 2.5	10.5 ± 1.2	17.3 ± 1.1	5.1 ± 1.8	5.5 ± 0.4	43.4 ± 9.5	59.6 ± 5.8
DO [mg O_2 L^{-1}]			6.3 ± 2.0	5.6 ± 1.8				
Temperature [°C]	39.8 ± 6.1	40.2 ± 5.7	20.8 ± 1.3	22.3 ± 1.4	22.7 ± 2.3	22.6 ± 1.8		
Potential [V]					4.1 ± 2.1	3.9 ± 0.6		
Current [A]					0.05 ± 0.03	0.06 ± 0.01		

2.3 Nitrification batch activity tests at different salinities

To determine the salt tolerance of ureolytic bacteria, AOB and NOB on the beads, Erlenmeyer flasks were filled with 200 mL beads and 300 mL mixed liquor from the bioreactor (operated using a 40% urine solution and an EC around 20 mS cm^{-1}). The EC was increased by adding a salt mix (1/5 NaCl and 4/5 $NaNO_3$ on a mass basis) to the Erlenmeyer flasks. Five different salt additions were tested: 0, 20, 40, 60 and 80 g salt L^{-1} mixed liquor resulting in an EC of 20, 39, 58, 77 and 96 mS cm^{-1}. The ureolysis rate was determined by adding urea (50 mg N L^{-1}) and allylthiourea (250 mg L^{-1}, to inhibit the ammonia oxidation) to the Erlenmeyer flasks. AOB activity was analysed by adding 2.5 mL of urine and 0.5 g L^{-1} $NaHCO_3$. NOB activity was determined by spiking the flasks with $NaNO_2$ (50 mg N L^{-1}). The tests were performed in triplicate in a room controlled at 28°C. The flasks were covered with parafilm to prevent evaporation and shaken at 130 rpm using an orbital shaker (Innova® 2300, New Brunswick, The Netherlands) to provide aeration. Samples were taken at a 30 minute time interval. Afterwards, they were filtered (0.20 µm Chromafil® Xtra filter, Macherey-Nagel, PA, USA) and stored in the fridge. Ammonium and nitrite concentrations were determined spectrophotometrically with a Tecan infinite plate reader (Infinite® F50 Absorbance Microplate Reader, Tecan Trading AG, Männedorf, Switserland) according to the Berthelot reaction (at 690 nm) and Montgomerey reaction (at 540 nm), respectively (Bucur et al. 2006, Montgomery and Dymock 1961). The experiments lasted between 3 (at low salinity) and 24 (at high salinity) hours. Activity rates were derived from the slope of the ammonium (ureolytic and AOB activity) or nitrite concentration (NOB activity) in function of time. A linear model was fitted to the data in R using the 'stats' package (version 3.4.0) (R Core Team 2017). Prior to formal hypothesis testing, the assumptions of homoscedasticity and normality of the residuals were visually assessed and confirmed with the Bartlett test and Shapiro test in R.

2.4 ED batch phosphate transport experiment

A batch experiment on the ED unit was performed in order to investigate the influence of the diluate pH on the phosphate transport to the concentrate. The diluate tank was filled with 2 L of UF filtrate with an EC around 20 mS cm^{-1} at the start of each experiment and the pH was adapted with HCl or NaOH to a pH of 6, 7 or 8. The concentrate tank was filled with 1.5 L of demineralised water. The current of the power supply was automatically controlled at 90% of the LCD based on the EC of the diluate. The current, voltage, pH of

the diluate and conductivity of the diluate and concentrate were continuously monitored throughout the experiment. Samples were taken at a 30 ot 60 minute time interval, at the beginning and end of the batch experiment, respectively. Afterwards, they were filtered (0.20 µm Chromafil® Xtra filter, Macherey-Nagel, PA, USA) and stored in the fridge (4°C) prior to analysis. The experiments were ended after 7 h (EC diluate < 2.3 mS cm^{-1}).

2.5 Analytical methods

Chloride, nitrite, nitrate, sulphate and phosphate were analysed with anion chromatography (930 Compact IC Flex with Metrosep A supp 5-150/4.0 column and conductivity detector, Metrohm, Herisau, Switzerland). Sodium, total ammonium nitrogen (TAN) and potassium were measured using cation chromatography (761 Compact IC with Metrosep C6-250/4.0 column and conductivity detector, Metrohm, Herisau, Switzerland). Calcium and magnesium concentrations were analysed by means of flame atomic absorption spectrometry (Shimadzu AA-6300, Shimadzu, Kyoto, Japan). The samples were diluted and acidified with 1% nitric acid and 2% of lanthanum solution. COD was determined with Nanocolor® test tubes (Nanocolor® COD 15000, Macherey-Nagel, PA, USA) in unfiltered samples. Total Kjeldahl nitrogen (TKN) was analysed according to Standard methods (Greenberg (1992)).

2.6 Microbial community analysis

Samples of the biofilm carriers from the cultivation reactor (CR) and pilot reactor (PR) were collected throughout the experiment for microbial community analysis. A distinction was made between “young” yellowish beads and “mature” brownish beads based on a visual colour difference. The “young” beads developed their color under the autotrophic conditions of the CR and this color typically matured into brownish upon several weeks exposure to the heterotrophic real urine conditions in the PR (Figure S4). The beads were stored at -80°C. For each sampling time point, three beads were pooled for DNA extraction using the method described by De Paepe et al. (2017). DNA extracts were sent out to LGC Genomics (Teddington, UK) for library preparation and sequencing of the V3-V4 region of the 16S rRNA gene on an Illumina Miseq platform. The sequence data are deposited at the NCBI (National Center for Biotechnology Information) database under accession number SRP111125. The data was processed using the mothur

246 software package (v.1.38.0) (Schloss et al. 2009) as outlined by De Paepe et al. (2017) and described in

247 section 1.2 in SI.

