Life Sciences in Space Research 18 (2018) 12–20

Contents lists available at ScienceDirect

Life Sciences in Space Research

journal homepage: www.elsevier.com/locate/lssr

# The influence of nitrogen concentration and precipitation on fertilizer production from urine using a trickling filter

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## ARTICLE INFO

*Keywords:*
Life support
Urine
Waste management
Biofilter
Nitrification

## ABSTRACT

Planetary habitation requires technology to maintain natural microbial processes, which make nutrients from biowaste available for plant cultivation. This study describes a 646 day experiment, in which trickling filters were monitored for their ability to mineralize nitrogen when loaded with artificial urine solutions of different concentrations (40, 60, 80 and 100% v/v). Former studies have indicated that increasing urine concentrations slow nitrogen conversion rates and induce growing instability. In the current experiment, nitrogen conversion rates, measured as nitrate production/day, did not differ between concentration levels and increasing instability was not observed. Instead, the buffering capacity of the mussel shells added as buffer system (~75% calcium carbonate) increased with increasing concentrations of synthetic urine possibly due to the higher phosphate content. The intensified precipitation of calcium phosphates seems to promote carbonate dissolution leading to improved buffering. For space applications, the precipitation of calcium phosphates is not desirable as for the phosphate to be available to the plants the precipitate must be treated with hazardous substances. With regard to terrestrial agriculture the process-integrated phosphate precipitation is a possibility to separate the macronutrients nitrogen and phosphate without addition of other chemicals. Thus, the described process offers a simple and cost-effective approach to fertilizer production from biogenic residues like slurry.

## 1. Introduction

Long-term planetary habitation requires biological life support system components with a high degree of closure (Drysdale et al., 2003, 2004). Special attention has to be paid to nutrient cycles, because these can only be closed to a high degree when plants as primary producers are involved (Binot et al., 1994; Gitelson et al., 1995; Hu et al., 2010; Tikhomirov et al., 2011). In a closed system all nutrients needed for plant growth are present in the waste streams produced by the crew and the plants themselves. Due to its high urea content urine is a good source of nitrogen but lacks e.g. phosphate and potassium. These have to be added, preferably by the use of other waste streams like faeces and the inedible parts of the plants. As human consumption of table salt is quite high, the resulting fertilizer contains more sodium chloride than common vegetable crops can tolerate without being adversely affected in growth. A possibility to recover the salt and tune the fertilizer for vegetable production is the cultivation of edible salt accumulating plants like *Salicornia europaea* (Tikhomirova et al., 2011).

The mineralization process converting biowaste to plant nutrients relies on the metabolism of a variety of microorganisms. For maintenance and utilization of such functional microorganisms in bioreactors, several approaches exist. A very simple and energy saving approach is the use of trickling filters, in which gravity ensures the distribution and aeration of the processed liquid. The only energy consuming element in the system is the pump which constitutes constant recirculation (Bornemann et al., 2015; Tchobanoglous et al., 2004). The mineralization of urea comprises three steps: (1) hydrolysis of urea to ammonium: $(NH_2)_2CO + H_2O \rightarrow CO_2 + 2\ NH_3$, (2) oxidation of ammonium to nitrite: $2\ NH_3 + 3\ O_2 \rightarrow 2\ NO_2^- + 2\ H^+ + 2\ H_2O$, and (3) oxidation of nitrite to nitrate: $2\ NO_2^- + O_2 \rightarrow 2\ NO_3^-$. Nitrate is the preferred nitrogen source of many plants and the desired product of the degradation process (Tchobanoglous et al., 2004; Stanier et al., 1986). The protons produced in the second step lead to a decrease in pH and a buffer as a source of alkalinity is needed to enable complete mineralization of urea (Tchobanoglous et al., 2004).

Trickling filters are fixed bed reactors. They contain filter material on the surface of which the microorganisms – mainly bacteria and archaea- can form a biofilm. When newly built filters are put into operation, function develops slowly with the growth of the necessary organisms. During the start-up period, it is typical for the accumulation of nitrogen compounds to be observed. First, the concentration of ammonium rises until ammonium oxidizing bacteria are established.

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https://doi.org/10.1016/j.lssr.2018.04.003
Received 4 December 2017; Received in revised form 27 February 2018; Accepted 30 April 2018

Second, the nitrite resulting from ammonium oxidation reaches a concentration maximum until the population of nitrite oxidizing bacteria is large enough to oxidize the nitrite to nitrate at the rate it is produced (Egli et al., 2003; Jubany et al., 2008).

For space applications, the crew time (labour) required for waste processing should be minimized and the microbial communities present in the filter have to be maintained. When trickling filters are applied, batch mode can be considered an operation mode with low maintenance effort and microbial wash-out (Tchobanoglous et al., 2004; Bornemann et al., 2015). Batch mode means that the filter units are fed with urine once, followed by a processing period during which the processed liquid is continuously cycled through the filter. After maturation, the batch is removed and replaced by a fresh batch. Earlier nitrification experiments with trickling filters that were operated in batch mode and loaded with synthetic urine diluted to 7% v/v and 20% v/v led to the hypotheses that (1) higher urine concentrations induce instability of operation and (2) higher urine concentrations slow down conversion rates (Bornemann et al., 2015). In wastewater with high ammonium concentrations, the degradation process is not limited by substrate availability. The threshold values given for moving bed and fixed bed reactors vary around 2.5 mg total ammonia nitrogen per litre. An increase in total ammonia concentration above this value does not lead to a further increase in the degradation rate (Greiner and Timmons, 1998; von Ahnen et al., 2015). The total ammonia concentration in hydrolysed urine significantly exceeds these values (480 mg/l in fresh urine; 8100 mg/l in stored urine (Udert et al., 2006)). Thus, moderate dilution should not cause substrate limitation resulting in lower degradation rates. Furthermore, dilution may prove to be beneficial in reducing the inhibitory effects of pH, nitrous acid, accumulation of intermediates or oxygen depletion (Gujer, 2010; Udert et al., 2003). On the other hand, dilution increases the volume of the treated solution, reducing the space available for e.g. plant cultivation in a life support system. This study presents an experiment in which nitrification rates and process stability within trickling filters loaded with synthetic urine in the concentrations 40, 60, 80 and 100% v/v were recorded. Concentrations lower than 7% v/v were considered not to be manageable due to the volume that would have had to be processed.

## 2. Material and methods

### 2.1. Filter units

The filters are built of opaque, plasticizer free, PVC tubes (Fig. 1, bulk volume of the filter material: ~6 l), which are vertically fixed to the wall. At the bottom end, a drain is formed using tube connectors that are common for sewage installation. In the middle of the tube, a branch piece is installed. It contains a plastic mesh pot (common in orchid cultivation, diameter: 100 mm) full of complete mussel shells ($CaCO_3$ content: 70–80% v/v, it is important to use complete shells, ground shells melt together due to calcium phosphate precipitation and block the tubes). The tubes above and beneath the branch piece are filled with pumice, which is held back by stainless steel grids (mesh width 10 mm). The upper end and the refill opening are sealed with caps. Each filter is connected to a 30 l PVC tank (Curver, Germany: Unibox 30 l with lid), which contains the filtrate. The tanks are fitted with centrifugal pumps for aquariums (Eheim, Germany: Eheim compact 1000 with 1000 l/h maximum head, power consumption: 23 W, pumping performance with a rise of 1860 mm: ~300 l/h) and the corresponding Eheim hoses (16/22 mm, PVC) to constitute permanent circulation of the liquid through the filters. During operation the tanks are covered with lids, which have been halved to facilitate the optical control of the test liquids. The rear half has openings for the drain, pump cable and hose. All filters were inoculated with 1 g of dried garden soil from the same thoroughly mixed sample. It was sampled from the garden of the German Aerospace Center in Cologne. Inoculation was followed by a preoperational run with tap water that lasted two days.

