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Natural microbial populations in a water-based biowaste management system for space life support

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ABSTRACT

The reutilization of wastewater is a key issue with regard to long-term space missions and planetary habitation. This study reports the design, test runs and microbiological analyses of a fixed bed biofiltration system which applies pumice grain (16–25 mm grain size, 90 m^2/m^3 active surface) as matrix and calcium carbonate as buffer. For activation, the pumice was inoculated with garden soil known to contain a diverse community of microorganisms, thus enabling the filtration system to potentially degrade all kinds of organic matter. Current experiments over 194 days with diluted synthetic urine (7% and 20%) showed that the 7% filter units produced nitrate slowly but steadily (max. 2191 mg NO_3–N/day). In the 20% units nitrate production was slower and less stable (max. 1411 mg NO_3–N/day). 84% and 76% of the contained nitrogen was converted into nitrate. The low conversion rate is assumed to be due to the high flow rate, which keeps the biofilm on the pumice thin. At the same time the thin biofilm seems to prevent the activity of denitrifiers implicating the existence of a trade off between rate and the amount of nitrogen loss. Microbiological analyses identified a comparatively low number of species (26 in the filter material, 12 in the filtrate) indicating that urine serves as a strongly selective medium and filter units for the degradation of mixed feedstock have to be pre-conditioned on the intended substrates from the beginning.

1. Introduction

1.1. Space life support

Present-day space exploration envisions a gradual transition from long-term orbital flights and the evolution to interplanetary flights and development of long-term base projects on the surface of Moon and Mars (International Space Exploration Coordination Group (ISECG), 2011). Bioregenerative life support systems are critical components for long-term and far-distance human space travel and habitation (Skoog and Brouillet, 1981; Skoog, 1984). Due to vast operating distances and the resulting long travel times the maintenance of the crew has to be fully accomplished on board (Lasseur et al., 1996; Salisbury, 1999; Gros et al., 2003), thus life support systems with a high degree of regenerativity are required (Binot et al., 1994; Gitelson et al., 1995).

Currently, several approaches to solve this problem are studied addressing the long-term objective to build a closed cycle in order to use biowaste for food production and atmosphere regeneration purposes (Schubert et al., 1984; Lobo and Lasseur, 2003; Hu et al., 2010; Tikhomirov et al., 2011). Next generation life support systems have to overcome consumption of resources, e.g., by solving the treatment and recycling of solid waste and wastewater on board thus reducing the need for food and water supply (Gros et al., 2003; Haque and Kreuzberg, 1993; Grant et al., 2012).

Nutrient recovery by bioregeneration works along the lines of terrestrial ecosystems and at the same time has to overcome one of their major characteristics: nutrient storage in the soil (Adey and Loveland, 2007). On the Earth carbon from the atmosphere and nutrients from the lithosphere are metabolized into biomass and fixed together with other nutrients in humus layers over millions of years. From these layers mineralization by microorganisms slowly releases nutrients and CO_2 for renewed plant growth. The plants produce the biomass and the oxygen many other organisms live on. Water is an essential part of this cycle being the reaction and transportation medium for many biochemical processes.

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Table 1
Waste management system (WMS) requirements, the corresponding design considerations, problems and solution approaches. MO = microorganisms.

WMS requirements	Design considerations	Method of choice	Problems	Solution approach
Low energy consumption	Simple bioregenerative system	Trickling filter	Operation in weightlessness	Gravity by acceleration Flow filter
Easy to handle/low maintenance	Low level of complexity			Gas exchange membranes Ceramics as filter material
Low space requirements	Flexible "all in one" system	Porous filter material Diverse community of soil MOs	Maintenance of diversity: In absence of substrate Despite varying growth rates Despite washout	Pumice to provide isolated habitats, different flow rates, good adsorption properties Batch mode Attached growth Make use of cometabolism
Robust performance/restartability	Near-natural community of MOs			
Detoxification	Microbial degradation of micropollutants			
Effectiveness	High influent concentrations High conversion rates	Community of soil MOs Trickling filter	Optimization	pH control Nitrate removal Addition of trace elements

Conversely, if water polluted with organic waste passes through the soil the contaminants will also be mineralized and reused for biomass production resulting in the recirculation of clean water.

The turnover rates of the soil ecosystem are much too slow for life support purposes, therefore the function of the soil has to be transferred into an environment in which mineralization is accelerated.

1.2. Design considerations for a water-based waste management system (WMS)

1.2.1. The C.R.O.P. project

C.R.O.P. (= Combined Regenerative Organic food Production) is a project of the German Aerospace Center (DLR) and aims at the development of a modular bioregenerative compartment for life support systems that directly combines the degradation of biowaste like plant residues, food remains, human wastes and wastewaters with soilless plant cultivation.

The C.R.O.P. waste management system is designed to reduce the metabolic pathways needed for the degradation of wastewater and other nutrient containing wastes to minimum length and to optimize conversion rates. It is envisaged to be a multifunctional fixed bed biofiltration system able to liquefy solids and to mineralize all kinds of organic wastes. Thus, with regard to the filter system the occurrence of solids is restricted to the early stages of the conversion process. The desired product is an aqueous nutrient solution, which can be directly fed into a soilless plant cultivation system to build up new biomass (see Table 1 for a summary of the design considerations described below). Currently several filter units are tested regarding their capability to degrade the urea contained in urine into nitrate. In the near future, research activity is planned to be extended on mixed feedstock to complement the nitrogen-rich urine with organic carbon sources.

1.2.2. Soil bacteria

Transferring the function of the soil to an artificial system mainly requires finding a way to culture the bacteria responsible for this function. While the generation of isolated laboratory strains is a demanding task, the culture of soil bacteria in their natural community only needs the provision of some substrate as a wide diversity of microbes is present in healthy soil. Besides their metabolic activity soil bacteria have another trait that is especially interesting for life support system design. Most species are able to form cysts, spores or other dormant stages to survive poor environmental conditions like starvation, extreme pH or aridity (Roszak and Colwell, 1987; Mondini et al., 2006; Blagodatskaya and Kuzyakov, 2013; Placella and Firestone, 2013). This implicates that systems inoculated with soil bacteria have the potential to be restarted after technical system failure.

1.2.3. Water as universal medium

Water can replace soil as carrier of nutrients for metabolic processes of microorganisms and plants. The advantage of water over soil is the much faster exchange of nutrients and gases when kept in constant flow. The enhanced exchange rates speed up metabolic processes and mineralization.

1.2.4. Natural communities versus pure cultures

In the discussion on the advantages and disadvantages of holistic and deterministic approaches in Bioregenerative Life Support System (BLSS) design the dynamic nature of ecosystems plays a central role. On the one hand dynamic stability has the potential to compensate for perturbations without changes in overall function; on the other hand the *in situ* evolution of a complex network is hard to predict. A deterministic design sets exogenous control of all biological processes against this unpredictability (see, e.g., Lasseur et al., 1996; Tamponnet and Savage, 1994). Long-term control, and thus predictability of an ecosystem can be achieved by eliminating selection pressures on organisms. Selection can be described as a process, which inevitably occurs when organisms with overlapping environmental demands share a habitat (Bornemann, 2012). Thus, a selection free state requires that organisms do not compete. A modern greenhouse in which each plant is individually provided with optimal conditions is a good example for a nearly selection free setting.