3. RESULTS

3.1 Precipitation on a 20% urine solution

To prevent scaling in the ED stack, removal of calcium and magnesium was targeted in the precipitation reactor. The fivefold dilution (20% urine solution) resulted in influent concentrations of 31 ± 10 mg Ca^{2+} L^{-1} and 11 ± 5 mg Mg^{2+} L^{-1}, respectively. Precipitation lowered the concentrations to 1.9 ± 1.0 mg Ca^{2+} L^{-1} (94% reduction) and 1.6 ± 0.4 mg Mg^{2+} L^{-1} (84% reduction). Concomittantly, 32% of the phosphate and 17% of the sulphate was precipitated. The sodium concentration increased threefold due to the dosage of NaOH to increase the pH. The ammonium concentration increased by a factor of 2.4, indicating that some ureolysis occurred in the influent tubing or in the precipitation reactor despite the high pH.

3.2 Nitrification bioreactor

3.2.1 Operation using a 20% urine solution

In the bioreactor, organic nitrogen and ammonium were converted into nitrate in order to stabilise the urine and to be able to capture the nitrogen in a non-volatile form in ED. On average, 1.2 L d^{-1} of urine with an average TKN concentration of 5.4 g N L^{-1} (before dilution) was treated, which corresponded to a volumetric nitrogen loading rate of 214 ± 85 mg N L^{-1} d^{-1}. Approximately one third of the TKN was already hydrolysed to ammonium before the urine entered the bioreactor (Figure 1A+B). On average 92% of the nitrogen present in the influent was converted into nitrate (Figure 1A+D). Some nitrogen (<1%) was not fully nitrified and was still present under the form of ammonium or nitrite in the effluent (Figure 1B+C). The missing, nitrogen (<8%) was most likely lost in the precipitation reactor and the bioreactor due to struvite precipitation, ammonia stripping and assimilation by the biomass, and also denitrification or N_2O production could not be excluded. The time series data, presented in Figure S6, showed effluent concentrations below 0.5 mg NH_4^+-N L^{-1} during 55% of the operation time and below 5 mg NH_4^+-N L^{-1} during 80% of the time. Only during reactor upsets (pH control failure or influent dosing problems), the concentration increased above 20 mg NH_4^+-N L^{-1}. Another purpose of the bioreactor was to remove the organic matter in order to prevent biofouling in ED. The chemical oxygen demand (COD) in the influent and effluent of the bioreactor was 818 ± 214 and 65 ± 13 mg L^{-1}, respectively, which corresponds to a removal percentage of 92%.

275 **FIGURE 1 (single column, no color)**

3.2.2 Operation using a 40% urine solution

The bioreactor was operated using a 40% urine solution to study the effect of a higher salinity on the nitrification. To maintain the same nitrogen loading rate of 215 mg N L^{-1} d^{-1}, the total influent rate was decreased from 5.8 to 3.5 L d^{-1}. As a consequence, the HRT increased from 5.0 to 8.6 days and the influent concentrations of the different ions doubled compared to the test on a 20% urine solution, increasing the EC in the bioreactor to 17.3 ± 1.1 mS cm^{-1} (compared to 10.5 ± 1.2 mS cm^{-1} in the test on a 20% urine solution). The fraction of organic nitrogen in the influent was considerably lower compared to the period on a 20% urine solution (Figure 1E), which is probably due to ureolysis as a result of the longer residence time in the influent line or in the precipitation reactor. No residual ammonium or nitrite were detected in the effluent of the bioreactor (Figure 1F+G), apart from accumulations on day 16 (21 mg NO_2^--N L^{-1}), day 27 (62 mg NO_2^--N L^{-1}) and day 28 (16 mg NH_4^+-N L^{-1} and 89 mg NO_2^--N L^{-1}) resulting from technical upsets. The nitrate concentration in the effluent reached 1477 ± 81 mg NO_3^--N L^{-1} (almost double compared to the test on a 20% urine solution) (Figure 1H). Again, 91% of the COD was removed but due to the higher influent concentration, the COD in the effluent was more than twice higher (158 mg COD L^{-1} instead of 65 mg COD L^{-1}) compared to the period on a 20% urine solution.

3.2.3 Halotolerance of the nitrifying biofilm

The short-term effect of a higher EC on maximum ureolysis, nitritation and nitratation rates was investigated in a batch experiment in which the beads were exposed to short-term salt stress by adding a salt mix of NaCl and $NaNO_3$. The ureolysis rate decreased by 52% from 1249 mg N L^{-1} d^{-1} at an EC of 20 mS cm^{-1} to 600 mg N L^{-1} d^{-1} at an EC of 96 mS cm^{-1} (Figure 2). The nitritation rate was barely affected at low EC (< 58 mS cm^{-1}) but decreased with 77% between an EC of 58 mS cm^{-1} and 96 mS cm^{-1}. The nitratation activity decreased almost linearly (p=3e-8, R^2 of 0.91) with more than 96% between 20 and 96 mS cm^{-1}. The ureolysis/nitritation ratio increased whereas the nitratation/nitritation ratio decreased at higher salt concentrations. Although the NOB activity was most severely affected at high EC, nitritation remained the rate limiting process.

FIGURE 2 (single column, no color)

3.2.4 Microbial community composition

The microbial community colonising the beads in the cultivation reactor (CR) and pilot reactor (PR) was followed up over time through next-generation 16S rRNA gene amplicon sequencing to track the influence of a synthetic urine solution *versus* the complex urine matrix. The microbial community at phylum level was dominated by *Proteobacteria* (70 ± 10%) and *Bacteroidetes* (23 ± 9%), while *Acidobacteria* gradually increased over time (up to 15% in the PR) (Figure S7). The most abundant families were *Moraxellaceae*, *Comamonadaceae*, *Xanthomonadaceae, Chitinophagaceae* and the most prevalent genera included *Acinetobacter*, *Luteimonas*, *Nitrosomonas* and *Comamonas* (Figure S8-S9). Approximately 30% of the community could not be classified at the genus level. The most dominant OTUs (Operational Taxonomic Unit) were related to *Acinetobacter venetianus* (OTU1), *Comamonas sp.* (OTU2), *Luteimonas aquatica* (OTU3) and *Nitrosomonas sp.* (OTU4) (Table S2, Figure S10).