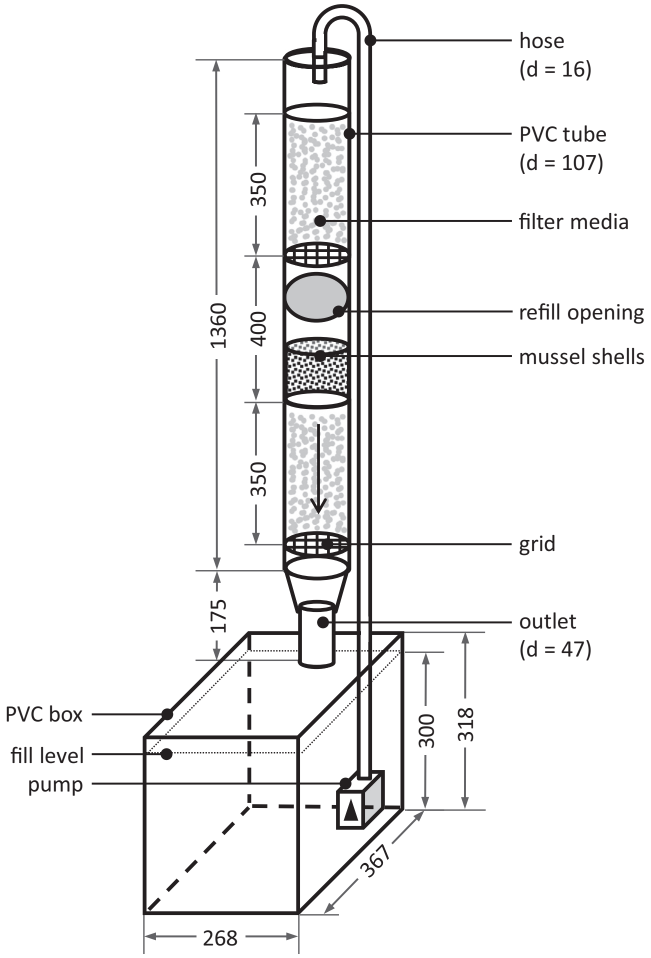

**Fig 1.** Schematic representation of a filter unit. Dimensions given as internal dimensions [mm]; *d* = diameter.

### 2.2. Filter material

The pumice used as filter material is called "Rote Eifellava" and is mined in quarries near Cologne. The average elemental composition is given elsewhere (Bornemann et al., 2015). Particle size ranges from 16 to 25 mm, bulk density ranges from 800 to 1400 g/l; specific weight ranges from 2.5 to 3.1 g/cm$^3$; porosity ranges from 0.2 to 0.5; specific surface is given as 90 m$^2$/m$^3$ (Grubert et al., 2007; Kasting, 2002).

### 2.3. Test run

The synthetic urine was prepared according to an earlier published protocol (Feng and Wu, 2006). It contains approximately 7780 mg of nitrogen per liter in total, which is mainly present as urea-N (approx. 7000 mg/l). In addition, there are small amounts of creatinine (380 mg N/l) and ammonium chloride (400 mg N/l). Phosphorus is present in the form of phosphate (890 mg PO4-P/l). The initial pH of the solution is 6.8.

For the test run, 12 filter units (units A – L) were grouped in triplicates. A, B, C received 30 l of synthetic urine (undiluted, 100% v/v). D, E, F received 24 l of the urine diluted with 6 l of tap water, thus the resulting urine solution contained approx. 80% v/v urine (≈ 6220 mg nitrogen per liter). G, H, I received 18 l of synthetic urine resulting in a 60% v/v urine solution with approximately 4670 mg nitrogen per liter. J, K, L received 12 l (40% v/v, 3110 mg N/l). Temperature and pH of