In microbial populations it is impossible to exclude selection, because single cells cannot be individually provided with an optimal environment. Therefore, selection in combination with high mutation rates indicates that microbial cultures will be subject to rapid evolutionary change. In the case of pure cultures such changes are likely to influence the desired function in the long run, while diverse communities can be assumed to provide more robust performance because they consist of a network built of trophic chains that includes several alternative pathways.

In addition, experience shows that species-poor monocultures are vulnerable to invasion, pests and diseases (Andow, 1983; Trujillo-Arriaga and Altieri, 1990; Zhu et al., 2000). Thus, with regard to a microbial waste management system, the choice of diverse near natural microbial populations can be considered beneficial.

Furthermore, high diversity unites microorganisms of various functions thus forming functional consortia. These can form the basis of a flexible all in one system which can degrade all kinds of organic matter and thus reduces space requirements to a minimum. The major challenge for realization of such a system is maintenance of the microbial diversity despite of the varying growth rates of the different microbial species, washout, and temporary absence of the substrate of some species, as waste usually varies in composition.

Table 2
Chemical composition of the biofilter material (pumice). Chemical analysis of refractory products by X-ray fluorescence spectroscopy via fused cast-bead method according to ISO 12677:2011 (International Standardization Organization (ISO), 2011).

Element	Mass percentage rate (mean ± SD)	Trace elements	Mass portion (µg/g)
SiO_2	42.9 ± 0.1	Mn	1536
		Ba	1047
Al_2O_3	13.9 ± 0.3	Sr	857
		Zr	346
Fe_2O_3	11.4 ± 0.1	V	289
		Cr	183
TiO_2	2.9 ± 0.1	Ni	108
		Nb	77
P_2O_5	0.6 ± 0.1	Co	75
		Rb	72
SO_3	0.2 ± 0.1	Cu	61
		Sc	30
CaO	12.2 ± 0.5	Ga	20
		Y	18
MgO	9.0 ± 0.2	As	12
		Th	11
K_2O	3.3 ± 0.1	Pb	4
		Mo	2
Na_2O	3.0 ± 0.2		
Total	99.3 ± 1.6		

1.2.5. Attached growth versus suspended growth processes

Literature concerning wastewater treatment lists the following advantages of attached growth processes over suspended growth processes: lower energy requirements, simpler operation and control, avoidance of bulking sludge, less equipment maintenance and better recovery from shock toxic loads (Tchobanoglous et al., 2004), which are all desirable characteristics for a WMS for life support purposes.

Furthermore, in attached growth processes the microorganisms responsible for conversion processes are fixed in a biofilm making additional separation and recycling of microbial biomass unnecessary and reducing bacterial loads in the effluent. Biofilm growth also contributes to the diversity in the system because oxygen availability decreases from the outer to the inner layers allowing for the growth of both aerobic and anaerobic bacteria. A serious disadvantage of the combined aerobic–anaerobic multispecies approach described above is the possibility that populations of denitrifying bacteria can develop in the anoxic zones of the biofilm. These would convert nitrogen compounds into gaseous nitrogen causing nitrogen loss. The most effective countermeasure is to remove the nitrate from the system as soon as it is produced.

A well proven method in wastewater treatment which involves attached growth is the use of trickling filters for nitrification or combined processes. Trickling filters are simple biological systems that meet the requirements for WMS in BLSS with regard to low energy consumption, easy handling and low maintenance effort.

For operation in weightlessness the trickling filters require certain adaptations. They have to be changed to membrane aerated flow filters in order to keep gas and liquid separated. Another solution is to operate the trickling filters under artificial gravity generated by acceleration.

1.2.6. Effectiveness

The effectiveness of the filtration system is determined by three measures:

(1) influent concentration of organic load (in the case of urine degradation: concentration of organic nitrogen), which can be processed at a constant rate over a long period; (2) rate at which organic loads are converted (nitrate production per day); (3) percentage of nitrogen converted into nitrate. As conversion rates and tolerance limits of the microorganisms with respect to concentrations of organic compounds and their degradation products strongly depend on environmental factors, conditions in the system have to be optimized to match the requirements of as many species as possible.

1.2.7. Filter material

The main task for the filter material is to provide an environment in which microorganisms with differing environmental demands can coexist, and in which the suppression of slow growing species is prevented. Pumice has special characteristics, which provide the conditions needed. The rough surface allows the biofilm to attach firmly to it. Thus, when parts of the biofilm are washed out it is likely that a fraction of the colonies remains from which they can regenerate. The pores present in the material offer isolated habitats which can work as protected growth areas for slow growing species. The varying composition of the waste and wastewater and therefore changing substrate concentrations for bacterial growth are moderated by the good adsorption properties of pumice. At times when a substrate is available in high concentrations it is partly adsorbed so that a reservoir for times in which the substrate is not available is formed. This mechanism is especially important for the maintenance of bacteria able to degrade micropollutants like pharmaceutical residues which are usually only present at intervals. The positive properties of pumice as filter material are intensified by the operation in batch mode instead of continuous flow. Bacterial cells that have been washed off the filter material are not immediately removed, but recirculated through the filter increasing their chance to resettle and maintain the species in the system.

In this study, we present the data generated in test runs of a water-based biological waste management system designed according to the considerations described above. It includes the results of microbiological analyses and prototype test runs with synthetic urine. Results are discussed with regard to the design considerations.

2. Material and methods

2.1. Filter material

The pumice used as filter material is called “Rote Eifellava” and is mined in quarries near Cologne. The average elemental composition is shown in Table 2. The particle size ranges from 16 to 25 mm; average porosity is 0.35 (range: 0.07–0.69; porosity measured as (wet weight – dry weight)/(wet weight – immersed

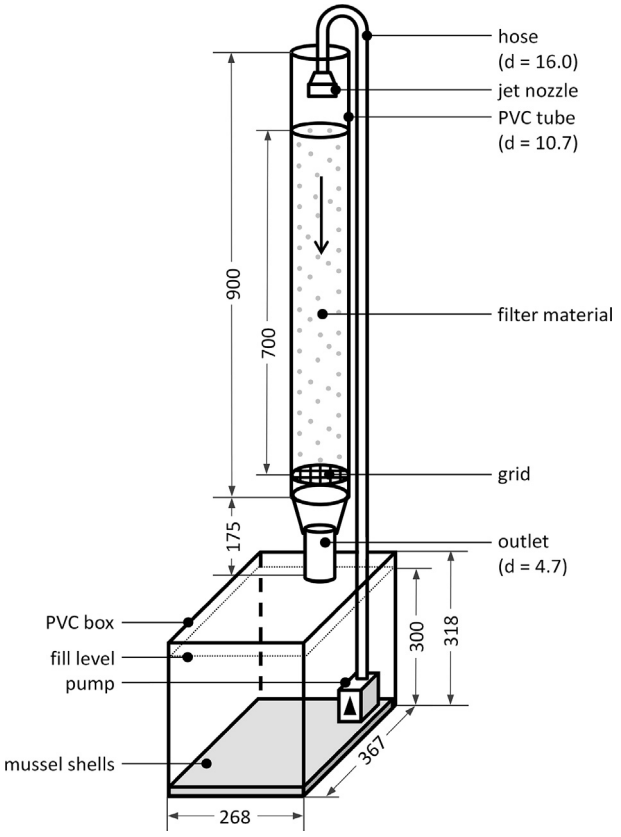

Fig. 1. Schematic representation of a filter unit. Dimensions given as internal dimensions [mm]; d = diameter.

weight)); bulk density (dry) averages 1000 g/l (own measurements). Technical data from other sources give a bulk density of 800–1400 g/l; a specific weight range 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).

