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Yield of dwarf tomatoes grown with a nutrient solution based on recycled synthetic urine

P. Zabel[a,*], G. Bornemann[b], M. Tajmar[c], D. Schubert[a]

[a] German Aerospace Center (DLR), Institute of Space Systems, Robert-Hooke-Str. 7, 28359 Bremen, Germany
[b] German Aerospace Center (DLR), Institute of Space Medicine, Cologne, Germany
[c] Technische Universität Dresden, Institute of Aerospace Engineering, Dresden, Germany

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ABSTRACT

Extended human spaceflight missions require not only the processing, but also the recycling of human waste streams in bio-regenerative life support systems, which are rich in valuable resources. The Combined Regenerative Organic food Production® project of the German Aerospace Center aims for recycling human metabolic waste products to produce useful resources. A biofiltration process based on natural communities of microorganisms has been developed and tested. The processed aqueous solution is, among others, rich in nitrogen present as nitrate. Nitrate is one of the main nutrients required for plant cultivation, resulting in strong synergies between the developed recycling process and plant cultivation. The latter is envisaged as the basis of future bio-regenerative life support systems, because plants do consume carbon dioxide, water and nutrients in order to produce oxygen, water, food and inedible biomass. This paper describes a series of plant cultivation experiments performed with synthetic urine processed in a bioreactor. The aim of the experiments was to investigate the feasibility of growing tomato plants with this solution. The results of the experiments show that such cultivation of tomato plants is generally feasible, but that the plants are less productive. The fruit fresh weight per plant is less compared to plants grown with the half-strength Hoagland reference solution. This lack in production is caused by imbalances of sodium, chloride, potassium, magnesium and ammonium in the solution gained from recycling the synthetic urine. An attempt on adjusting the produced bioreactor solution with additional mineral fertilizers did not show a significant improvement in crop yield.

1. Introduction

A space greenhouse is often envisioned to take over a number of functions of a future life support system such as air revitalization, food production and water recycling. Although a large number of plant growth chambers have been built and launched in the past (Zabel et al., 2016), there are still challenges to overcome in order to build a reliable space greenhouse. In commercial greenhouses, the nutrient solutions are usually prepared by mixing crystalline and/or liquid fertilizers with water. This procedure guarantees an optimal supply of all required minerals in the correct amount. Adapting this procedure for a space greenhouse is not trivial. Either the nutrient salts have to be supplied from Earth or produced in-situ at the location of the space greenhouse. The first might work for plant growth chambers and small greenhouses and short mission durations, but is inconvenient for large greenhouses and long mission durations. The in-situ production of nutrient salts is only possible if the minerals are present and easily accessible at the location of the greenhouse. Nevertheless, this production is work and energy intensive.

There have been a number of similar attempts in the past on recycling urine and other liquid wastes with a bioreactor and then using the product solution for plant cultivation. During the experiments conducted in the Bios-3 facility (Gitelson et al., 2003), unprocessed human urine has been supplied to wheat plants (Lisovsky et al., 1997). Experiments in cultivating potato plants with a nutrient solution produced from plant biomass processed in a bioreactor have been conducted in NASA's Biomass Production Chamber (Mackowiak et al., 1997a,b; Garland et al., 1997a,b). Researchers at the Texas Tech University and NASA's Johnson Space Center developed and tested a membrane-aerated biological reactor for treating liquid waste streams (Meyer et al., 2015; Christenson et al., 2015; Sevanthi et al., 2014).

DLR's C.R.O.P.® (Combined Regenerative Organic food Production)

* Corresponding author.
E-mail addresses: paul.zabel@dlr.de (P. Zabel), gerhild.bornemann@dlr.de (G. Bornemann), martin.tajmar@tu-dresden.de (M. Tajmar), daniel.schubert@dlr.de (D. Schubert).

project investigates the production of a plant nutrient solution out of biological waste produced by the crew and the greenhouse itself in order to recycle valuable nutrients. The goal of C.R.O.P.® is to develop a bio-regenerative compartment for life support systems that combines biological waste (e.g. food residuals, urine, plant material) treatment with soilless plant cultivation.

The waste treatment system is under development at the DLR Institute of Aerospace Medicine in Cologne, Germany. The plant cultivation tests are done at the DLR Institute of Space Systems in Bremen, Germany, where the experiments reported in this study were conducted.

2. Materials and methods

2.1. Urine derived nutrient solution

The urine solution used in the experiments was the product of the C.R.O.P.® biofiltration process of synthetic urine during which the contained urea is nitrified. The production process is described in detail in Bornemann et al. (2018). The synthetic urine was made according to the recipe of Feng and Wu (2006), which can be found in the Annex. The composition of the C.R.O.P.® solution is given below. The TOC of the C.R.O.P.® solution averages 150 mg/l, the COD averages 210 mg/l after 4 h pasteurization at 70 °C.