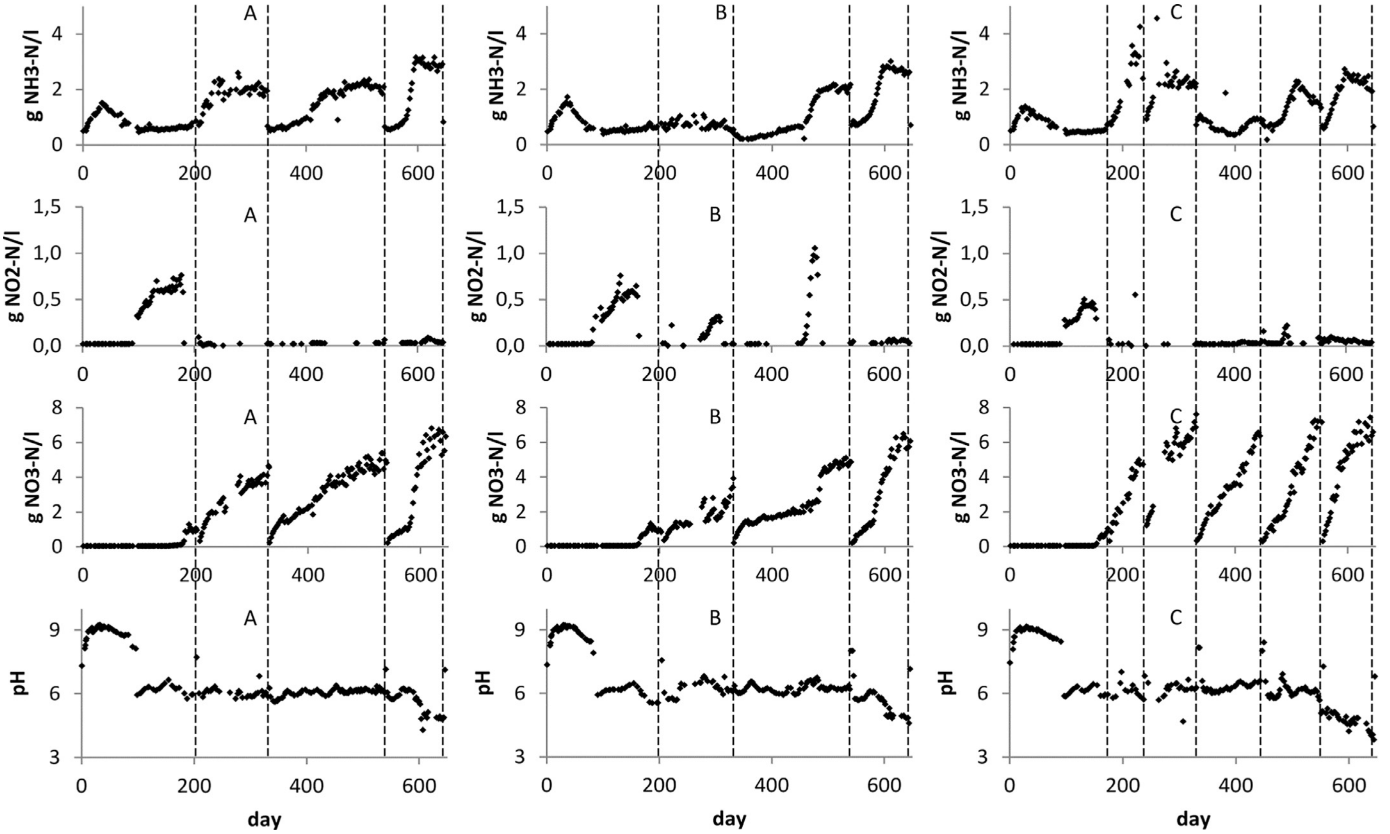

**Fig. 2.** Operation history of filter units A–C (100% v/v) showing the concentrations of the three nitrogen compounds and pH. Dashed vertical lines mark the days of refilling and start of a new batch. The first processing period is the start-up period.

the solutions in all tanks were measured three times a week (temperature: WTW Multi 1970i with TetraCon 325; pH: Sentix 41; all WTW, Germany). The 646 day measuring period presented here started with the setup of the tubes. The first processing period is counted as the start-up period of the systems. All ions ($NH_4^+$, $NO_2^-$, $NO_3^-$, $PO_4^{3-}$, $Ca^{2+}$, $SO_4^{2-}$, $K^+$, $Na^+$, $Cl^-$, $Mg^{2+}$) were measured in mg/l using an ion exchange chromatograph (Metrohm, Germany: IC-System Professional 850 – Anions + Cations; anion column: Metrosep A Supp 5 – 150 × 4.0 (carbonate eluent), precolumn: Metrosep A Supp 4/5 Guard, Metrosep RP 2 Guard 3.5). The long gap in measurement between the 254th and 275th day is due to a failure of the IC system and the time needed for repair work. During the test period, the filter units were run in batch mode, i.e. the 30 l of processed urine was completely removed and replaced by a fresh batch of synthetic urine in the corresponding concentration when nitrate concentration was near maximum. Often, the filtrate of several filter units was exchanged in one working step to reduce the maintenance effort and simulate realistic conditions. Therefore, the percentage of nitrogen, in the form of nitrate given in the results, does not necessarily reflect the maximum possible value. Temperature was held constant, some seasonal variation occurred (mean: 28.9 °C; SD: 1.1).

### 2.4. Statistics

Figures were created with Excel 2010 (scatterplots) or SPSS 21 (IBM SPSS Statistics 21 1989) (boxplots) and modified in PowerPoint 2010. The schematic representation of the filter units was done with PowerPoint 2010. Due to the occurrence of small sample sizes ($n = 3$), average values are given as medians. Additionally, the range is given (outliers and extreme values included). The first hypothesis provided in the introduction concerns the stability of filter performance. Stability is measured as the variance of the nitrate production rates of the filters within one concentration level. Differences in variances are tested with the SPSS 21 Levene's Test of homogeneity of variances. This test was also applied for comparing the variances in pH. To test the second hypothesis concerning the decrease in nitrate production rates with increasing urine concentration, SPSS 21 was utilized for linear regression. The end of the start-up period is defined as the time when the nitrate concentration starts to increase. It is measured as the number of days elapsed since the first start of the newly built filter. Its relationship to urine concentration is also analyzed with linear regression. The results of the statistical tests are given with the following parameters: $n$ = sample size, β = standardized regression coefficient, $R^2$ = model fit and $P$ = significance level. The measurement data of each trickling filter unit were evaluated for their own. Values from units of the same triplicate were not summarized, because the increase in information loss caused by the use of sum parameters was considered higher than the gain of simplicity.

The processing time per batch is given in days. The overall nitrogen content (g NO3-N) of a batch is calculated on the basis of the nitrogen content of the synthetic urine in g/l (given in Section 2.3.) multiplied by 30 to account for the initial 30 l the runs started with. Due to evaporation, at the end of the processing period the volume was reduced to 28 l (when the loss exceeded 2 l the units were refilled to 28 l with deionized water). Consequently, the nitrate produced in a batch (g NO3-N) is calculated using the nitrate concentration on the day of exchange in g/l multiplied by 28.

As nitrate production curves varied, curve fitting was abandoned and nitrate production rate was calculated as grams of NO3-N produced divided by the processing time in days (g NO3-N/no. of days).

## 3. Results

### 3.1. Start-up period

In an experiment lasting 646 days, triplicates of filter units were operated with 100% v/v, 80% v/v, 60% v/v and 40% v/v artificial urine solutions. Figs. 2–5 show the pH and the concentrations of the nitrogen compounds (ammonium, nitrite and nitrate) over the complete duration of the experiment. The filter units were not used prior to the start-up period. The first processing period included the start-up period, during which the microbial populations were established in the filter. The dynamics often start with an accumulation of ammonia (urea hydrolysis) followed by a peak in nitrite (first step of nitrification). Then nitrate production (second step of nitrification) begins (Tchobanoglous et al., 2004; Stanier et al., 1986). The 100% v/v units all showed these dynamics with early ammonium peaks and moderate nitrite peaks as time went on. Nitrate production started late, around the 160th day. The increase in pH caused by urea being hydrolyzed to ammonium, followed by a drop to pH 5 – 6 caused by ammonia oxidation is well visible (Fig. 2). The 80% v/v urine units also started with similar ammonium and nitrite peaks. Compared to the 100% v/v units, ammonium accumulation started later, while nitrate production began earlier (~120th day). The late ammonium peak corresponds with an earlier drop in pH (Fig. 3). The 60% v/v units showed differences in their start-up periods with G and I having ammonium peaks at the same time as the 80% v/v units. This was combined with high nitrite values and the initiation of nitrate production at approximately the same time as the 80% v/v units. Unit H showed a differing pattern to G and I. Ammonium accumulated early, followed by a low nitrite peak and no onset of nitrate production in the first processing period, even though the pH indicates ammonia oxidation. The batch was exchanged on the 300th day and a second start-up period with peaks in ammonium and nitrite followed before nitrate production was observed (Fig. 4). All the 40% v/v units had an ammonium peak at the 100th day, which is in-between the times of the 100% v/v and the 80% v/v units. Units K and L accumulated nitrite early and in low concentration. Nitrate production started around the 75th day. Unit J accumulated nitrite in higher concentrations and nitrate production started on the 75th day but stagnated at a low level until the 150th day (Fig. 5). The duration of the start-up periods for the individual filters is given in Table 1. Duration increased with urine concentration (Fig. 6).