A principle coordinate analysis (PCoA) of the microbial community on the beads of the CR and PR at the OTU level (Figure 3) revealed how the reactor-specific microbial community developed over time. After start-up and stabilisation, samples originating from the same reactor (CR or PR) are clustered together. CR, operated using a synthetic influent, was characterized by the dominance of *Acinetobacter*. On the other hand, *Comamonas, Luteimonas* and *Ferruginibacter* were more characteristic for PR (Figure S9). Samples of PR were more scattered and the observed shifts coincided with shifts in the influent composition, as demonstrated by the arrows in Figure 3. *Acinetobacter*, *Comamonas* and *Ferruginibacter* became more estabilished on the beads when the influent was shifted from the urea solution to real urine, whereas *Azoarcus* and *Dokdonella* disappeared. During operation using a 20% urine solution, the relative abundance of *Luteimonas* and *Nitrosomonas* gradually increased. Changing the influent back to a synthetic $(NH_4)_2SO_4$ solution between the two experiments (to maintain the microbial culture), resulted in a decreased relative abundance of *Acinetobacter* and further increased abundance of *Luteimonas*. Feeding the PR with a 40% urine solution afterwards, led to an increased enrichment of *Comamonas* on the beads.

Nitrosomonas was the sole known AOB genus present in the CR and PR, with a total relative abundance around 7%. Further classification using NCBI BLAST, RDP SeqMatch and a Maximum Likelihood phylogenetic

tree indicated that the key players in the AOB community were closely related to *N. aestuarii*, *N. marina*, *N. europaea* and *N. ureae* (Table S2, Figure S11-S12). OTU4, which showed the highest sequence identity to *N. aestuarii/marina*, was the most abundant AOB with relative abundances reaching up to 90% in some samples. Interestingly, OTU24, most similar to *N. europaea,* which is only moderately halotolerant, was replaced by more halophilic *N. aestuarii/marina/ureae*-like organisms in the PR when the influent was shifted from a synthetic influent to real urine. When the influent was changed back to a synthetic $(NH_4)_2SO_4$ solution, OTU81 and OTU91 (most similar to *N. halophila* and *N. ureae,* respectively) proportionally increased but disappeared when the reactor was operated again on real urine (40%).

158 *Bradyrhizobiaceae* (family including NOB) related OTUs were found in the microbial community, but individual OTUs were <1% of the sequenced community (Figure S8). Neither qPCR gave sufficient results to draw conclusions from.

FIGURE 3 (single column, color)

3.3 Electrodialysis

3.3.1 Operation using a 20% urine solution

The effluent of the bioreactor, with an EC of 10.5 ± 1.2 mS cm^{-1}, was fed into the ED to concentrate the nutrients. The predominant ions in the feed stream of the ED were sodium (of which >75% originates from the NaOH dosage in the precipitation reactor and nitrification reactor), nitrate (due to nitrification), chloride and potassium (Figure 4A). By applying an electric field, on average 4.1 L d^{-1} of diluate with an EC of 5.1 ± 1.8 mS cm^{-1} and 0.7 L d^{-1} of concentrate with an EC around 50 mS cm^{-1} was produced. The average recovery efficiencies (Figure 4C) varied between 40% and 74% with the highest efficiency for sulphate (74%), followed by potassium (71%) and nitrate (70%). Only 40% of the phosphate in the feed stream was transported to the concentrate stream. Sodium and nitrate made up the largest fraction of the transported ions, corresponding to respectively 47% and 35% of the total molar transport, whereas phosphate and sulphate contributed less than 1% to the absolute molar transport. In general, the concentrations in the concentrate were 4-5 times higher than the concentrations in the feed stream (Figure 4D), while only phosphate had a lower concentration factor (2.6).

3.3.2 Operation using a 40% urine solution

Increasing the urine concentration from a 20% to 40% urine solution, almost doubled the ion concentrations and the EC of the feed stream to the ED, but the volumetric salt loading rate remained the same (Figure 4B, Table S3). ED produced on average 2.8 L d^{-1} diluate and 0.7 L d^{-1} concentrate with an EC value of around 5.5 and 60 mS cm-1, respectively. The recovery efficiencies increased by 7 to 14% but the concentration factors of each ion decreased (Figure 4C+D). The coulombic efficiency is compared in Table S3.

FIGURE 4 (double column, full width, no color)

3.3.3 Influence of pH on phosphate transport in ED

The recovery efficiency of phosphate in ED was significantly lower compared to the other ions. Transfer of phosphate through membranes is challenging due to its large size (high molecular mass and large hydration shell resulting in a high Stokes radius) and low diffusion rate in water (Table S5). It was hypothesized that the phosphate transfer to the concentrate could be enhanced by increasing the pH in the diluate since the dominant phosphate species shifts from $H_2PO_4^-$ to HPO_4^{2-} between a pH of 6 and 8 (Table S6). Generally, the higher the charge of an ion, the more the ion is susceptible to the electric field and thus the larger the driving force in the bulk solution (Nernst-Planck). This hypothesis was addressed in a batch experiment in which three different pH values were tested. Since nitrate and chloride removal should not be pH dependent, these ions were used as a reference to confirm whether the difference in recovery efficiency of phosphate could be attributed to the pH difference and not to technical variability between the tests. Phosphate transfer was clearly influenced by the pH as opposed to nitrate and chloride transfer (Figure 5, S14). After an electric charge of 2500 Coulomb (C) had passed through the ED, 29%, 54% and 31% of phosphate was removed from the diluate at a pH of 6, 7 and 8, respectively, yielding pH 7 as optimum.

FIGURE 5 (single column, no color)

3.4 Recovery of N, P and K

The aim of this study was to determine the recovery potential of the nutrients in urine based on the performance of the pilot installation. During operation using a 20% urine solution, on average 32% of the phosphate-P was captured in precipitates. In total, 64% of the nitrogen, 22% of the phosphorus and 70% of

the potassium were captured in the concentrate. On average, 29% of the nitrogen, 33% of the phosphorus and 30% of the potassium remained in the diluate (Figure 6A-C, S13). About 8% of the nitrogen was not recovered, as mentioned in Section 3.2.1. The concentrate contained on average 3.7 g NO_3^--N L^{-1}, 1.5 g K^+ L^{-1} and 0.06 g PO_4^{3-}-P L^{-1}. On a 40% urine solution, 29% of the phosphate-P was captured in precipitates. In total, 70% of the nitrogen, 38% of the phosphate-P and 83% of the potassium were captured in the concentrate. On average, 17% of the nitrogen, 32% of the phosphate-P and 17% of the potassium remained in the diluate (Figure 6D-F). About 13% of the nitrogen was lost. The concentrate contained on average 5.1 g NO_3^--N L^{-1}, 2.3 g K^+ L^{-1} and 0.06 g PO_4^{3-}-P L^{-1}.