The experiment compared the C.R.O.P.® bioreactor solution with a reference nutrient solution known as Half-Strength Hoagland Solution (Hoagland and Arnon, 1950). This solution is commonly used as a baseline for soilless plant cultivation. Micro-Tina dwarf tomato (Scott et al., 2000) was selected as crop used in the experiments, because of the small size suitable for small plant growth chambers in space.

2.2. Experiment hardware

2.2.1. Overview

All experiments were conducted in four custom-built growth chambers (GC), see Fig. 1. The chamber structure is made out of aluminum profiles. The wall elements are compressed hard plastic with a white coating. Each chamber is 1.0 m wide, 0.5 m deep and 1.0 m high. This results in a cultivation area of 0.5 m^2 and a volume of 0.5 m^3 per chamber.

There is a high-power LED lamp with customizable spectrum in each chamber. The left two chambers (GC 1 and GC 2) and the right two chambers (GC 3 and 4) share the same nutrient solution tank. Installed on the backside of the chambers are fans for air circulation and the connections to the centralized atmosphere management system of the laboratory. All four chambers are connected to a stand-alone control and data acquisition system based on a programmable logic controller, which is mounted on the right wall of the experiment setup.

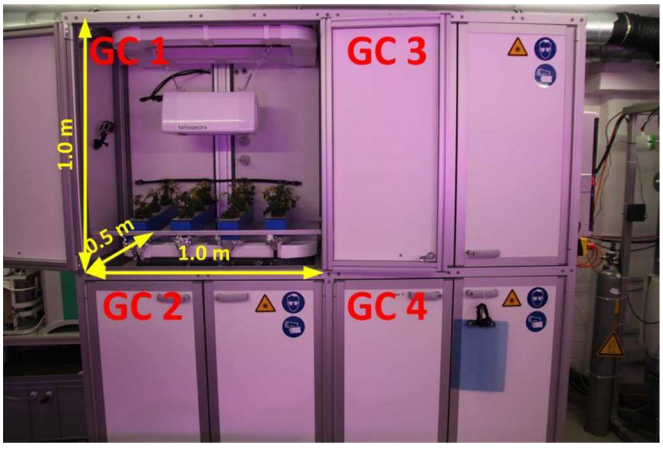

Fig. 1. Overview of GC 1–4 hardware setup.

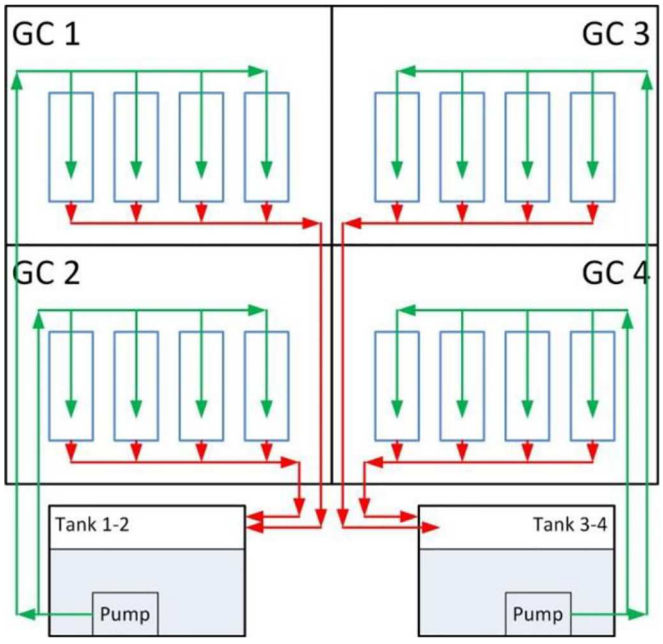

Fig. 2. Nutrient delivery system schematic. A pump in each tank supplies nutrient solution (green lines) to the growth channels (blue boxes). Excess fluids return to the tanks (red lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2.2. Illumination system

The illumination system consists of one LX601 C-plate lamp of the Swedish company Heliospectra AB per chamber. The lamp is designed to illuminate an area of 1.2 × 1.2 m at a distance of 0.5 m and is air-cooled. The LX601 has 240 LEDs of four different wavelengths: blue LEDs (450 nm), red LEDs (660 nm), far-red LEDs (735 nm) and white LEDs (5700 K). Each wavelength can be controlled separately. When the lamp is set to 100% for the full spectrum it has a photon flux of 862–1011 µmol/s and a power demand of 630 W.

2.2.3. Nutrient delivery system

The nutrient delivery system consists of two 80 l plastic tanks each containing a submersible aquarium pump capable of pumping water up to two meters height. One tank supplies the left two chambers (GC1-2) and one tank supplies the right two chambers (GC3-4) through a system of pipes and manual valves, as shown in Fig. 2. The supply line of each chamber is then split into smaller pipes feeding the plants. The number of growth channels and the number of plants per channel can be adapted for different plants and different experiments. The current setup consists of four growth channels per chamber each holding three plants. The small pipes end in three drippers, one for each plant, and are commercially available gardening components. The growth channels itself are made out of plastic and can be outfitted with different lids. They are mounted inside the chamber with a small inclination towards the doors to allow water flow towards the return water collection tube. This tube transports the nutrient solution back towards the supply tank. The complete layout of the fluid lines is shown in Fig. 2. The growth channels are large enough to contain the 80 × 80 × 60 mm Rockwool blocks in which the plants are growing.