### 3.2. Nitrate production

The 100% v/v units (A and B) produced nitrate comparatively slowly while the third 100% v/v unit (C) produced nitrate at nearly double rate. In B, slow nitrate production was accompanied by nitrite peaks that indicated a still developing population of nitrite oxidizers. The pH values averaged 6.5, 6.6 and 6.5, respectively, in the units A,B and C and were comparatively constant over processing time (Fig. 2). The 80% v/v units had uniform processing periods. Nitrite peaks occurred in the first two periods. The pH was slightly more unstable compared to the 100% v/v units (Fig. 3) and averaged 5.8, 5.9 and 6.0 in the units D,E and F, respectively. The 60% v/v units (G and I) also showed considerable uniformity in their processing behaviour including nitrite peaks in the first processing period. In unit H, the failed starting period was followed by a successful starting period after the first refill. After that, the unit began normal operation. In the 60% v/v units G,H and I, pH was increasingly unstable averaging 5.8, 6.0 and 6.0, respectively (Fig. 4). All 40% v/v units showed anomalies. In unit J, the irregularities of the starting period continued. Two normally processed batches were followed by a third batch, which completely failed. After batch exchange, a complete second starting period could be observed. In units K and L, the eighth batch did not reach the expected

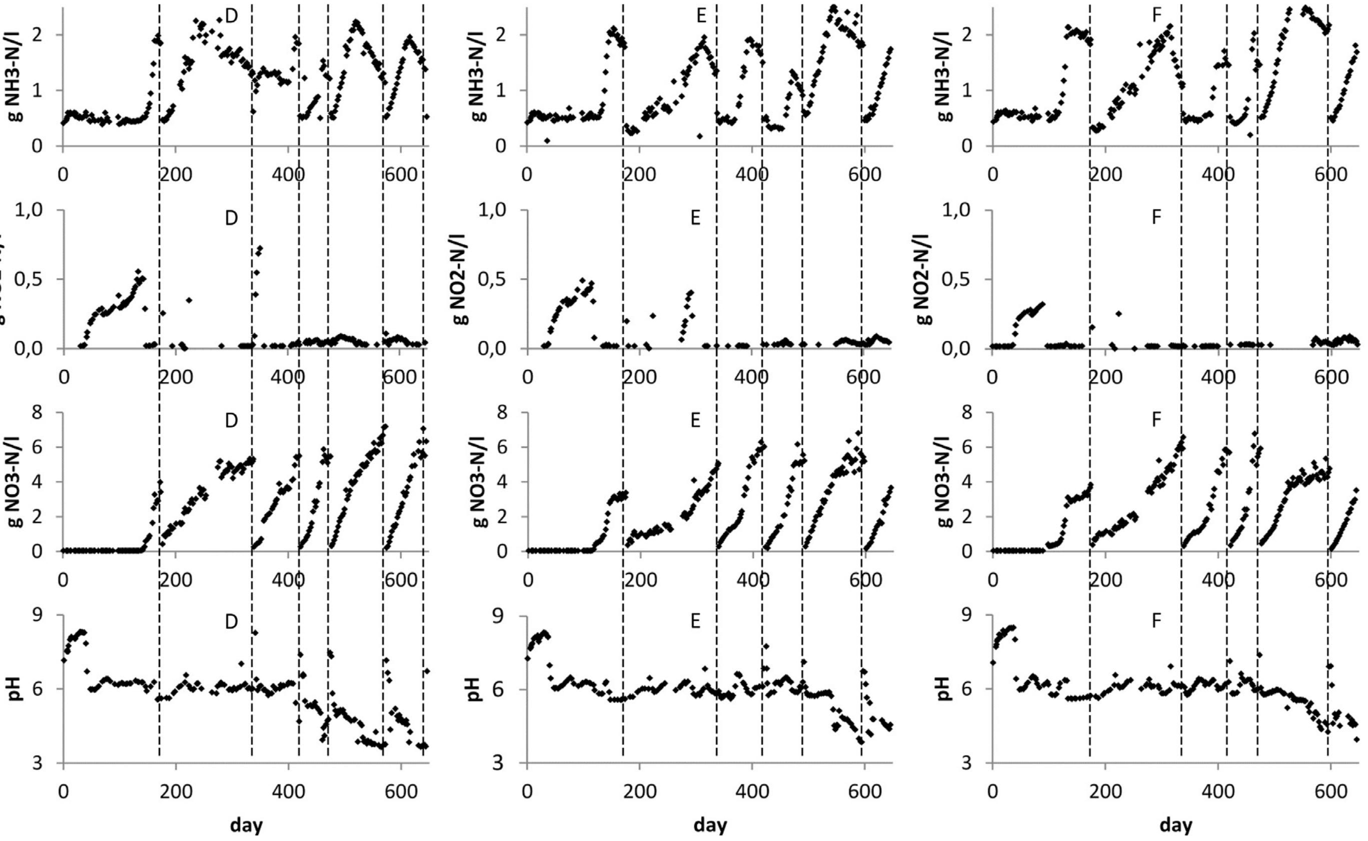

**Fig. 3.** Operation history of filter units D–F (80% v/v) showing the concentrations of the three nitrogen compounds and pH. Dashed vertical lines mark the days of refilling and start of a new batch. The first processing period is the start-up period.