FIGURE 6 (single column, no color)

4. DISCUSSION

4.1 Precipitation and nitrification minimize scaling and biofouling in ED

In this study, an integrated system combining precipitation, nitrification and ED was evaluated to refine and concentrate nutrients from fresh urine. Operation of this three-stage system was successfully demonstrated in a relevant environment, corresponding to level 6 on the technology readiness level (TRL) scale. The use of a precipitation reactor proved to be an effective strategy to safeguard the ED from excessive scaling. More than 90% of the calcium and 80% of the magnesium was precipitated and no scaling was observed in the ED unit. Both the precipitation and nitrification reactor require base addition. In the precipitation reactor, approximately 215 mmol NaOH L^{-1} urine was dosed in the precipitation reactor to increase the pH to 11. In the nitrification reactor, base is needed to counteract the acidification caused by nitrification. Without base addition, maximally ~50% of the nitrogen in urine can be converted into nitrate (Udert et al. 2003a). In principle, base addition in the precipitation reactor does not increase the total base consumption, as a more alkaline influent entering the bioreactor reduces the amount of alkaline equivalents required in the bioreactor to counteract the acidification. In total, about 300 mmol NaOH L^{-1} urine was needed to convert all urea into nitrate, of which only one third was dosed in the nitrification reactor.

The use of a bioreactor proved to be an effective strategy to limit biofouling in ED by oxidizing the COD. Moreover, uncharged urea was converted into nitrate in order to stabilise the urine and to capture the nitrogen in a non-volatile form in ED. Although the total nitrogen concentration in the influent fluctuated due to the variable composition of urine, stable nitrification was achieved in the bioreactor with an average volumetric nitrogen loading rate of 215 mg N $L^{-1}_{reactor}$ d^{-1}, and a salinity of 10.5 mS cm^{-1}. Only after reactor disturbances (due to failure of the pH control or influent dosing problems), ammonium was detected in the bioreactor effluent. Nitrifiers quickly recovered once the optimal conditions were restored. Bacteria grown in a biofilm are generally more protected to transient stressors, such as a low pH and high free ammonia concentration due to diffusion limitations (Ikuma 2013). Moreover, nitritation, which causes the acidification, is generally hampered by a low pH, presumably due to a limited ATP generation (Fumasoli et al. 2015). In addition, besides precipitation and protection of the subsequent ED, the precipitation reactor

was also used to increase the alkalinity of the nitrification reactor influent. As a consequence, we never observed the pH dropping below 5.5, in case of pH control failure. It is in this respect also noteworthy that the cultivation reactor was pH controlled solely by providing sufficient alkalinity in the feed and not by a pH-controller.

ED was used to concentrate nutrients in an energy-efficient manner. The concentrate flow was generated by osmotic and electro-osmotic water transport across the membrane and contained about 70% of the ions of the feed stream. The increase in concentrate EC and obtained concentration factors of 3-5 are in line with results obtained by Pronk et al. (2007). Periodic cleaning in place (~once a month) with an acid and alkaline solution was sufficient to keep the ED stack operational >7 months with the same membranes and a stable performance. Such cleaning can easily be automated. However, the anion exchange membranes turned brown (Figure S15), which was also reported by Aponte and Colon (2001). This is probably due to irreversible adsorption of negatively charged organic molecules (Aponte and Colon 2001).

4.2 Nitrification and COD oxidation remained unaffected at a decreased urine dilution

The high salt content of urine and base addition leads to a high EC in the bioreactor. Living in a salty environment is an energetically costly process for bacteria since more energy is required to balance the cell osmotic pressure (Oren 1999). This limits the energy available for nitrification, which typically results in lower activity rates at high EC. The salt tolerance of the nitrifying community on the beads was assessed in a batch experiment in which beads were exposed to a short-term salt shock. The maximum ureolysis, nitritation and nitratation rates of the beads decreased with 52%, 82% and 96%, respectively, when the EC was increased from 20 mS cm^{-1} to 96 mS cm^{-1}. Interestingly, the rate limiting nitritation and hence, the overall nitrification rate, remained unaffected up to 40 mS cm^{-1}. This also explains why nitrification was not affected during continuous operation using a 40% urine solution, despite the higher EC (17.3 mS cm^{-1} instead of 10.5 mS cm^{-1}). The results of the batch experiment suggest that the bioreactor can operate on undiluted urine (expected EC between 45-60 mS cm^{-1}) but at a lower volumetric rate, although this should be tested on the long-term. The relatively high salt resistance of the beads is probably a result of the inoculum selection (Coppens et al. 2016). The higher drop in nitratation rate indicates that the NOB on the beads were more sensitive to the short-term salt stress than the AOB. Literature is inconclusive concerning

the salt tolerance of AOB *versus* NOB, due to differences in experimental set-up, operational conditions and test duration, salt dosage and AOB and NOB community composition (Moussa et al. 2006). Moussa et al. (2006) and Coppens et al. (2016) reported that AOB were more sensitive to salt stress. On the other hand, Bassin et al. (2011), Dincer and Kargi (1999), Cui et al. (2009) and Cortes-Lorenzo et al. (2015) concluded, based on the accumulation of nitrite in their experiments, that NOB were more affected by salinity.