2.2.4. Atmosphere management system

The atmosphere management system of GC1–4 consists of two parts: circulation fans and the centralized system of the EDEN laboratory. Both parts are linked to the air inlet and outlet tubes inside the different

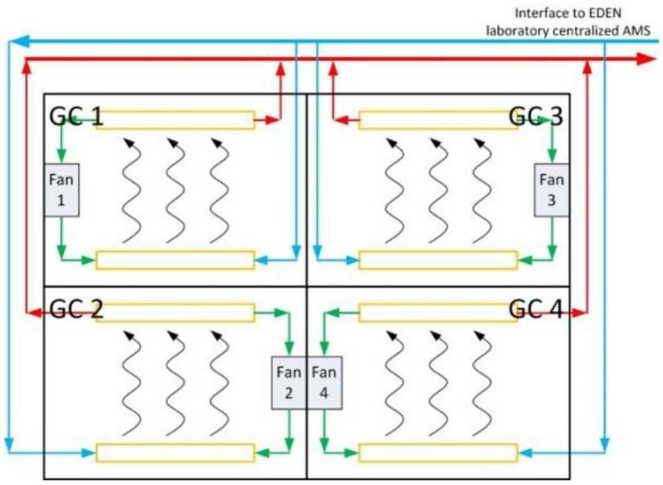

Fig. 3. Atmosphere management system schematic. Fan 1–4 are used to circulate air (green lines). Additionally GC 1–4 are connected to the EDEN laboratory centralized AMS to receive cool air (blue lines) and to get rid of warm air (red lines). Orange boxes symbolize the air distribution channels inside each chamber.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

chambers. Fig. 3 shows how the different parts of the atmosphere management system are connected to each other.

The circulation fans are of the type InlineVent RR EC 125 of the company Helios and mounted on the backside of the chambers. They have a maximum air-flow rate of 610 m^3/h and are controllable with a 0–10 VDC input. The purpose of these fans is to guarantee a proper air mixture inside the chambers. Within GC 1 and GC 3, sensors for temperature, relative humidity and carbon dioxide concentration are installed. The data is collected every minute and saved on a flash drive.

2.3. Nutrient solutions

2.3.1. Half-Strength Hoagland solution

The following three step process was used to makeup a Half-Strength Hoagland solution (Hoagland and Arnon, 1950):

Step 1: Prepare six different solutions á 1 l from crystalline fertilizer components,

Step 2: Prepare a 4 l stock solution bottle by adding defined amounts of each of the six liquid components made in step 1,

Step 3: Prepare bulk solution by mixing 4 l of stock solution (as made in step 2) with 46 l deionized (electrical conductivity < 0.01 mS/cm) water and fill into nutrient tank.

Table 1 shows the nutrient concentration of a 4 l half-strength Hoagland stock solution used in the experiment. The stock solution is a concentrated solution in this case 4 l of stock solution for a 50 l nutrient solution tank. The values are also used as control for the evaluation of the C.R.O.P.® nutrient solutions explained in the following two sections.

2.3.2. Raw C.R.O.P.® bioreactor nutrient solution

The nutrient concentrations of the raw C.R.O.P.® bioreactor nutrient solution are shown in Table 2. The table shows values of a 4 l stock solution which is mixed with deionized water to make up 50 l of nutrient solution. The raw C.R.O.P.® bioreactor solution is used directly without any other treatment as a nutrient solution during the experiments described in the following sections. The solution used in the experiments is the product of the experiments described in Bornemann et al. (2018).

2.3.3. Adjusted C.R.O.P.® bioreactor nutrient solution

The idea behind adjusting the raw C.R.O.P.® bioreactor nutrient solution was to improve the nutrient composition of the solution by adding certain minerals up to the point where the concentration is similar to the half-strength Hoagland solution. Consequently, potassium, magnesium, sulfate and sometimes also calcium were added to the raw C.R.O.P.® bioreactor solution. The nutrient concentrations in the raw C.R.O.P.® bioreactor solution however vary between supply batches. An ion-chromatography measurement was always required to determine the exact concentrations of the produced batch.

An excel sheet was used to calculate the deficit of the C.R.O.P.® pure solution compared to the half-strength Hoagland solution. Since the nutrient minerals always come as a pair of a cation and an anion, determining the acceptable amount of added minerals was challenging.