2.2. Filter units

The filters are built of opaque plasticizer free PVC tubes (length: 90 cm, diameter: 11 cm, bulk volume of the filter material: ~6 l), which are vertically fixed to the wall. The bottom end is blocked with a metal grid and a drain is formed using tube connectors common for sewage installation. 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 flow rate, power consumption: 23 W) and the corresponding Eheim hoses (16/22 mm, PVC) to constitute permanent circulation of the liquid through the filters (see Fig. 1). The upper end of the hoses is fitted with a jet nozzle (Messner, Germany: Volcano Jet special G1/2″) that disperses the water jet over the complete surface of the filter material. In 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 and the pump cable and hose. Before use, all filters were incubated with 1 g of dried garden soil sampled in the garden of the German Aerospace Center in Cologne followed by a preoperational run with tap water that lasted two days. When the tanks were loaded with fresh synthetic urine, 500 g of mussel shells (coarsely ground, feed supplement for chicken purchased at the local farm shop, $CaCO_3$ content: 70–80%) as a bioregenerative source of calcium carbonate were added for acid neutralization.

2.3. Test run

The synthetic urine was prepared according to the protocol of Feng and Wu (2006). It contains approximately 7780 mg 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). The initial pH of the solution is 6.8.

The microbial degradation of the urea contained in urine (nitrification) comprises three steps: (1) hydrolysis of urea to ammonium, (2) oxidation of ammonium to nitrite, and (3) oxidation of nitrite to nitrate, which is the preferred nitrogen source of many plants and thus the desired product of the degradation process. In the course of the degradation process pH values first increase due to the production of alkaline ammonium, and then drop due to acid accumulation during ammonium oxidation. Electric conductivity increases with increasing concentrations of ionic nitrogen compounds.

For the test run three filter units (units 1–3) received 2 l of synthetic urine diluted with 28 l of tap water, thus the resulting urine solution contained approx. 7% of urine (≈0.520 mg nitrogen per liter). Three other filter units (units 4–6) received 6 l of synthetic urine resulting in a 20% urine solution with approximately 1555 mg nitrogen per liter. Temperature, EC and pH of the solutions in all tanks were measured daily except for holidays and weekends (temperature and EC: WTW Multi 1970i with TetraCon 325; pH: Sentix 41; all WTW, Germany). The filter units had already been in continuous batch mode operation for two years. The 194 days measuring period presented here started with the setup of the ion exchange chromatograph (Metrohm 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). As an indicator of filter performance nitrate production and change of EC were monitored. During the measuring period the filtrate in filter unit 1 was exchanged eight times (eight filtrate maturation periods numbered A–H), the filtrate in filter units 2 and 3 seven times (seven maturation periods numbered A–G) when nitrate concentrations reached maximum values. The filtrate in units 4–6 was exchanged six times (A–F).

2.4. Statistics

Figures were created with Excel 2010. Curves for the 7% filter units were fitted with the Matlab curve fitting tool using the exponential equation: $a * \exp(b * x) + c * \exp(d * x)$. The dynamics of the 20% filter units could not be fitted with the same equation. For them the cubic polynomial $a * x^3 + b * x^2 + c * x + d$ was used. Average maximum nitrate concentration ($NO_{3\max}$ in mg NO_3–N per l) was determined by computing the maximum of the function. The corresponding value for nitrate production is $NO_{3\max} * 30$ (for 30 l bulk liquid volume). Nitrate production rate was computed as $v_{\max} = NO_{3\max} * 30/d_{\max}$. As the function approaches the maximum asymptotically and maturation periods were prolonged due to the necessity to observe the maximum nitrate concentration the units produce, we also computed the production rate in the point $NO_{3\max}/2$ in parallel to the computation of the Michaelis Menten constant to get a better measure for the average nitrate production rate ($v_{1/2} = (NO_{3\max}/2 * 30)/d_{1/2}$) of a filter unit (see Fig. 2).

2.5. Microbiological analyses

2.5.1. Microscopy

For microscopy a few pieces of pumice were taken out of the filter units. The biofilm on the surface and in the pores was stained

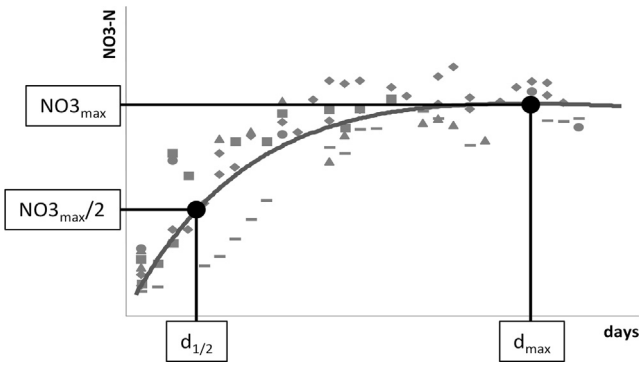

Fig. 2. Statistical parameters for the evaluation of filter performance.

with LIVE and DEAD dye (Fluorenscence dye: Molecular Probes/Invitrogen L7007 LIVE/DEAD® BacLight™ Bacterial Viability Kit, red = dead bacteria stained with propidium iodide; green = live bacteria stained with SYTO® 9. In the black and white pictures live bacteria appear white, dead bacteria light gray). Microscopic pictures were taken using a laser microscope (Nikon eclipse 80i with Nikon Detector D-eclipse C1, laser wavelength 488 nm, detection filter FITC 515–555 nm, software: Nikon EZ-C1 3.50 build 724).

2.5.2. Collection of rock and filtrate samples

For microbiological analyses 50–100 ml of the filtrate and 25–50 g of pumice was aseptically taken from a C.R.O.P. filter unit in our testing facility by the end of the test run. In addition, samples from fresh unused pumice gravel and the tap water in our laboratory were taken. The pumice was ground into pieces of less than 5 mm in diameter, mixed in a tube, and used as samples.

2.5.3. Extraction of DNA and generation of PCR-SSCP genetic profiles

Total DNA was taken from filtrate and pumice samples as described in detail by Peters et al. (2000) and Schwieger and Tebbe (1998). Different primer systems were used to amplify 16S rRNA genes from total community DNA (Peters et al., 2000; Schwieger and Tebbe, 1998). The single stranded DNA molecules were purified and separated on a MDE polyacrylamide gel according to the previously mentioned protocol (Schwieger and Tebbe, 1998). Selected products ("bands") identified in the MDE polyacrylamide gels after silver staining were excised with razor blades, and single stranded DNA was eluted from the gel by a crush and soak procedure (Schwieger and Tebbe, 1998; Sambrook et al., 1989). The single-stranded DNA molecules were reamplified by PCR using the same primers and conditions as for the respective SSCP analysis. For cloning, DNA from the reamplified PCR products was used as template for 16S rRNA gene amplification by standard PCR, using the universal bacterial primers. Alignments and database identifications of the consensus sequences were carried out using BLASTN (Schwieger and Tebbe, 1998; Gévaudan et al., 2012).

3. Results

3.1. Test run

In an experimental run over a period of 194 days, three filter units were operated with 7% urine solution and three filter units with 20% urine solution. The corresponding nitrate production rates are shown in Fig. 3 and Fig. 4. On an average the filter units 1, 2 and 3, which were fed with 7% urine solution, converted 84% of the contained nitrogen into NO_3–N, which corresponds to an average maximum NO_3–N concentration of 435 mg NO_3–N/l (1927 mg NO_3/l). Production rates v_{max} averaged 516 mg NO_3–N per day (2286 mg NO_3/d or 996 mg of processed urea per day).