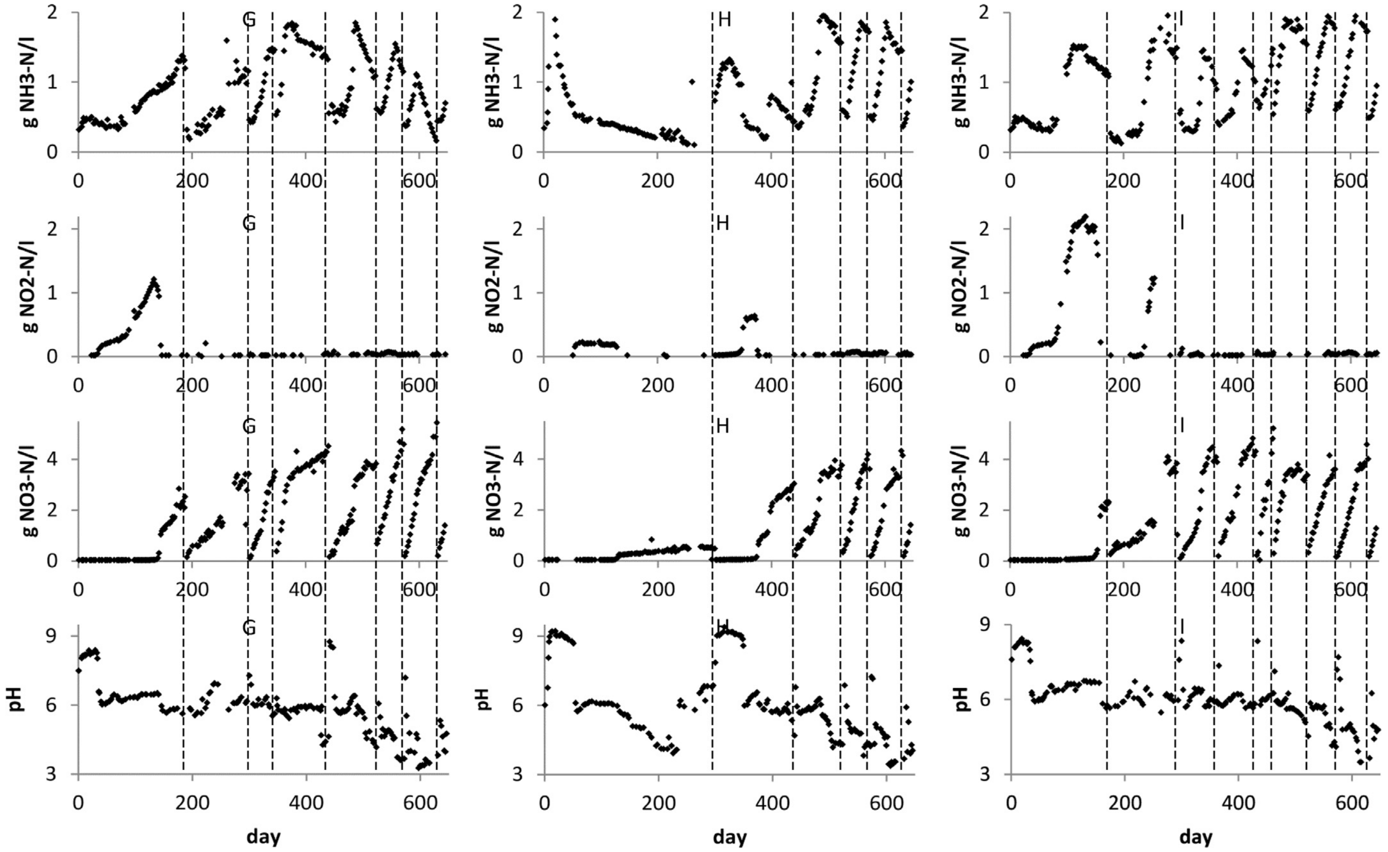

**Fig. 4.** Operation history of filter units G–I (60% v/v) showing the concentrations of the three nitrogen compounds and pH. Dashed vertical lines mark the days of refilling and start of a new batch. The first processing period is the start-up period.

nitrate concentration. As both curves are completely similar, this failure can be explained by a handling error: Both units were obviously filled with urine in lower concentration than intended. In all 40% v/v units the variation in pH was quite high (Fig. 5). The pH values averaged 6.2, 5.5 and 5.1 in units J, K and L, respectively.

Table 1 summarizes the operation parameters of the filter units. With decreasing urine concentration, the number of batches that were processed increased and the duration of the processing periods decreased. A decrease in nitrate production rate with increasing urine concentration could not be found (Fig. 7). Variances of nitrate production rates among concentration levels were not significantly different (Fig. 8).

### 3.3. pH and phosphate precipitation

Figs. 2–5 illustrate the increasing instability in pH with decreasing urine concentration. The variances of pH among concentration levels were significantly different showing increasing ranges with decreasing urine concentration (Fig. 9). Fig. 10 illustrates the relationship between phosphate and calcium concentrations. Phosphate concentration decreased with increasing calcium concentration. This is because during processing, calcium phosphates continuously precipitated (Maurer and Boller, 1999) and the mussel shells provided new calcium together with the carbonate needed for buffering. In the 40% v/v unit, the phosphate was quickly depleted below detection level and calcium concentration rapidly increased. In the 100% v/v unit, more phosphate was available and precipitation kept the concentration of calcium constant for more than 60 days. The concentrations of $SO_4^{2-}$, $K^+$, $Na^+$, $Cl^-$ and $Mg^{2+}$ ions did not change during processing (not shown).

## 4. Discussion

The study described in this paper is based on the hypotheses drawn from an earlier experiment, in which filter units of the same type were run with 7% v/v and 20% v/v artificial urine solution (Bornemann et al., 2015). The earlier results indicated that higher urine concentrations induce instability of operation and slow conversion rates. These hypotheses were tested in an experiment with urine concentrations from 40% v/v to 100% v/v. It was assumed that instable operation causes higher variability in nitrate production rates. The corresponding test clearly showed that instability did not increase with the urine concentration applied (Fig. 8). With regard to conversion rates, a continuous decrease with increasing urine concentration was expected. The regression test showed that there is no linear relationship among the conversion rates of different concentration levels (Fig. 7). Fig. 8 indicates that 60% v/v–80% v/v urine might be an optimal concentration level with higher nitrate production rates, but the differences between average rates were not significant. Clear differences among concentration levels were only found in the duration of the start-up periods. These increased with increasing urine concentration (Fig. 6). Unexpected behaviour during the start-up period as observed in one 60% v/v and one 40% v/v unit led to a considerable prolongation of start-up.

Considering the entire operation history of each filter unit, filter units within the same concentration level showed similar behaviour. Exceptions are the units which showed contrasting behaviour immediately from start-up. The irregularities continued throughout the complete operation period.

From the results of the phenomenological analysis of trickling filter behaviour, hypotheses about the microorganisms active in the filters can be drawn. The results of other studies indicate that each nitrogen concentration has its own specific community of nitrifyers and biofilm