For the adjusted C.R.O.P.® bioreactor nutrient solution used in the experiments the following mineral combinations were used:

- Potassium dihydrogen phosphate KH_2PO_4
- Magnesium sulfate heptahydrate $MgSO_4 \cdot 7H_2O$
- Calcium nitrate tetrahydrate $Ca(NO_3)_2 \cdot 4H_2O$

Potassium phosphate and magnesium sulfate were added to all used batches of raw C.R.O.P.® bioreactor solution. For those batches 1.5 l of raw C.R.O.P.® bioreactor solution was mixed with 2.5 l of deionized water and the required amount of additional minerals. A few batches had a lower calcium concentration (up to 50% less) than normal. This was most likely caused by precipitation in the raw bioreactor solution due to long storage times. 1 l raw C.R.O.P.® bioreactor solution and 3 l deionized water were used for the batches which needed additional calcium. Afterwards calcium nitrate was added. Due to the higher dilution these batches also required a higher amount of potassium phosphate and magnesium sulfate to be added. However, the final concentrations of all adjusted C.R.O.P.® bioreactor solutions were very similar. Table 3 shows the nutrient concentrations of the adjusted C.R.O.P.® bioreactor nutrient solution for the 4 l concentrated bottles and the final nutrient solution in the tanks of the growth chambers.

2.3.4. Nutrient solution comparison

The most significant difference between the three solutions was the high concentration of sodium and chloride in the two C.R.O.P.® bioreactor nutrient solutions, as shown in Fig. 4. The microorganisms in the filter units did not reduce the amounts of sodium chloride present in the artificial urine of a human. The half-strength Hoagland solution on the other side had basically no sodium chloride, because it is not necessary for plant cultivation. Sodium cations are competing with potassium cations in root uptake and chloride anions are competing with the uptake of nitrate. Both effects are associated with impeding plant development and reduced yield.

The concentrations of calcium and nitrate were in the same range for all three nutrient solutions. Ammonium was more present in the

Table 1
Nutrient concentration in mg/l in the half-strength Hoagland solution.

Substance	K^+	Ca^{2+}	Mg^{2+}	Cl^-	NO^{3-}	SO^{4-}	NH^{4+}	PO^{4-}	Na^+
4 l concentrated stock solution[a]	1466	1002	304	4	5425	1203	106	594	13
50 l bulk solution in growth chamber tanks[b]	117.3	80.2	24.3	0.3	436.2	96.2	8.5	47.5	1.0

[a] Values determined with ion-chromatography.
[b] Calculated from the measured values.

Table 2
Nutrient concentration in mg/l in the original and diluted C.R.O.P.® pure nutrient solution.

Substance	K^+	Ca^{2+}	Mg^{2+}	Cl^-	NO^{3-}	SO^{4-}	NH^{4+}	PO^{4-}	Na^+
4 l raw C.R.O.P.® bioreactor solution[a]	459	1048	46	1168	5853	433	733	459	669
50 l raw C.R.O.P.® bioreactor solution in growth chamber tanks[b]	36.7	83.8	3.7	93.4	468.2	34.6	58.6	36.7	53.5

[a] Values determined with ion-chromatography.
[b] Calculated from the measured values.

C.R.O.P.® bioreactor solutions. This was again a result of the micro-organism culture in the filter units and the use of urine as the base material. The magnesium and sulfate concentrations were much lower in the raw C.R.O.P.® bioreactor solution than in the two others. The adjusted C.R.O.P.® bioreactor solution had similar values compared to the half-strength Hoagland solution, due to the added magnesium sulfate.

The lack of potassium in the raw C.R.O.P.® bioreactor solution was problematic. Increasing the potassium concentration in the adjusted C.R.O.P.® bioreactor solution to the same level as the control solution was not possible without increasing the concentration of anions beyond the level of the control solution. A trade between too low concentrations of potassium and too high concentrations of phosphate was made. This led to the adjusted C.R.O.P.® bioreactor solution having only two third of the potassium of the control solution, but still twice as much as the raw C.R.O.P.® bioreactor solution. However, due to the addition of potassium phosphate the concentration of phosphate in the adjusted C.R.O.P.® bioreactor solution was around three times as high as in the half-strength Hoagland solution.

2.4. Environmental conditions

2.4.1. Illumination system

The LED lamps were set to a photoperiod of 16 h per day. The photoperiod started at 08:00 each day and lasted until 23:59. All lamps were switched on simultaneously and followed the same cycle. The distance between the bottom of the lamps and the top of the growth channels was around 315 mm and the distance between lamp and the top of the plant canopy around 200 mm. Plants grown in the two center growth channels directly below the lamp received around 1000 µmol/(m^2 s) respectively 57.6 mol/(m^2 d). The plants in the outer growth channels close to the left and right walls of the chamber received less light (500 µmol/(m^2 s), respectively 28.8 mol/(m^2 d) than the plants in the middle. This was caused by the short distance between the plant canopy and the lamp. The difference in the distribution of the light was similar among all four chambers.

The light intensity is relatively high and the lamps deliver more energy than the plants need. This was caused by a malfunction of the lamp control system, which was only discovered late in the experiments.