$v_{1/2}$ averaged 2191 mg NO_3–N per day (9699 mg NO_3/d or 4850 mg of processed urea per day). Filter units 4, 5 and 6, which were fed with 20% urine solution, converted 76% of the available nitrogen into nitrate. The corresponding average maximum NO_3–N concentration was 1172 mg NO_3–N/l (5190 mg NO_3/l). The average production rate v_{max} was 1076 mg NO_3–N per day (4763 mg NO_3/d or 2382 mg of processed urea per day). $v_{1/2}$ averaged 1411 mg NO_3–N per day (6246 mg NO_3/d or 3123 mg of processed urea per day) (Table 3).

Corresponding temperatures and pH values are given in Fig. 5 and Fig. 6. Temperature was sufficiently constant. pH values reached a minimum around the tenth day of the maturation periods. The increase in electric conductivity was completed after ten days in units 1–3 and after 20 days in units 4–6 (Fig. 7). No sloughing occurred during the described test runs with synthetic urine.

3.2. Microbiology

For microscopy some pumice particles were taken out of a running filter unit. Fig. 8 shows the microscopic images of the filter material and of the biofilm growing on it. The biofilm is especially dense inside the pores of the material. The culture-independent 16S rRNA gene-based study of the bacteria community provided insights into the microbial diversity in the biofilm on the filter material and in the filtrate of a filter unit. The analysis also showed that there were some bacteria species present in fresh pumice particles and tap water used to build and operate the filter units (Table 4). The microflora of the filter unit comprised bacteria from bacteroidetes, flavobacteria, sphingobacteria, bacilli, nitrospira, α-, β- and γ-proteobacteria genera. Besides the autotrophic species commonly involved in the degradation of organic and inorganic nitrogen compounds, some heterotrophic denitrifiers and nitrogen fixing species, a variety of species living on other substrates was found. The bacteria identified from the sequences showed an average identity of $\geq$90% to known phylotypes.

4. Discussion

The main objective of the work presented in this paper is to develop a multifunctional water-based biowaste management system, which is robust, easy to maintain and produces a fertilizer solution applicable in hydroponic plant cultivation.

Table 3
Comparison of conversion rates and maximum values of nitrate concentrations of filter units operated with 7% and 20% synthetic urine.

	7%			20%		
	unit 1	unit 2	unit 3	unit 4	unit 5	unit 6
% N in NO_3–N	81%	90%	80%	81%	79%	67%
d_{max}	26 d	27 d	23 d	34 d	31 d	34 d
NO_3–N_{max}	422 mg/l	466 mg/l	418 mg/l	1252 mg/l	1228 mg/l	1037 mg/l
v_{max}	496 mg/d	510 mg/d	543 mg/d	1107 mg/d	1194 mg/d	926 mg/d
$d_{1/2}$	3 d	3 d	4 d	13 d	13 d	12 d
$v_{1/2}$	2445 mg/d	2394 mg/d	1734 mg/d	1436 mg/d	1464 mg/d	1334 mg/d

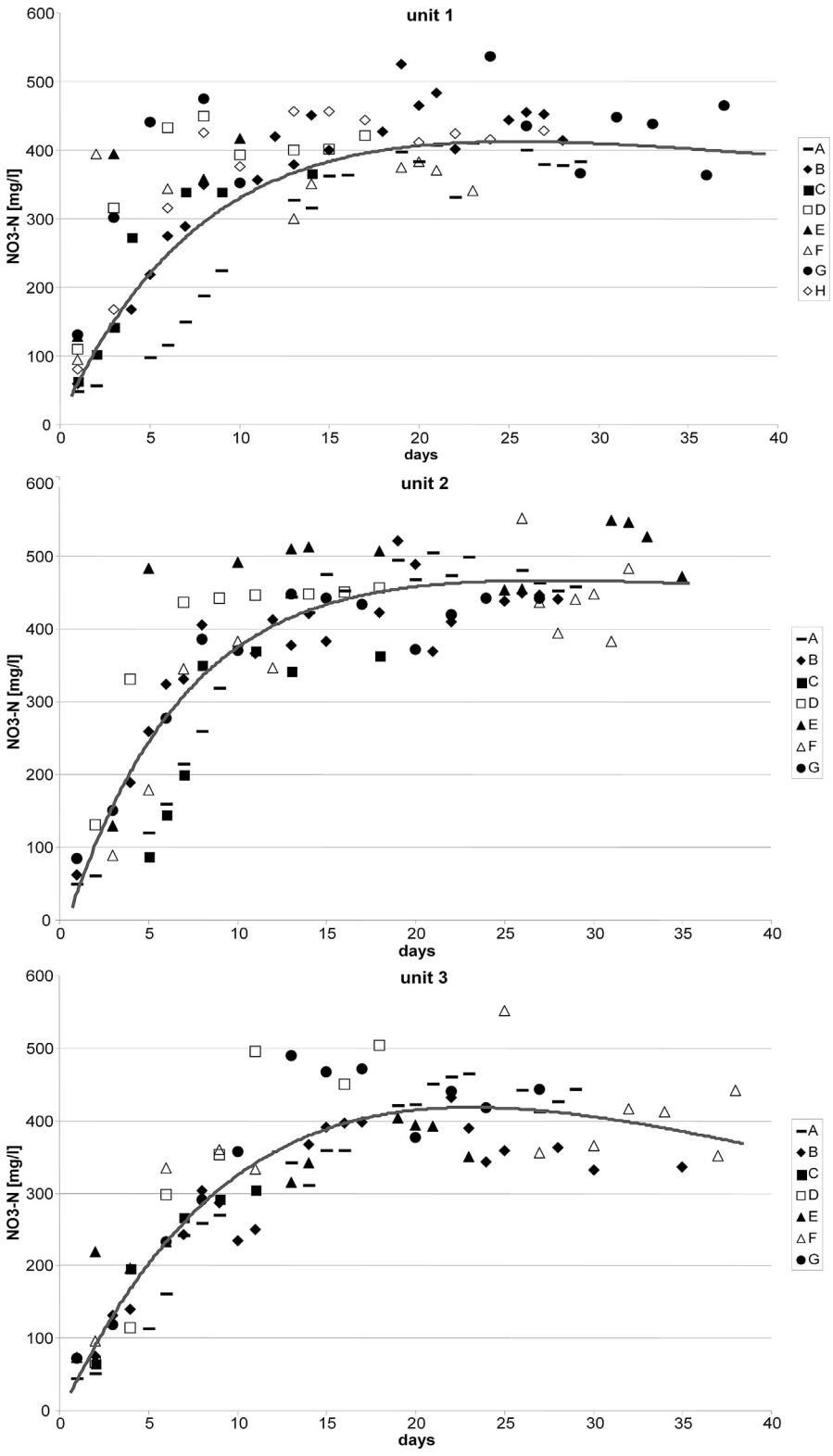

Fig. 3. Nitrate production rates of three filter units loaded with 7% urine solution over a test period of 194 days. Letters label the consecutive maturation periods. The line was estimated with an exponential fitting function.