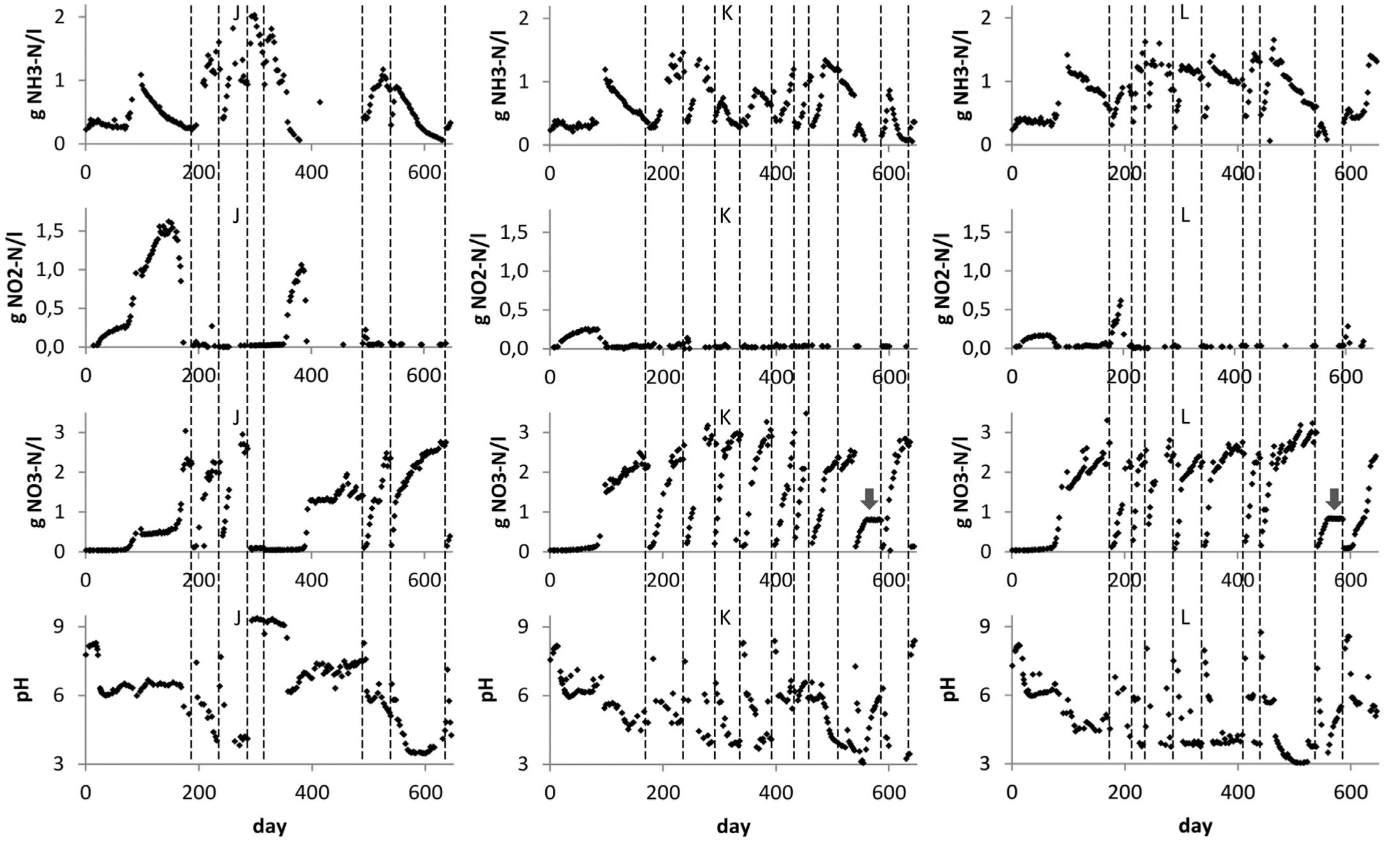

**Fig. 5.** Operation history of filter units J–L (40% v/v) showing the concentrations of the three nitrogen compounds and pH. Dashed vertical lines mark the days of refilling and start of a new batch. The first processing period is the start-up period. The processing periods with urine of lower concentration are marked with arrows. The corresponding data are left out of all analyses.

**Table 1**
Key parameters of filter performance during the 646 day test run. Proc. = processing, *d* = days, *v* = conversion rate, parameters marked with* do not include the data of irregular runs. Irregular runs in parentheses are due to handling errors.

| | 100% | | | 80% | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | F |
| Starting period [d] | 173 | 159 | 154 | 138 | 110 | 103 |
| No. of batches | 3 | 3 | 5 | 5 | 4 | 4 |
| No. of irregular runs | 0 | 0 | 0 | 0 | 0 | 0 |
| Processing time [d] | 128 | 128 | 95 | 82 | 95 | 103 |
| Proc. time [range] | 105–209 | 105–209 | 61–113 | 55–164 | 71–164 | 55–164 |
| v [mg NO3-N/d] | 999 | 859 | 2024 | 2049 | 1706 | 1531 |
| v [range] | 648–1691 | 652–1619 | 1575–2241 | 905–2776 | 865–2056 | 1084–3007 |
| V* [mg NO3-N/d] | | | | | | |
| v* [range] | | | | | | |
| % N in NO3-N* | 63 | 59 | 78 | 89 | 81 | 86 |
| mg urea degraded/d* | 2385 | 2235 | 4213 | 4314 | 3394 | 3832 |

| | 60% | | | 40% | | |
|---|---|---|---|---|---|---|
| | G | H | I | J | K | L |
| Starting period [d] | 138 | 367 | 142 | 188 | 82 | 72 |
| No. of batches | 6 | 4 | 7 | 6 | 9 | 9 |
| No. of irregular runs | 0 | 1 | 0 | 2 | (1) | (1) |
| Processing time [d] | 71 | 71 | 60 | 50 | 51 | 49 |
| Proc. time [range] | 46–112 | 49–140 | 31–120 | 29–175 | 31–76 | 26–100 |
| v [mg NO3-N/d] | 1743 | 1624 | 1774 | 1024 | 1510 | 1234 |
| v [range] | 856–2625 | 607–2063 | 895–4715 | 88–1477 | 472–4152 | 486–2746 |
| v* [mg NO3-N/d] | | 1998 | | 1303 | 1565 | 1315 |
| v* [range] | | 920–4152 | | 784–1477 | 920–4152 | 838–2746 |
| % N in NO3-N* | 84 | 97 | 81 | 69 | 91 | 73 |
| mg urea degraded/d* | 3880 | 3171 | 4435 | 1850 | 3590 | 3020 |

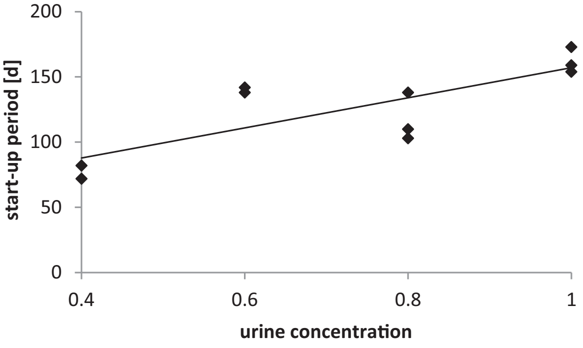

**Fig. 6.** Influence of urine concentration on the duration of the start-up period. The black line is a regression line ($R^2 = 0.583$; $\beta = 0.793$; $P < 0.05$). Start-up periods of H and J have been left out of the analysis due to the irregular behaviour of these units.

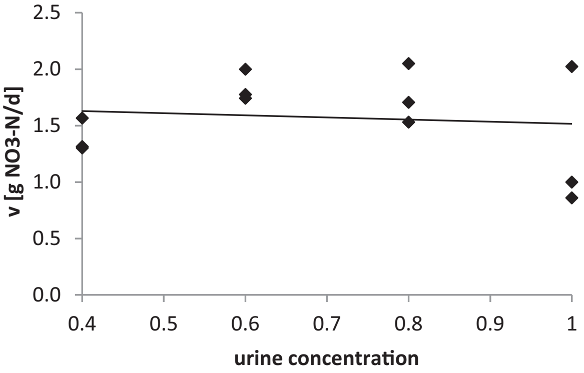

**Fig. 7.** Influence of urine concentration on nitrate production rate v (medians, data of irregular runs not included). The black line is a regression line ($R^2 = -0.086$; $\beta = -0.113$; $P > 0.05$).