2.4.2. Nutrient delivery system

The parameters of the nutrient delivery system were pH and electrical conductivity (EC) of the nutrient solution, the supply interval and the supply duration. Table 4 shows the experiment set points for the nutrient delivery system. Both nutrient supply cycles were set to the same parameters, the pumps started to work at the same time. The EC value of the nutrient solution was set to 1.0 for the first weeks, when the plants were still small. For the rest of the growth cycle the EC value was increased to 2.0.

2.4.3. Atmosphere management system

The ability to control the atmosphere inside the growth chambers was rather limited. The circulation fans of GC1–4 were set to 40% of their maximum capacity of 610 m^3/h. The temperature and relative humidity of the air could not be controlled actively, because they were affected by the air stream coming from the centralized air conditioning of the laboratory. However, the sensors inside the chambers allowed monitoring of the temperature and the relative humidity as well as the carbon dioxide concentration. Table 5 shows typical average values during the experiment runs.

2.5. Statistics

The parameters fruit dry weight, plant dry weight and the resulting harvest index (fruit dry weight/total dry weight) measured in the experiments were compared using the ANOVA function (aov(y ~ treatment + experiment)) of the statistics software R. Pairwise comparison was conducted post-hoc with the pairwise *t* test. P-values were adjusted by the Benjamini–Hochberg method (pairwise.t.test (y, treatment, p.adjust.method = "BH")).

3. Results

3.1. Overview of experiment growth cycles

The following chapters describe in detail the four experiment growth cycles conducted for this study. Table 6 summarizes the growth cycles showing the date, name of the experiment, grown crop and the nutrient solutions used for each cycle. The Micro-Tina No. 1 growth cycle is not part of the evaluation, because it was only used to validate the chamber hardware and experiment procedures.

3.1.1. Micro-Tina No. 2 description

The Micro-Tina No. 2 experiment started after the test growth cycle (Micro-Tina No. 1). The plants were sown on March the 9th 2016 and the experiment was terminated on the 26th of July 2016. On March the 18th, 48 seedlings were transferred into the growth chambers. GC 1-2 were supplied with a half-strength Hoagland nutrient solution, while those in GC3-4 were supplied with the raw C.R.O.P.® bioreactor nutrient solution.

All plants grew well and developed the first flowers around April 18th. No differences between the plants fed by the different nutrient solutions could be observed during the first months of the experiment

Table 3
Nutrient concentration in mg/l in the C.R.O.P.® tuned nutrient solution.

Substance	K^+	Ca^{2+}	Mg^{2+}	Cl^-	NO^{3-}	SO^{4-}	NH^{4+}	PO^{4-}	Na^+
4 l adjusted C.R.O.P.® bioreactor[a]	879	1213	315	1080	6176	1434	650	1782	621
50 l adjusted C.R.O.P.® bioreactor solution in growth chamber tanks[b]	70.3	97.0	25.2	86.4	494.1	114.7	52.0	142.6	49.7

[a] Values determined with ion-chromatography.
[b] Calculated from the measured values.

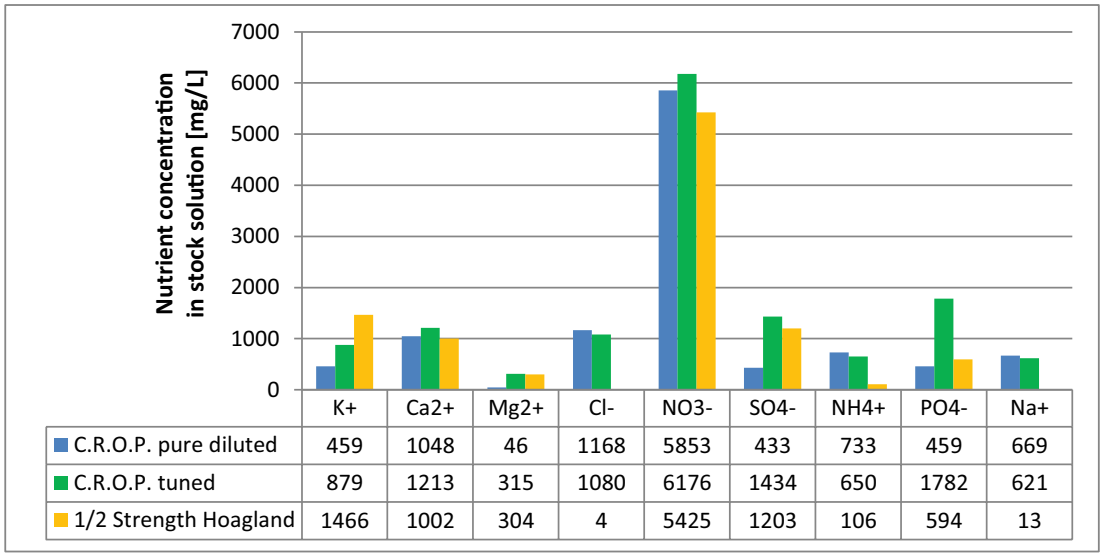

Fig. 4. Comparison of the nutrient concentrations in all three solutions. The diagram compares the concentrations of 4 l concentrated stock solutions which are used to make up 50 l of nutrient solutions.