The trickling filters chosen as processing units proved to be easy to handle and energy-saving because operation based on only one pump each. The choice of pumice as filter material was mainly due to its porosity. The microscopic examination of the attached biofilm suggests that single pores can serve as microhabitats for bacteria colonies and thus successfully simulates the heterogeneity of soil habitats. A community of 26 bacterial species was identified on the filter material and 12 species in the filtrate. This indicates that bacteria are effectively retained in the filter material. On fresh unused pumice and in the water from the laboratory taps only a few species were found substantiating the assumption that inoculation with soil enables the culture of a community of soil bacteria

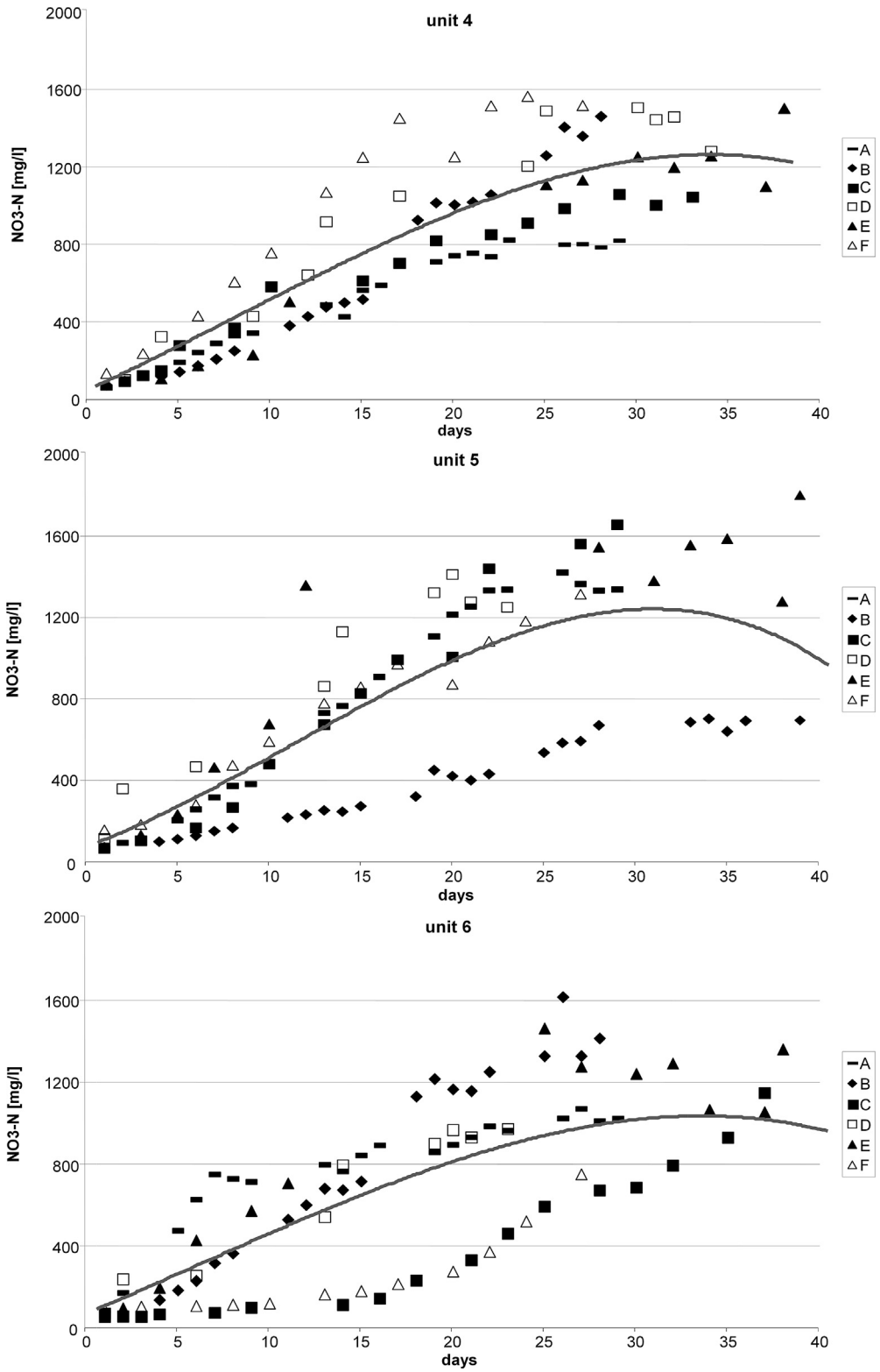

Fig. 4. Nitrate production rates of three filter units loaded with 20% urine solution over a test period of 194 days. Letters label the consecutive maturation periods. The line was estimated with a polynomial fitting function.

in the filters. The fact that several species found in the tap water were absent in the filter unit indicates that the growth of cells introduced with the feedstock is inhibited.

Soil can be addressed as a universal culture medium which provides all kinds of substrates for bacteria and other microbes to grow on together with all kinds of different habitats. This is reflected by the high number of microbial species found in most soils. To inoculate a trickling filter with soil means to make it omnipotent with regard to the substrates that can be degraded. Running such a filter with urine only, means to apply a highly