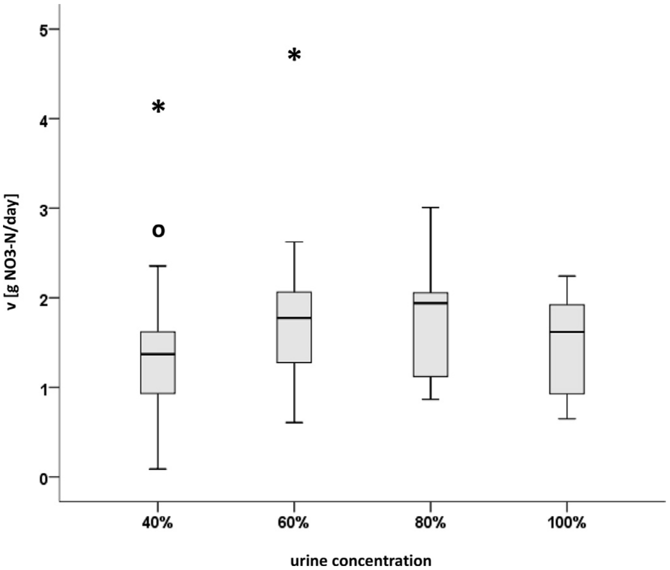

**Fig. 8.** The distributions of the nitrate production rates of the different urine concentrations (100% v/v: $n = 11$; 80% v/v: $n = 13$; 60% v/v: $n = 17$; 40% v/v: $n = 22$; Levene'sTest: $P > 0.05$; SPSS Median Test: $P > 0.05$; data of irregular runs included). Bold line = median, top and bottom edge of the box = 25% and 75% percentile, whiskers = range, circles = outliers (distance to box > 1.5 × box height), asterisk = extreme value (distance to box > 3 × box height).

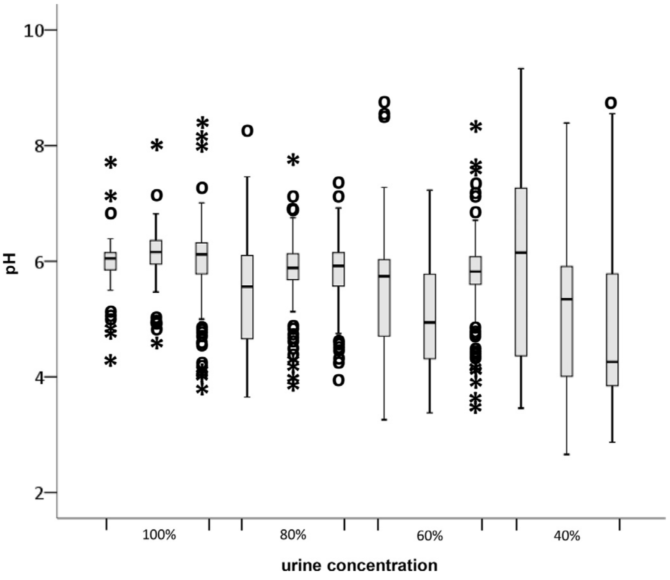

**Fig. 9.** The distributions of pH values of the different urine concentrations (Levene's Test; 100% v/v: $n = 422$; 80% v/v: $n = 426$; 60% v/v: $n = 355$; 40% v/v: $n = 417$; $P < 0.001$; data of irregular runs not included). Bold line = median, top and bottom edge of the box = 25% and 75% percentile, whiskers = range, circles = outliers (distance to box > 1.5 × box height), asterisk = extreme value (distance to box > 3 × box height).

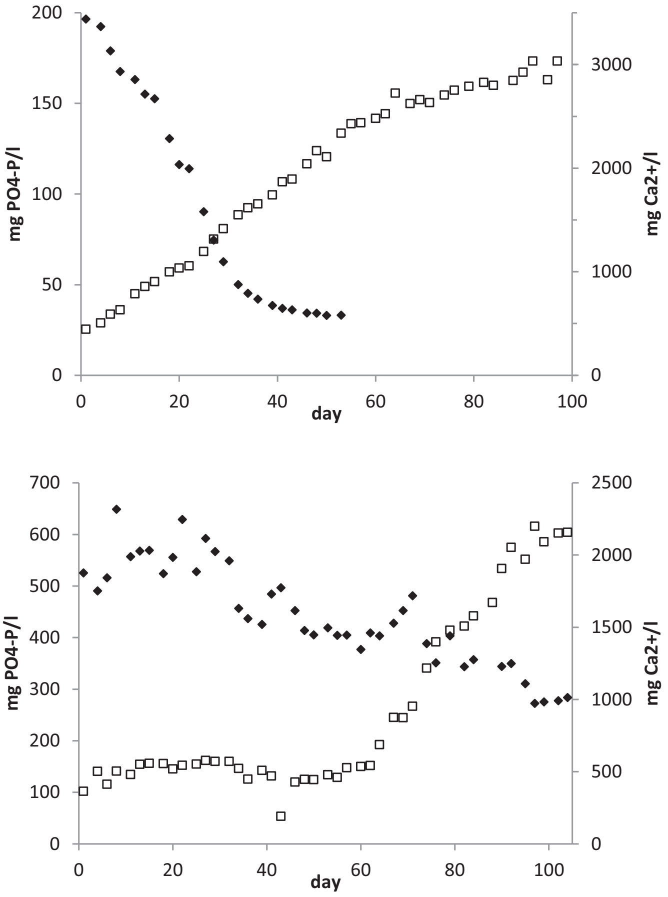

**Fig. 10.** Examples of the development of PO4-P concentration (black diamonds) and $Ca^{2+}$ concentration (white squares) during processing periods of one 40% v/v unit (top) and one 100% v/v unit (bottom).

structure (Zielińska et al., 2012; Wijeyekoon et al., 2004). The variation in the start-up period duration implies that communities adapted to high substrate concentrations might have lower growth rates, or are of lower abundance in the soil used to inoculate the filters and therefore the populations had to develop from a very low initial number of individuals or even came from other sources, such as ambient air or water. As soon as the communities were established, nitrate production rates did not differ. As substrate availability was not limited and environmental conditions did not differ among concentration levels, it can be concluded that the nitrate production rate was limited mainly by population size, i.e. enzyme concentration and thus is sensitive to changes in surface area, shear forces induced by flow rate and grazing.

Regarding the buffering system constituted by the presence of calcium carbonate (mussel shells) in the filter tube, the amplitude of pH changes increasing with decreasing concentration was counterintuitive. In the case of insufficient buffer capacity, significant pH variation was expected to occur in the filter units with high urine concentrations, because these produce more acidity and carbonate delivery from the dissolving shells could be too slow. Considering the development of phosphate and calcium concentrations, it can be hypothesized that the dissolution of the mussel shells was not only driven by acidity but was influenced by phosphate concentration. Precipitation of calcium phosphates seems to promote dissolution and the presence of more phosphate in units of high urine concentrations led to better buffering performance. It was concluded above, that nitrate production rate was significantly determined by population size. As the presence of alkalinity is essential for process progress, an effect of mussel dissolution rate cannot be excluded.

## 5. Conclusions

The long-term nitrification experiment with trickling filters showed that synthetic urine concentration does not influence filter performance in terms of stability and nitrate production rate and therefore dilution is not necessary. Filter units work reliably when the start-up period proceeds undisturbed. The phosphate precipitation induced by the calcium from the mussel shells, which were introduced into the system as a buffer, seems to improve buffering capacity. Considering these findings, it can be hypothesized that nitrate production rate is the result of a complex interplay of precipitation equilibrium, dissolution equilibrium and population sizes. Even though the synthetic urine used in this study simulates the composition of natural human urine, the results of the study have to be substantiated by experiments with natural urine to determine the influence of the microflora usually present in natural substrates. The highest nitrate production rates observed corresponded to urea degradation rates of approximately 4 g urea per day. Assuming that one crew member excretes 15–20 g of urea per day, the filter volume per crew member would be considerably high. In future investigations it has to be tested, if nitrification rate can be accelerated by changes in operation conditions.