Table 4
Summary of nutrient delivery system set points.

pH	EC [mS/cm]	Time between supply intervals	Supply duration
6.1	1.0/2.0	15 min	1 min

cycle. However, after the first harvest differences between the plants in GC1-2 and GC3-4 became more and more visible. While the plants fed with the Hoagland solution in GC1-2 remained strong and healthy, the plants fed with the raw C.R.O.P.® bioreactor solution stopped developing new leaves and flowers. The remaining leaves started to wither. After the second harvest the first plants in GC3-4 died.

3.1.2. Micro-Tina No. 3 description

The second experiment run and the third in total, Micro-Tina No. 3, started in July 2016 and went on for around five months until end of December 2016. The transfer from the germination boxes to the plant growth chambers happened ten days later than scheduled, due to technical issues with the plant growth chambers. Consequently, the seedlings were significantly larger when transferred. The plants in chambers GC 1-2 were supplied with the raw C.R.O.P.® bioreactor nutrient solution, while the plants in GC 3-4 were fed with the adjusted C.R.O.P.® bioreactor nutrient solution.

One plant in GC 1 died within days after the transfer. All other plants grew well and developed flowers and fruit. No differences in the appearance of the plants fed with both nutrient solutions have been visible during the growth cycle. All plants appeared healthy and strong until the end of the experiment run.

3.1.3. Micro-Tina No. 4 description

The Micro-Tina No. 4 experiment run began immediately after the previous run at the end of 2016. After 16 days the seedlings were transferred from the germination boxes to the plant growth chambers. All plants developed flowers in early February 2017. The first harvest was performed at the end of March 2017.

All plants grew well and developed flowers and fruit. No differences in the appearance of the plants fed with both nutrient solutions have been visible during the growth cycle. All plants appeared healthy and strong throughout the whole experiment.

3.2. Timing of first flowers and first harvest

There was no significant difference in the timing of the first flowers and first harvest between the three experiment runs. Fig. 5 shows a timeline of the three experiment runs. The first flowers appeared on day 40, 45 and 39 after sowing for the experiment runs No. 2, No. 3 and No. 4. The first ripe fruit were harvested on day 91, day 95 and day 90 respectively. The plants of the Micro-Tina No. 3 experiment took a few days longer to develop the first flowers and consequently the first harvest was delayed as well. This was most likely caused by the delayed transfer from the germination greenhouse into the plant growth chambers due to technical issues with the experiment hardware.

3.3. Harvest data

Upon each harvest the numbers of fruit per plant and the fresh weight (FW) of each fruit per plant were determined. A sample of fruit was dried at 60 °C for at least 72 h to measure the dry weight (DW) of the fruit. Additionally, the DW (without roots) of each plant (stems and leaves) was measured at the end of each experiment run. The DW of the roots could not be assessed, because most roots were contained inside the growth substrate. With the aforementioned measurement values other parameter such as the DW/FW ratio of the fruit, the fruit DW per plant and the harvest index were calculated. The harvest index is the ratio of the produced edible biomass to the whole biomass grown by the plants, in this case without the mass of the roots. The combined total production results of all experiment runs are shown in Table 7. The standard error for all average values were calculated and included. The harvest values of the failed raw C.R.O.P. bioreactor solution of

Table 5
Typical temperature, relative humidity and carbon dioxide concentration.

	Photoperiod temperature	Darkperiod temperature	Photoperiod relative humidity	Darkperiod relative humidity	Carbon dioxide concentration
Average	24.6 °C	17.9 °C	44.7%	65.3%	600 ppm[a]

[a] With peaks of up to 2500 ppm, due to humans inside the laboratory environment.

Table 6
Overview of experiment growth cycles.

Date	Experiment name	Crop	Nutrient solutions
March–July 2016	Micro-Tina No. 2	48× Micro-Tina dwarf tomato	GC1-2 with 1/2 strength Hoagland solution. GC3-4 with raw C.R.O.P. bioreactor solution.
July–Dec. 2016	Micro-Tina No. 3	48× Micro-Tina dwarf tomato	GC1-2 with raw C.R.O.P. bioreactor solution. GC3-4 with adjusted C.R.O.P. bioreactor solution.
December 2016–May 2017	Micro-Tina No. 4	48× Micro-Tina dwarf tomato	GC1-2 with raw C.R.O.P. bioreactor solution. GC3-4 with adjusted C.R.O.P. bioreactor solution.