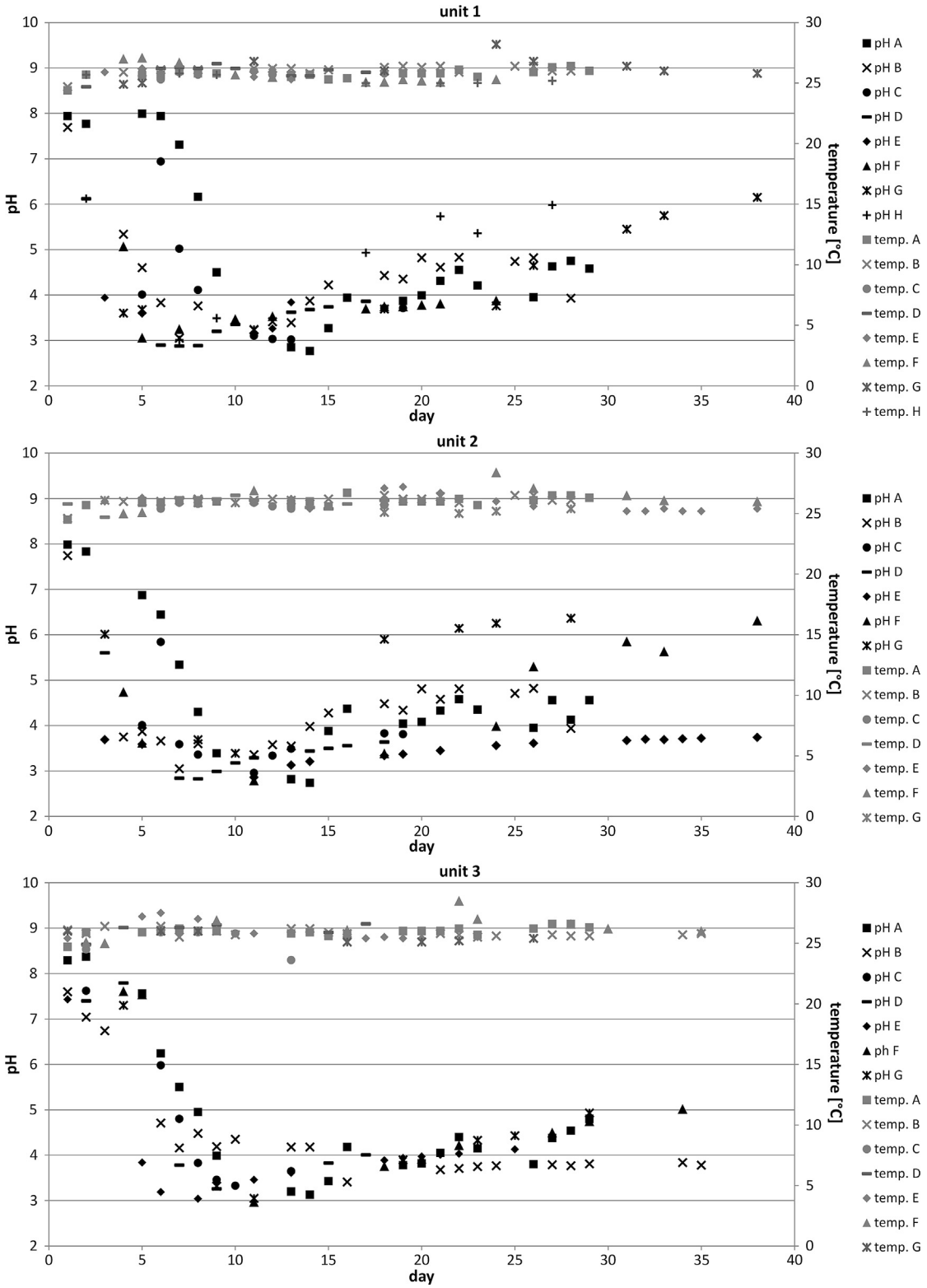

Fig. 5. Temperature (right vertical axis) and pH (left vertical axis) of the filtrate in three filter units loaded with 7% urine solution during a 194 days test run. Letters label the consecutive maturation periods.

selective grow medium with low amounts of organic carbon and high amounts of organic nitrogen, which is well aerated and favors the growth of aerobic autotrophic bacteria.

In accordance with the selective environmental conditions, the microbiological analysis identified six aerobic autotrophic nitrifiers in the filter material, two of which were also found in the filtrate. Compared to the low amount of organic carbon which was introduced into the system in the form of urea and creatinine, a large number of heterotrophic bacteria of various kinds settled in the system (20 of 28 found in the system). It can be assumed that many of these species at least partly live on dead cells from the biofilm which would also explain the absence of excess biomass.

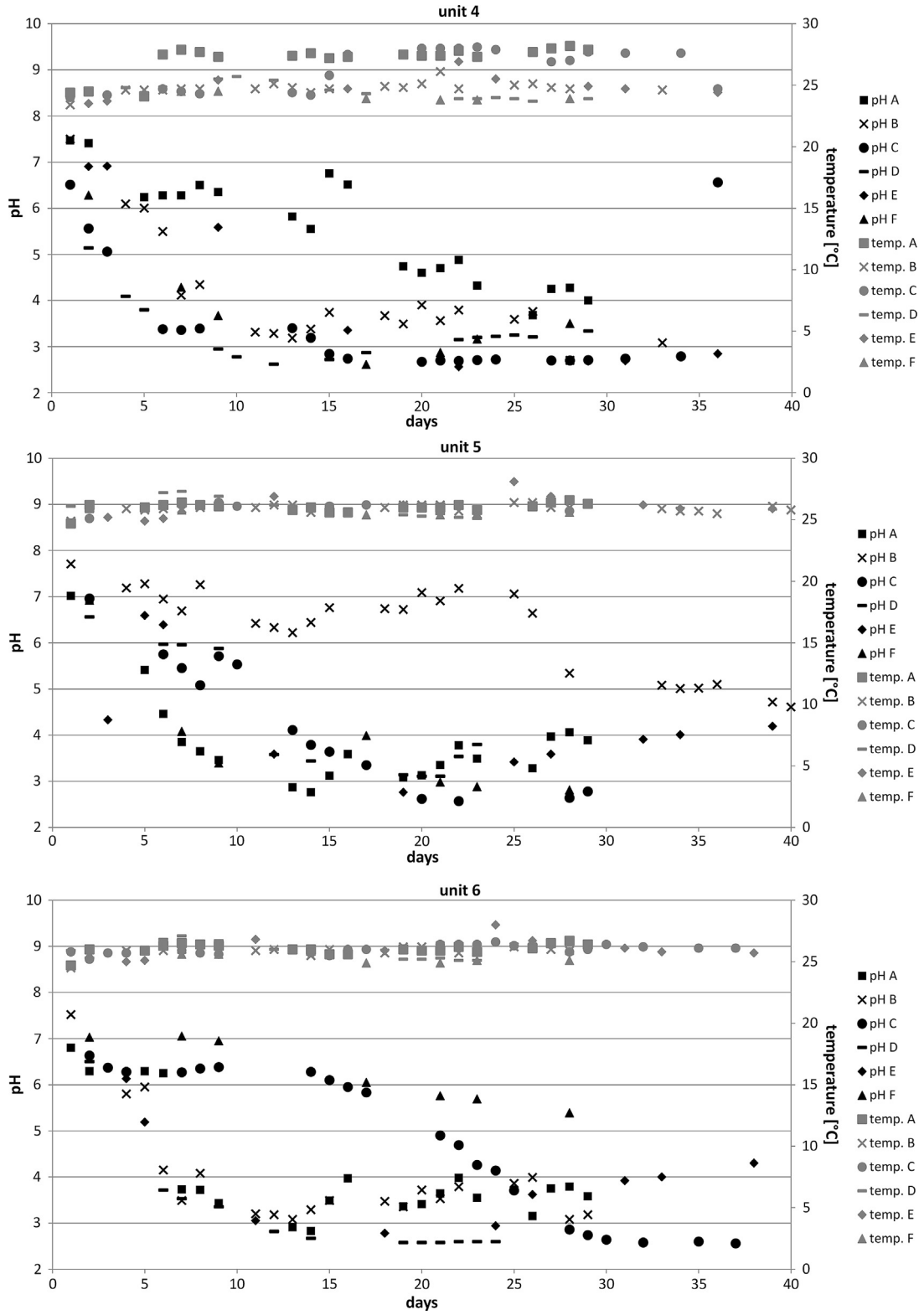

Fig. 6. Temperature (right vertical axis) and pH (left vertical axis) of the filtrate in three filter units loaded with 20% urine solution during a 194 days test run. Letters label the consecutive maturation periods.

The occurrence of anaerobic bacteria demonstrates the establishment of anoxic microhabitats in the system. In these, anaerobic or facultative anaerobic denitrifiers could be active causing nitrogen loss in the form of gaseous nitrogen. As 70–90% of the available nitrogen is present as nitrate at the end of a maturation period, nitrogen leakage can be considered comparatively low. It is possible that the nitrogen fixing bacteria found in the filter material contribute to the amount of converted nitrogen by fixing gaseous nitrogen produced by denitrifiers and returning it to the solution.