4.3 Shifts in microbial community composition parallel shifts in influent composition

The shifts in microbial community composition in the pilot reactor coincided with changes in the influent composition, as shown by PCoA analysis (Figure 3) and may be attributed to the nature of the organic fraction in the influent (acetate in synthetic medium *versus* complex COD matrix in urine). In addition, salinity is known to affect the microbial community composition in nitrification reactors (Bassin et al. 2012, Coppens et al. 2016, Cortes-Lorenzo et al. 2015, Gonzalez-Silva 2016, Moussa et al. 2006). Classification using NCBI BLAST, RDP SeqMatch and a maximum likelihood phylogenetic tree indicated that the AOB community was dominated by species closely related to *N. aestuarii* or *N. marina*. Both species are obligate halophilic and described in marine environments (Koops et al. 1991). *N. aestuarii* was also found to be the dominating AOB species in reactors operated on seawater (Gonzalez-Silva 2016, Sudarno et al. 2010). The presence of these halophilic AOB species can also explain why the nitritation activity was so well preserved at high EC in the batch experiment. Besides salinity, the shifts in the AOB community in the pilot reactor corresponded to the nitrogen source (urea-N or ammonium-N). OTUs related to the urease-negative *N. europaea* and *N. halophila* (Koops et al. 1991), only thrived when the reactor was operated using a synthetic ammonium sulphate solution.

4.4 Lowering the urine dilution leads to a higher recovery efficiency but a lower concentration factor in ED

Increasing the urine concentration (from 20% to 40%), decreased the volume ratio of diluate to concentrate from 5.8 to 4. Due to the higher EC gradient between the diluate and concentrate and the increased transport of ions from diluate to concentrate, the osmotic and electro-osmotic water transport increased. Hence, relatively more concentrate was produced on a 40% urine solution. Depending on the ion, the recovery efficiency increased by 7 to 14%, which is probably due to the higher residence time in the diluate

tank (14h instead of 8h). Despite the higher end concentrations and overall EC of the concentrate on a 40% urine solution, the concentration factors decreased, as a result of the higher ion concentrations in the feed stream. To conclude, more nutrients were captured in the concentrate, but in a comparatively larger concentrate volume.

4.5 Strategies to increase nutrient recovery efficiencies

Respectively 70% and 80% of the ions were captured in 15% and 20% of the initial volume when the pilot installation was operated using a 20% or 40% urine solution. It is almost impossible to capture all nutrients in the concentrate. As a result of the equilibrium established between the diluate and concentrate stream, part of the nutrients remain in the diluate. Additionally, relatively low concentration factors (ion concentration in the concentrate stream devided by the concentration in the feed stream) present a second limitation inherent to ED due to the osmotic and electro-osmotic water transport. A high concentration gradient between the diluate and the concentrate, leads to increased osmotic water transport from diluate to concentrate and back diffusion of ions from concentrate to diluate, which in turn limits the maximum achievable concentration factor.

In case of phosphate, the large size and low diffusion rate of the ion limits the recovery efficiency to 40%, as opposed to 70%-75% for all other ions, corresponding to a concentration factor of only 2.6. It was hypothesized that the phosphate transport would increase by increasing the pH in the diluate since more phosphate ions are dissociated at a higher pH, which could facilitate the transport, as the ions are more susceptible to the electric field. The recovery efficiency of phosphate in the batch test at pH 7 was indeed higher compared to pH 6. The slower phosphate transport at pH 8 compared to pH 7 can possibly be attributed to competition in migration with carbonate ions. Like phosphate, the charge of carbonate depends on the pH. Most of the carbonate is uncharged (H_2CO_3) at pH 6 while more than 90% of the carbonate species have a charge of -1 (HCO_3^-) at pH 8 (Table S7). Further research is needed to confirm the hypothesis of reduced phosphorus transport due to carbonate competition at elevated pH.

Another way to recover more phosphorus, is to precipitate all the phosphorus in the precipitation reactor. Phosphorus precipitation is limited by calcium and magnesium due to the excess of anions (phosphate, sulphate and carbonate) in urine. By substituting NaOH by $Ca(OH)_2$ or $Mg(OH)_2$ to increase the pH in the

precipitation reactor or adding another calcium or magnesium source, up to 90% of the phosphate can be precipitated (Etter et al. 2011). However, excess calcium or magnesium dosage should be avoided to prevent scaling issues in the ED unit.

Decreasing the concentrate volume by means of distillation is an option to concentrate the nutrients in a smaller volume. Distillation was already applied by Udert and Wachter (2012) in combination with nitrification to concentrate nutrients in urine. All nutrients were recovered in a dry solid. However, the high energy demand of distillation (~700 $Wh_{primary\ energy}\ L^{-1}$ urine) presents a major drawback of the process. The energy consumption of ED is significantly lower. The electrode power consumption of ED in this study equalled only 4.3 $Wh_{electrical\ energy}\ L^{-1}$ urine or 14 $Wh_{primary\ energy}\ L^{-1}$ urine (Table S4). However, ED removed only ~88% of the water, whereas distillation can remove almost all water. Udert and Wachter (2012) suggested that the energy demand could be significantly reduced by first removing 80% of the water with reverse osmosis and subsequently operating distillation with vapor compression (~100 Wh L^{-1} urine). Alternatively, the required 80% water removal could be obtained by ED instead of RO, as demonstrated in this study. To conclude, a combination of precipitation, nitrification, ED and distillation could offer a maximal concentration of nutrients with a minimal input of energy.

4.6 Resource reuse possibilities for precipitate, ED concentrate and ED diluate

The output of the integrated system consists of the precipitates formed in the precipitation reactor and the diluate and concentrate stream of the ED unit. The precipitates could be used as a solid phosphorus fertilizer in agriculture. About 30% of the phosphate in urine was incorporated in the precipitates, which corresponds to values reported in literature (Udert et al. 2006). The use of urine derived precipitates as fertilizer has been demonstrated by Bonvin et al. (2015). Moreover, it was reported by Escher et al. (2006) and Ronteltap et al. (2007) that urine precipitates were clean and safe fertilizers since the micropollutants in urine (pharmaceuticals, hormones…) mainly remained in the liquid phase.