Mussel shells have been introduced into the system as a bioregenerative buffer system. These proved to be a sufficient buffer when undiluted urine was processed. Dilution led to inadequate buffer performance due to the reduced phosphate content. The option to keep and breed mussels as source of carbonate and protein in space is currently explored. The occurrence of phosphate precipitation induced by the calcium delivered by the shells can be considered problematic for space applications. The precipitated phosphate is not available for plants and can only be dissolved using hazardous substances like sulfuric acid. Thus, operation parameters have to be changed to establish conditions which reduce precipitation, or an alternative regenerative buffer providing a better doseable source of alkalinity has to be used. On the other hand, phosphate precipitation is a possibility to separate the macronutrients nitrogen and phosphate without the addition of further chemicals. In terrestrial agriculture, this may prove to be useful on farms with large livestock, on which the amount of slurry produced is higher than the amount, which can be spread in the fields. The precipitate and the nitrate solution can be dried separately and used for industrial fertilizer production.

## Acknowledgements

The authors are very grateful to Birgit Bromeis for her excellent handling of the C.R.O.P.® samples. The authors thank Matt Bamsey, Connor Kiselchuk and Ralf Anken for proof reading. We also thank the unknown reviewers for helpful comments.

## Disclosure statement

No competing financial interests exist.

The described study was part of the project C.R.O.P.®, which is supported by grants from the DLR program topic “Research under Space Conditions”.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.lssr.2018.04.003.

## References

Binot, R.A., Tamponnet, C., Lasseur, C., 1994. Biological life support for manned missions by ESA. Adv. Space Res. 14, 71–74.

Bornemann, G., Waßer, K., Tonat, T., Möller, R., Bohmeier, M., Hauslage, J, 2015. Natural microbial populations in a water-based biowaste management system for space life support. Life Sci. Space Res. 7, 39–52.

Drysdale, A.E., Ewert, M.K., Hanford, A.J, 2003. Life support approaches for Mars missions. Adv. Space Res. 31 (1), 51–61.

Drysdale, A.E., Rutzke, C.J., Albright, L.D., LaDue, R.L, 2004. The minimal cost of life in space. Adv. Space Res. 34, 1502–1508.

Egli, K., Langer, C., Siegrist, H.-R., Zehnder, A.J.B., Wagner, M., van der Meer, J.R, 2003. Community analysis of ammonia and nitrite oxidizers during start-up of nitritation reactors. Appl. Environ. Microbiol. 69, 3213–3222.

Feng, D., Wu, Z, 2006. Culture of *Spirulina platensis* in human urine for biomass production and $O_2$ evolution. J. Zhejang Univ. Sci. B 7 (1), 34–37.

Gitelson, J.I., Blum, V., Grigoriev, A.I., Lisovsky, G.M., Manukovsky, N.S., Sinyak, Y.E., Ushakova, S.A, 1995. Biological-physical-chemical aspects of a human life support system for a lunar base. Acta Astronaut. 37, 385–394.

Greiner, A.D., Timmons, M.B, 1998. Evaluation of the nitrification rates of microbead and trickling filters in an intensive recirculating tilapia production facility. Aquacul. Eng. 18, 189–200.

Grubert, A., Haefner, F. Haneke, J., Kuhn, W., Lang, R., Schmelmer, K., Wehninger, A. and Weidenfeller, M. Near-surface mineral raw products in Rhineland-Palatinate. State Government of Rhineland-Palatinate (ed.), 2007, (German).

Gujer, W, 2010. Nitrification and me – a subjective review. Water Res. 44, 1–19.

Hu, E., Bartsev, S.I., Liu, H, 2010. Conceptual design of a bioregenerative life support system containing crops and silkworms. Adv. Space Res. 45, 929–939.

IBM SPSS Statistics 21, 1989. Command Syntax Reference. IBM Corporation 2012. ftp://public.dhe.ibm.com/software/analytics/spss/documentation/statistics/22.0/en/client/Manuals/IBM_SPSS_Statistics_Command_Syntax_Reference.pdf.

Jubany, I., Carrera, J., Lafuente, J., Baeza, J.A., 2008. Start-up of a nitrification system with automatic control to treat highly concentrated ammonium wastewater: experimental results and modeling. Chem. Eng. J. 144, 407–419.

Kasting, U, 2002. Cleaning Capacity of Central Treatment Plants for Heavily Contaminated Rainwater from Street Sewage Systems,. PhD Thesis. University of Kaiserslautern (German).

Maurer, M., Boller, M, 1999. Modelling of phosphorus precipitation in wastewater treatment plants with enhanced biological phosphorus removal. Water Sci. Tech. 39, 147–163.

Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., Painter, P.R., 1986. General Microbiology, fifth ed. Macmillan Education Ltd, Houndsmills, Basingstoke, Hampshire, London.

Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2004. Wastewater Engineering: Treatment and Reuse, fourth ed. Metcalf & Eddy Inc./McGraw-Hill, New York.

Tikhomirov, A.A., Ushakova, S.A., Velichko, V.V., Tikhomirova, N.A., Kudnko, Yu.A., Gribovskaya, I.V., Gros, J.-B., Lasseur, C, 2011. Assessment of the possibility of establishing material cycling in an experimental model of the bio-technical life support system with plant and human wastes included in mass exchange. Acta Astronaut. 68, 1548–1554.

Tikhomirova, N.A., Ushakova, S.A., Kudenko, Yu.A., Gribovskaya, I.V., Shklavtsova, E.S., Balnokin, Yu.V., Popova, L.G., Myasoedov, N.A., Gros, J.-B., Lasseur, Ch, 2011. Potential of salt-accumulating and salt-secreting halophytic plants for recycling sodium chloride in human urine in bioregenerative life support systems. Adv. Space Res. 48, 378–382.

Udert, K.M., Fux, C., Münster, M., Larsen, T.A., Siegrist, H., Gujer, W, 2003. Nitrification and autotrophic denitrification of source-separated urine. Water Sci. Technol. 48 (1), 119–130.

Udert, K.M., Larsen, T.A., Gujer, W, 2006. Fate of major compounds in source-separated urine. Water Sci. Technol. 54 (11–12), 413–420.

von Ahnen, M., Pedersen, L.-F., Bovbjerg Pedersen, P., Dalsgaard, J, 2015. Degradation of urea, ammonia and nitrite in moving bed biofilters operated at different feed loadings. Aquacul. Eng. 69, 50–59.

Wijeyekoon, S., Mino, T., Satoh, H., Matsuo, T, 2004. Effects of substrate loading rate on biofilm structure. Water Res. 38, 2479–2488.

Zielińska, M.Bernat, Cydzik-Kwiatkowska, K., Sobolewska, J., A., Wojnowska-Baryla, I, 2012. Nitrogen removal from wastewater and bacterial diversity in activated sludge at different COD/N ratios and dissolved oxygen concentrations. J. Environ. Sci. 24 (6), 990–998.