Even though a variety of microbial species could be established in the filter unit, it is especially pre-conditioned on the degradation of urine. Any change of feedstock would need new con-

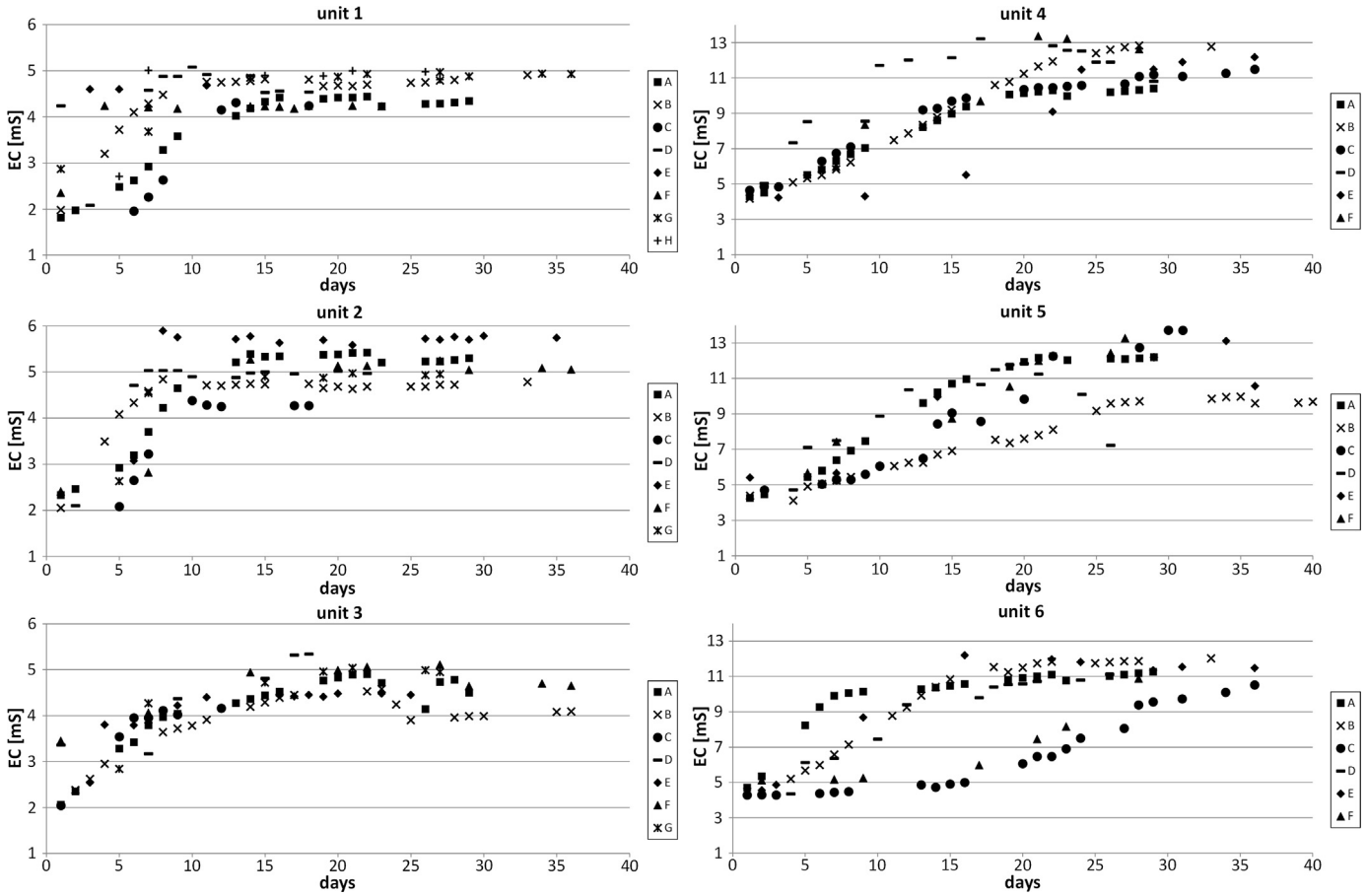

Fig. 7. EC of the filtrate in six filter units loaded with 7% (1–3) and 20% (4–6) urine solution during a 194 days test run. Letters label the consecutive maturation periods.

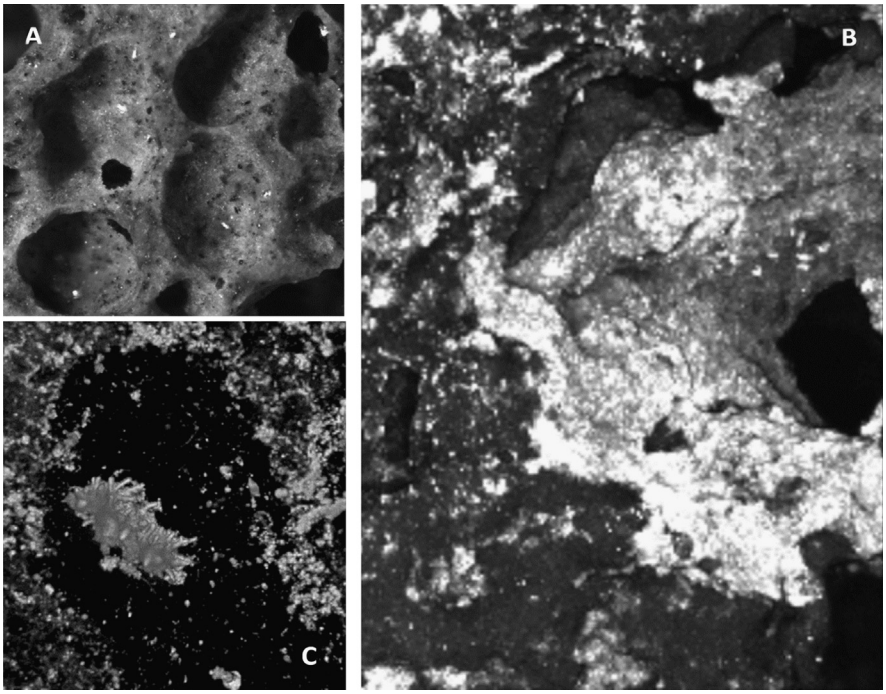

Fig. 8. (A) Overview over the pores in a pumice particle (multifocal, z-stack: 320 µm in steps of 16 µm, lower edge = 593 µm). (B) Biofilm growth in the pores of a pumice particle taken out of a working filter unit (fluorescence 488 nm, live = white, dead = light gray, z-stack: 300 µm in 15 µm steps, right edge = 240 µm). (C) A colony of Rhodopseudomonas palustris growing isolated in a pore of a pumice particle taken out of a running filter unit (fluorescence 488 nm and 535 nm, live = white, dead = light gray, z-stack 320 µm in steps of 16 µm, right edge = 130 µm).

Table 4
Bacterial species identified in the samples from the filter material, bulk liquid (filtrate), fresh pumice and tap water from the laboratory, and their functions.