The ED concentrate is rich in nutrients, predominantly nitrogen (NPK = 85.9/0.7/13.4 mole%), which is present under the form of nitrate. Nitrate, in contrast to urea, ammonia or ammonium nitrate, is a thermally stable, non-volatile molecule and, in a lot of cases, the preferred nitrogen source for plants (Marschner 1995, Udert and Wachter 2012). Besides nitrogen, plant growth requirements are also met for

potassium whereas phosphorus will be the limiting macronutrient in the ED concentrate due to the partial incorporation in the precipitates (Larsen et al. 2013). The excessive salt concentration, particularly sodium, resulting from the NaOH demand by precipitation and nitrification, might present another barrier for fertilizer application. In this regards, partial nitrification is a promising new lead to reduce the sodium load, simultaneously saving chemicals (Udert and Wachter 2012). The dense nature of the ED membranes typically results in a high retention of pathogens and micropollutants in the diluate (Escher et al. 2006, Pronk et al. 2006b, Pronk and Kone 2009). However, Pronk et al. (2006a) showed that breakthrough of certain micropollutants (e.g., propranolol and ibuprofen) can occur over time. To guarantee a micropollutant-free product, a post-treatment, such as ozonation or adsorption on activated carbon, can be included to remove the residual micropollutants in the concentrate (Dodd et al. 2008, Larsen et al. 2013, Pronk et al. 2007, Udert et al. 2016). Ozonation is even more effective in combination with a nitrate based, COD low stream given the ozone scavenging potential of ammonium and competition between COD and micropollutants for the chemical oxidant (Larsen et al. 2013, Pronk et al. 2007).

Finally, the ED diluate is low in nutrients and salts, which makes it a suitable stream for water recovery through membrane filtration, as particularly relevant for regenerative life support systems for human Space exploration (Clauwaert et al. 2017, Lindeboom et al. 2015).

5. CONCLUSION

- An electrodialysis (ED) stack was operated for ~7 months on real, diluted urine. The use of a precipitation reactor and aerobic bioreactor proved to be an effective strategy to minimize scaling and biofouling in ED.
- More than 90% and 95% of incoming COD and urea were converted in the bioreactor at salinities of 10 to 20 mS cm^{-1}. Shifts in the microbial community coincided with changes in the influent composition of the bioreactor. The AOB community was dominated by species closely related to *Nitrosomonas aestuarii* and *Nitrosomonas marina.*
- Respectively 70% and 80% of the ions were captured in 15% and 20% of the initial volume when the pilot installation was operated using a 20% or 40% urine solution.
- The P-rich precipitates and N+K-rich ED concentrate can be valorised as fertilizers, whereas water can be recovered from the ED diluate. Further research on the fate of micropollutants and pathogens in the three-stage system is necessary to valorise the recovered fertilizers

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7. SUPPLEMENTARY INFORMATION

Supplementary information related to this article can be found at [http link].

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FIGURE CAPTIONS

Figure 1. **Nitrogen concentrations in the influent and effluent of the bioreactor on 20% and 40% urine solutions.** Total nitrogen (TN) is calculated as the sum of total Kjeldahl nitrogen (TKN), NO_2^--N and NO_3^--N. Organic N is calculated by subtracting NH_4^+-N from TKN. Inorganic N equals the sum of NH_4^+-N, NO_2^--N and NO_3^--N. Samples were analysed over a period of 100 days on a 20% urine solution (n=35) and over a period of 40 days on a 40% urine solution (n=16). The box-and-whisker plots depict the minimum, first quartile, median, third quartile, maximum and outlying points (>1.5 x interquartile range).

Figure 2. **Maximum ureolysis, nitritation and nitratation rates and ratios as a function of electrical conductivity (EC).** The error bars display the standard deviation (n=3).

Figure 3. **Principle coordinate analysis (PCoA) biplot of the microbial community composition on the beads in the cultivation reactor (CR) and pilot reactor (PR) at the OTU level, based on the Jaccard distances as determined by next-generation 16S rRNA gene amplicon sequencing.** Each sample is indicated by a symbol with a shape according to the reactor and bead and a colour according to the influent composition. A visual distinction was made between mature and young beads based on the difference in colour of the beads (Figure S4). The numbers (1-10) correspond to the sampling day (day 1, 37, 72, 86, 100, 114, 135, 156, 218 and 259). Day 1 refers to the day of the first sample, corresponding to, respectively, 150 and 1 days after start-up of CR and PR.

Figure 4. **Performance of the electrodialysis (ED) unit on 20% and 40% urine solutions.** Each plot displays average values and standard deviations, based on 35 samples over 100 days using a 20% urine solution and 16 samples over 40 days operating using a 40% urine solution.

A) Concentration in the ED influent and diluate during operation using a 20% urine solution.

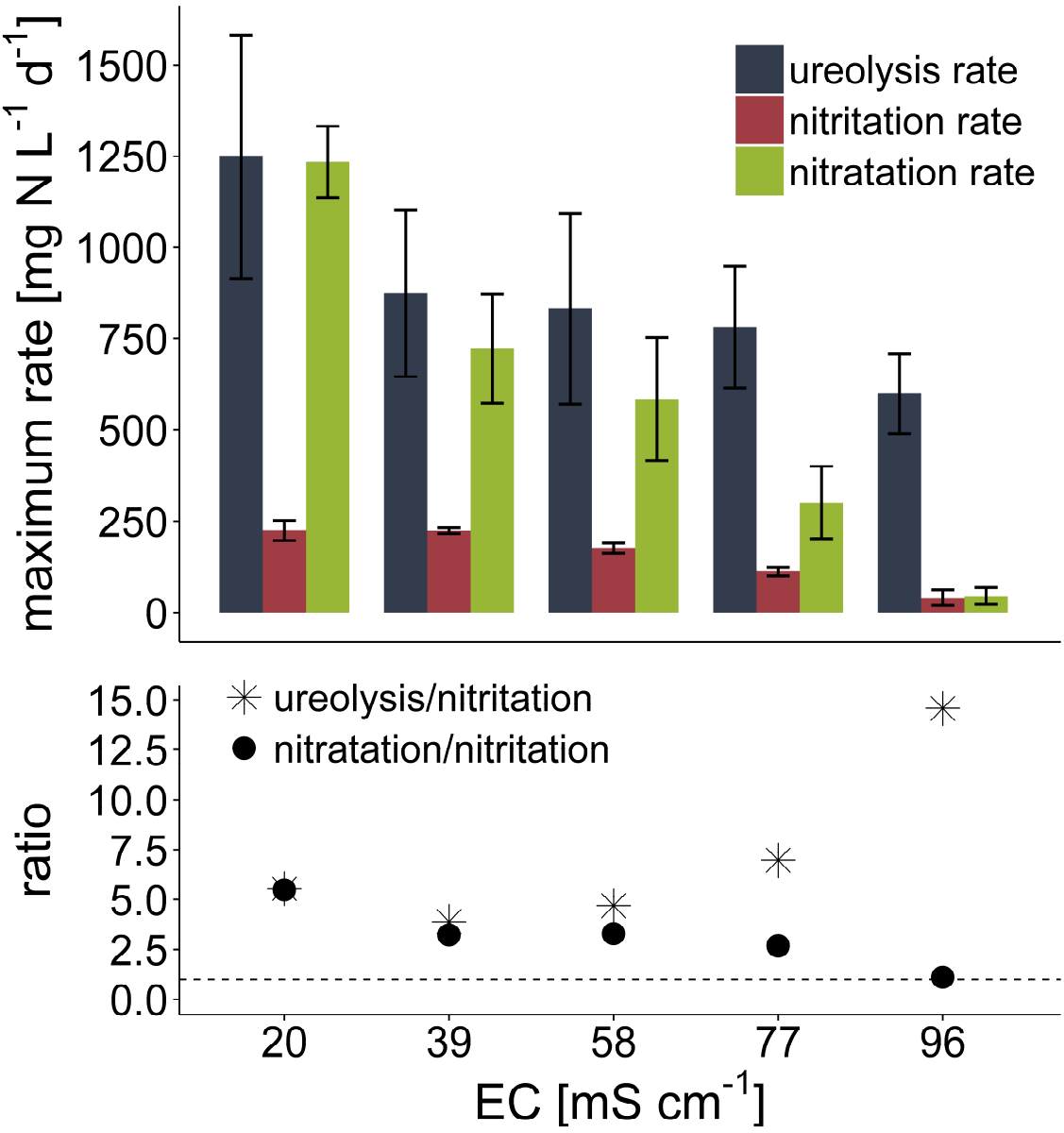
B) Concentration in the ED influent and diluate during operation using a 40% urine solution.