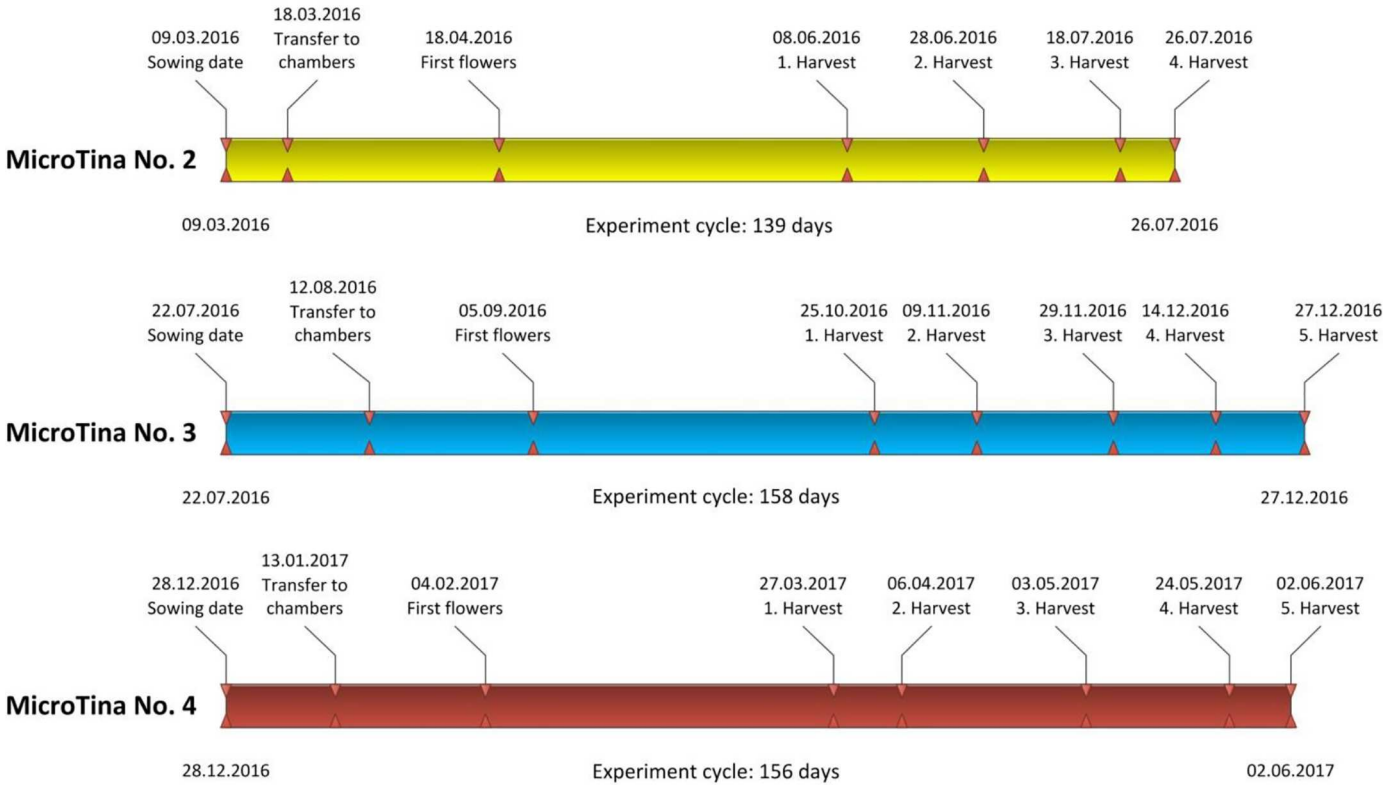

Fig. 5. Overview of the timing of all three experiment runs.

Table 7
Summary of experiment harvest data for the reference half-strength Hoagland solution and the two C.R.O.P. solutions. Average values are shown with standard error.

	1/2 Strength Hoagland solution	Raw C.R.O.P. solution	Adjusted C.R.O.P. solution	Raw C.R.O.P. solution	Adjusted C.R.O.P. solution
Experiment run	2	3	3	4	4
Growth cycle [d]	139	158	158	156	156
Total fruit FW [g/m^2]	3360.20	3065.30	3004.30	2507.32	2685.19
Average fruit per plant	60.79 ± 3.60	59.43 ± 3.57	47.96 ± 2.99	47.79 ± 2.53	49.67 ± 2.55
Average fruit FW per plant [g]	140.01 ± 8.49	133.27 ± 6.88	125.18 ± 7.58	104.46 ± 4.42	112.01 ± 5.26
Average fruit FW per plant over growth cycle [g/d]	1.01 ± 0.06	0.81 ± 0.05	0.79 ± 0.05	0.67 ± 0.03	0.72 ± 0.03
Average fruit FW [g]	2.30 ± 0.20	2.24 ± 0.18	2.61 ± 0.23	2.19 ± 0.15	2.26 ± 0.16
Average fruit DW/FW [%]	11,37 ± 0.15	11,69 ± 0.15	10.61 ± 0.12	11.37 ± 0.15	10.39 ± 0.10
Average fruit DW per plant [g]	15.91 ± 0.64	15.58 ± 0.52	13.28 ± 0.52	11.88 ± 0.29	11.64 ± 0.28
Average plant DW without fruit and roots [g]	12.38 ± 0.40	14.40 ± 0.60	20.39 ± 0.69	15.22 ± 0.59	20.25 ± 0.66
Average harvest index per plant [%]	56.24 ± 1.77	51.96 ± 1.74	39.44 ± 1.72	43.85 ± 1.55	36.50 ± 1.41

experiment run 2 were included in the analysis. Figs. 6 and 7 show the main differences between experiment 3 and 4. The results of experiment 2 are included in the figure, but not in the statistical tests.