Genus	Species	Function	Aerobic	Anaerobic	Autotrophic	Heterotrophic	Filter material	Filtrate	Fresh pumice	Tap water	References
Rudaea	*cellulosilytica*[m]	hydrolysis cellulose, urea	X			X	X	X			(Weon et al., 2009)
Nitrosospira	*multiformis*[k]	ammonia → nitrite	X		X		X				(Norton et al., 2008)
Nitrosovibrio	*tenuis*[k]	ammonia → nitrite	X		X		X				(Harms et al., 1976)
Nitrosomonas	*ureae*[k]	ammonia → nitrite	X		X		X				(Koops et al., 1991)
Nitrosomonas	*aestuarii*[k]	ammonia → nitrite	X		X		X				(Koops et al., 1991)
Nitrospira	*defluvii*[h]	nitrite → nitrate	X		X		X	X			(Attard et al., 2010)
Nitrospira	*moscoviensis*[h]	nitrite → nitrate	X		X		X	X			(Blackburne et al., 2007)
Mesorhizobium	*tianshanense*[j]	nitrogen fixation	X			X	X				(Jarvis et al., 1997)
Mesorhizobium	*mediterraneum*[j]	nitrogen fixation	X			X	X				(Nour et al., 1995)
Mesorhizobium	*temperatum*[j]	nitrogen fixation	X			X	X				(Gao et al., 2004)
Diaphorobacter	*nitroreducens*[k]	denitrification	X	X		X	X		X		(Khan and Hiraishi, 2002)
Acidovorax	*ebreus*[k]	denitrification		X		X	X		X		(Byrne-Bailey et al., 2010)
Comamonas	*denitrificans*[k]	denitrification	X	X		X			X		(Andersson et al., 2009)
Comamonas	*testosteroni*[k]	steroid degradation	X			X	X			X	(Gumaelius et al., 2001)
Rhodopseudomonas	*faecalis*[j]	ammonium degradation		X	X	X	X				(Zhang et al., 2010)
Rhodopseudomonas	*palustris*[j]	ammonium degradation		X	X	X	X				(Ramana et al., 2012)
Flavobacterium	*granuli*[c]	urease positive	X			X				X	(Aslam et al., 2005)
Terrimonas	*lutea*[b]	nitrate reduction	X			X	X				(Xie and Yokota, 2006)
Flavobacterium	*pectinovorum*[c]	nitrate reduction	X			X				X	(Aslam et al., 2005)
Delftia	*tsuruhatensis*[k]	nitrate reduction, lipase hydrol.	X			X				X	(Shigematsu et al., 2003)
Rhodanobacter	*lindaniclasticus*[m]	lindane degradation	X			X	X	X			(Nalin et al., 1999)
Rhodanobacter	*spathiphylli*[m]	gelantine degradation	X			X	X	X			(De Clercq et al., 2006)
Rhodanobacter	*terrae*[m]	hydrolysis of casein	X			X	X	X			(Weon et al., 2007)
Lysobacter	*brunescens*[m]	hydrolysis of petrate, starch	X			X	X				(Christensen and Cook, 1978)
Lysobacter	*ginsengisoli*[m]	aesculin hydrolysis	X			X	X				(Jung et al., 2008)
Lysobacter	*panaciterrae*[m]	aesculin hydrolysis	X			X	X				(Ten et al., 2009)
Luteimonas	*aquatica*[m]	hydrolysis: starch, casein	X			X	X				(Chou et al., 2008)
Flavobacterium	*sp.*[c]	hydrolysis: starch, casein	X	X		X	X				(Bernardet et al., 1996)
Flavobacterium	*succinicans*[c]	degradation of diff. compounds	X			X				X	(Zhang et al., 2006)
Bacillus	*circulans*[f]	fermentation		X		X		X		X	(Nakamura and Swezey, 1983)
Bacillus	*nealsonii*[f]	fermentation		X		X		X		X	(Venkateswaran et al., 2003)
Delftia	*acidovorans*[k]	hydrolysis of acetamide	X			X				X	(Horowitz et al., 1990)
Pseudomonas	*fragi*[m]	dairy spoilage	X			X	X				(Pereira and Morgan, 1957)
Hydrotalea	*flava*[d]	hydrolysis of diff. compounds	X			X		X			(Kämpfer et al., 2011)
Stenotrophomonas	*sp.*[m]	hydrogen sulfide prod.	X			X				X	(Palleroni and Bradbury, 1993)
Xanthomonas	*sp.*[m]	plant pathogen	X					X			(Vauterin et al., 1995)
Bacteroides	*sp.*[b]	gastrointest. flora		X				X			n/a
Lysobacter	*pocheonensis*[m]									X	n/a
Gynumella	*flava*[m]						X	X			n/a

[b] Bacteroidetes/Bacteroidetes.
[c] Bacteroidetes/Flavobacteria.
[d] Bacteroidetes/Sphingobacteria.
[f] Firmicutes/Bacilli.
[h] Nitrospirae/Nitrospira.
[j] Proteobacteria/Alpha proteobacteria.
[k] Proteobacteria/Beta proteobacteria.
[m] Proteobacteria/Gamma proteobacteria.

ditioning. Regarding multifunctionality, the most effective way to build up a flexible filter system would be to operate the filter with mixed substrates from the beginning to pre-condition it for the desired function.

During the test runs filter units loaded with the same urine concentration showed similar conversion kinetics while there were clear differences between filter units loaded with different concentrations. Compared to filter units operated with 7% urine solution, which showed robust performance over the period of 194 days, the units operated with 20% urine showed a higher variance in maximum nitrate concentrations. This is also reflected in the lower percentage of nitrogen converted into nitrate and indicates that higher loading rates induce increasing instability in terms of operational safety. The 20% units had higher overall conversion rates v_{max} than the 7% units. In contrast to this the half-maximal rate $v_{1/2}$ was lower in the 20% units. One reason for this contradiction might lie in the differing curve fitting procedures. Considered by the graphical representation in Fig. 3, the duration of the maturation periods in the 7% units can easily have been overestimated due to the asymptotic approximation to the maximum. Therefore, the half-maximal rate can be considered more reliable indicating that higher loading rates slow down conversion rates. One reason for increased instability and slowed conversion might be the composition of the microbial population. High concentrations of nitrogen compounds and other ions as found in urine could inhibit growth and metabolic activity of many species thus slowing down conversion.

Generally, conversion rates were quite low. It can be assumed that maximum performance was not reached, because maturation periods were prolonged due to the necessity to determine the maximum concentration of nitrate the filters can produce. In addition, the decrease of pH around the tenth day of the maturation periods indicates that calcium carbonate dissolves too slowly out of the mussel shells. Although slight acidity (pH 6) is desirable to minimize nitrogen loss in the form of ammonia, too low pH inhibits nitrification (Tchobanoglous et al., 2004). This means that filter performance can still be optimized by making calcium carbonate readily available and by shortening of maturation periods. Another factor that very likely contributes to the retardation of conversion is the high flow rate of 1000 l/h. It keeps retention time short and the biofilm thin but also ensures optimal oxygen supply and consequently reduces nitrogen loss by denitrification to a minimum.

5. Conclusions

Human urea excretion averages 15–30 g per day. The ideal configuration of a filter unit for integration into a modular life support system would be one unit per person to facilitate scaling according to crew number and to ensure sufficient redundancy. The necessary nitrate production rates to degrade the urine of one person per day were not achieved during the test run, but this does not necessarily mean that the filter units need to be enlarged. There are several other entry points for the optimization of conversion rates. First of all pH control has to be improved by making calcium carbonate better available or choosing another buffer. With regard to urine concentration the results indicate that higher concentrations slow down conversion. This has to be examined in further experiments with higher concentrations. When higher concentrations are to be processed shifting from the batch process applied in this test run to a fed batch process might prove beneficial. Particular attention will be paid to the influence of flow rates on biofilm growth and the effect of increased biofilm thickness on conversion rates and nitrogen loss. It has already been discussed that the low amount of leakage and low conversion rates were due to the thin but well aerated biofilm layer. Based on this assumption, it can be hypothesized that there is a trade off between rate and leakage. It has to be checked whether lower rates should be accepted for the sake of a higher degree of closure.

In summary, results to date have shown that trickling filter units involving pumice populated with a community of soil microorganisms are capable of producing a nitrate solution from highly diluted urine at a constant rate over long operational periods. After the optimization of nitrate production from urine the impact of the introduction of carbon compounds, like carbohydrates and cellulose from plant waste, on the system has to be determined and evaluated with regard to the desired multifunctionality. When the optimal conditions for filter operation are determined, plants will be introduced into the system.

Disclosure statement

No competing financial interests exist.

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