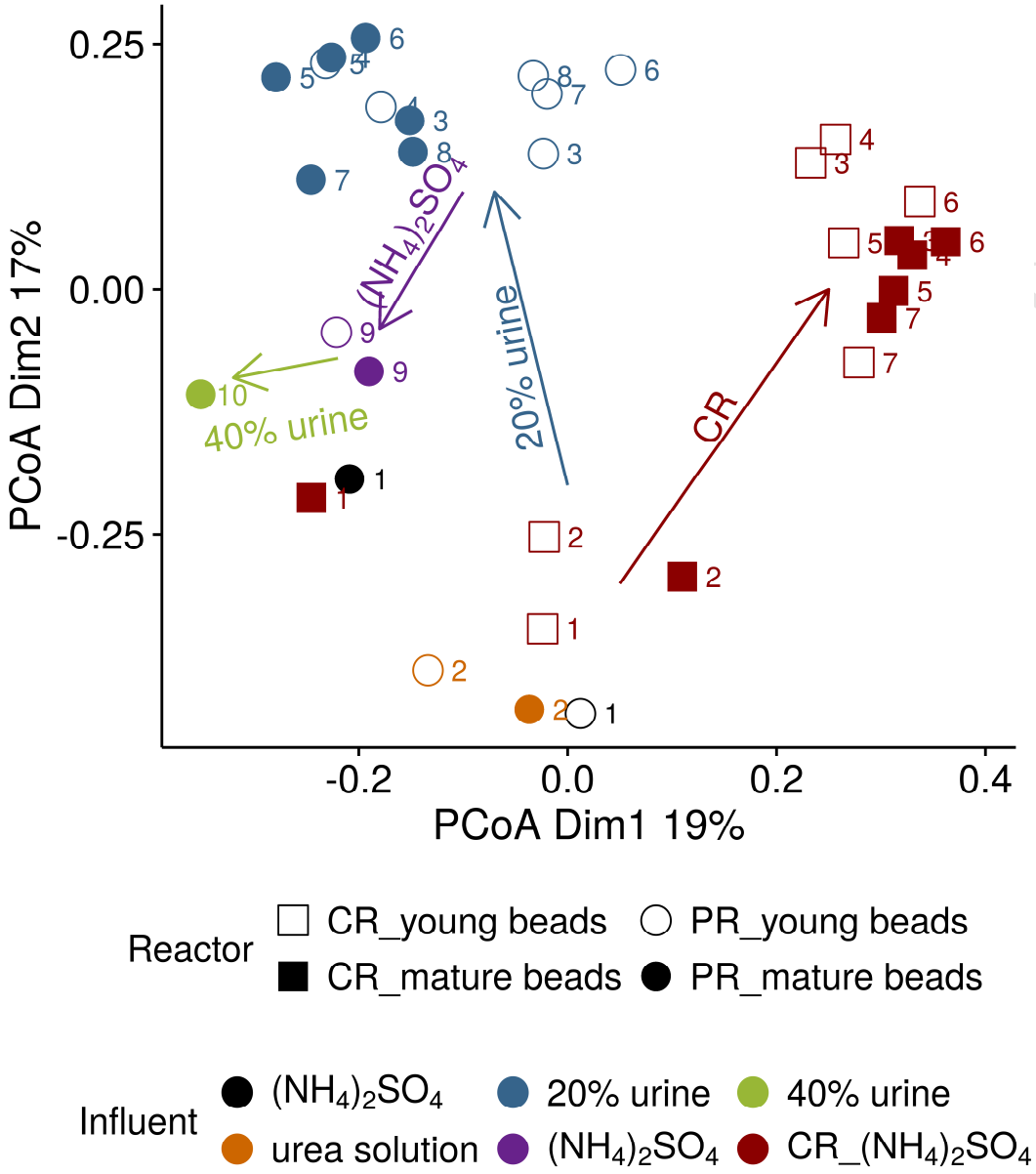
C) Recovery efficiency, calculated by dividing the difference in mass of an ion between the feed stream (ED influent) and diluate by the mass of the ion in the feed stream.

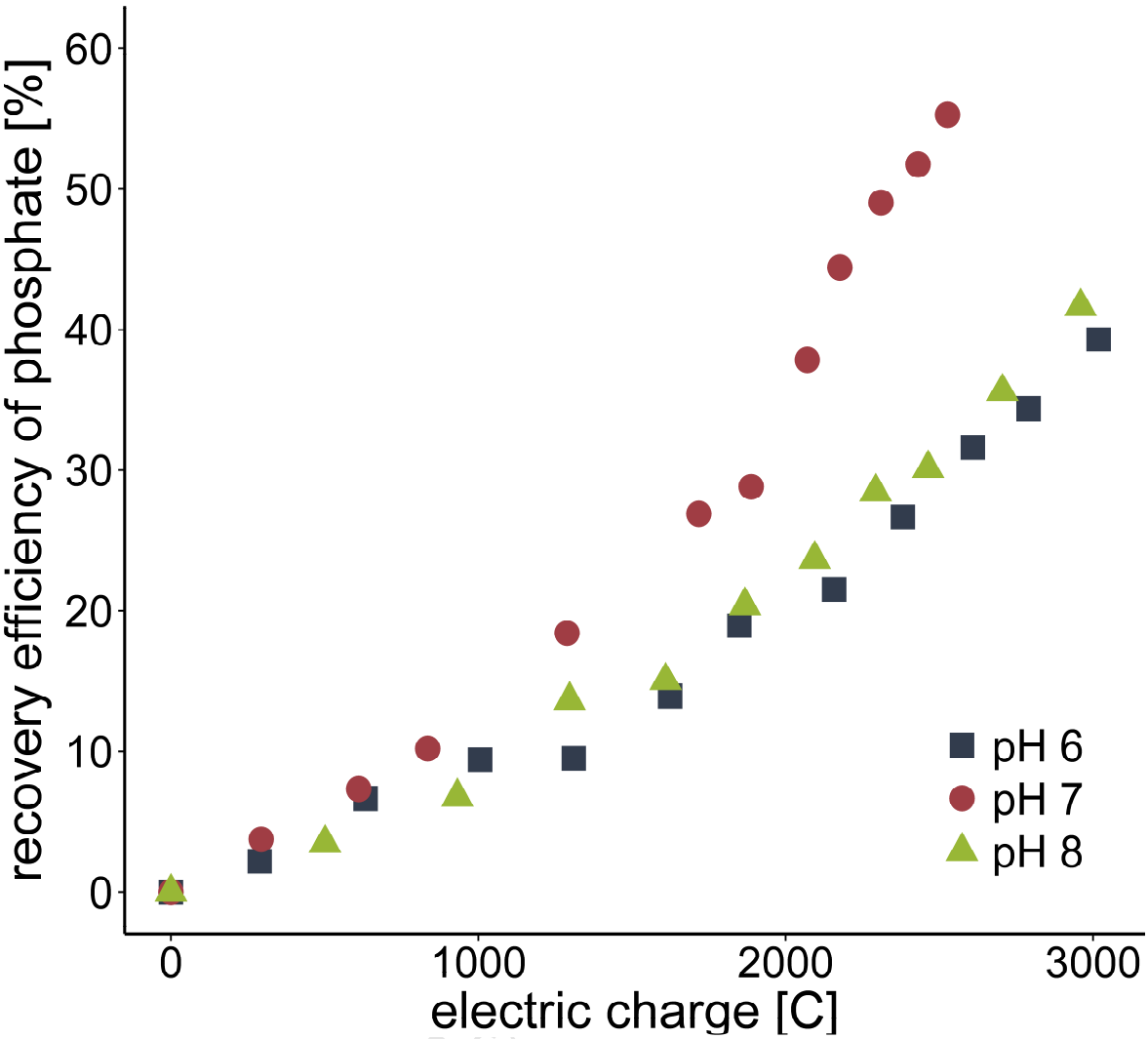
D) Concentration factor, calculated by dividing the ion concentration in the concentrate stream by the concentration in the feed stream (ED influent).

Figure 5. **Recovery efficiency of phosphate in function of the cumulative electric charge that passed through the stack in the ED batch experiment at pH 6, 7 and 8.** The cumulative electric charge (in Coulomb) was calculated by multiplying the applied current and time ($Q_{i+1}=Q_i + I_{i+1}*(t_{i+1}-t_i)$). The recovery efficiency was calculated by dividing the difference in phosphate concentration in the diluate at time t and t_0 by the phosphate concentration at t_0.

Figure 6. **Nitrogen, phosphorus and potassium concentration in the different stages of the pilot installation during operation using 20% and 40% urine solutions.** Samples were analysed over a period of 100 days on a 20% urine solution (n=35) and over a period of 40 days on a 40% urine solution (n=16). The nitrogen concentration in the influent is calculated as the sum of total Kjeldahl nitrogen (TKN), NO_2^--N and NO_3^--N concentrations. The nitrogen concentration after nitrification, in the diluate and concentrate is the sum of NH_4^+-N, NO_2^--N and NO_3^--N.


maximum rate [mg N L-1 d-1]
1500
1250
1000
750
500
250
0
ureolysis rate
nitritation rate
nitratation rate
ratio
15.0
12.5
10.0
7.5
5.0
2.5
0.0
ureolysis/nitritation
nitratation/nitritation
20
39
58
77
96
EC [mS cm-1]


0.25
0.00
-0.25
PCoA Dim2 17%
-0.2
0.0
0.2
0.4
PCoA Dim1 19%
(NH4)2SO4
20% urine
40% urine
CR
Reactor
CR_young beads
PR_young beads
CR_mature beads
PR_mature beads
Influent
(NH4)2SO4
20% urine
40% urine
urea solution
(NH4)2SO4
CR_(NH4)2SO4


recovery efficiency of phosphate [%]
60
50
40
30
20
10
0
0
1000
2000
3000
electric charge [C]
pH 6
pH 7
pH 8

HIGHLIGHTS

1. Pre-treatment enables ED operation on real urine with minimal scaling & biofouling
2. 64-70% of N, 54-67% of P, 70-83% of K was captured in 15-20% of the initial volume
3. Base dosage for precipitation equally lowered base demand for full nitrification
4. More than 90% and 95% of incoming COD and urea were converted at 10-20 mS cm^{-1}
5. Nitrifying sludge cultivated at 10 mS cm^{-1} still demonstrated activity >90 mS cm^{-1}