The plants supplied with the half-strength Hoagland solution outperformed the plants cultivated with the two C.R.O.P.® bioreactor solutions. This result was to some degree anticipated, because the C.R.O.P.® bioreactor solutions had significant deficits and imbalances in their nutrient compositions. Each plant grown with the half-strength

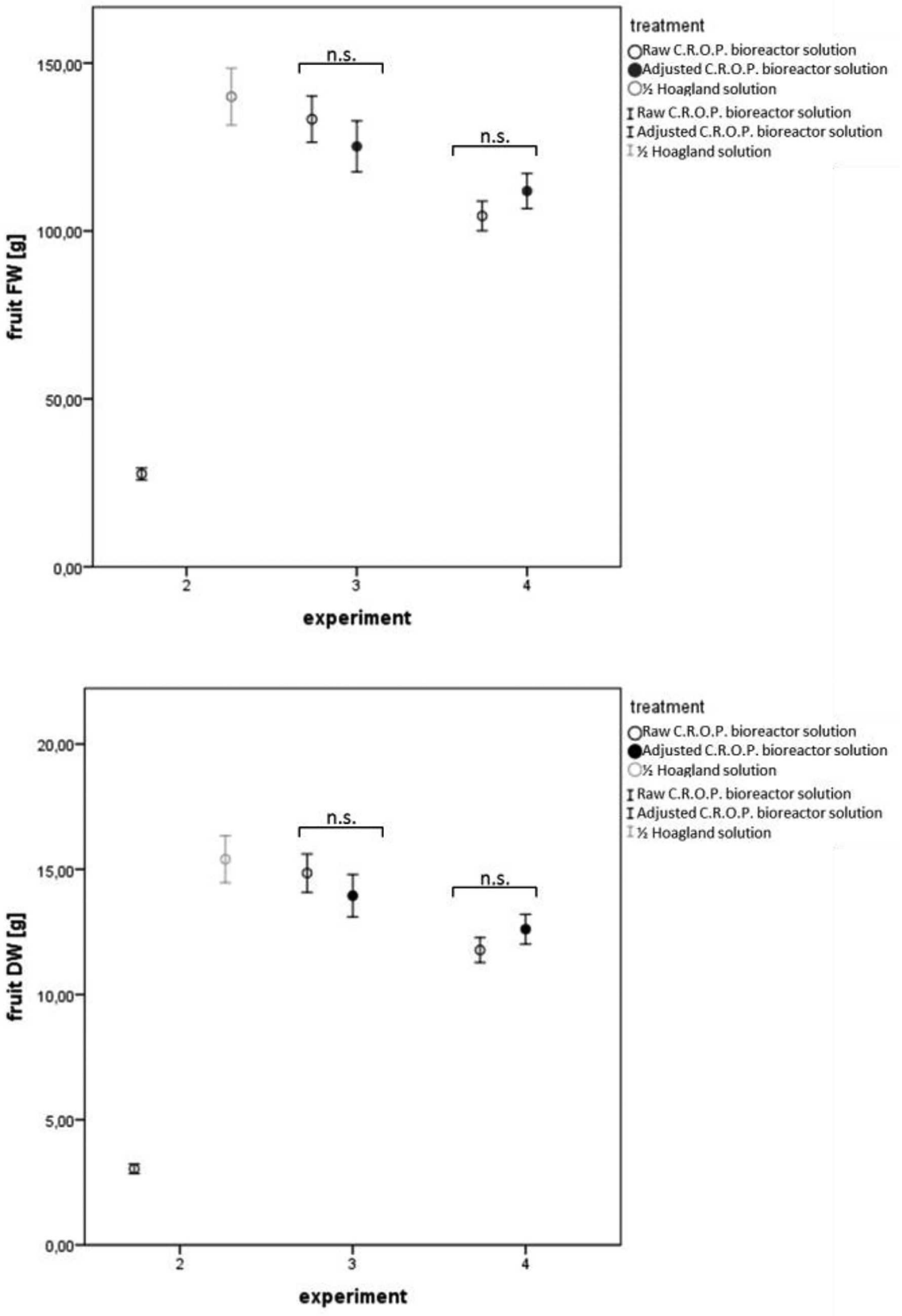

Fig. 6. Mean ± SE of fruit fresh weight (fruit FW) and fruit dry weight (fruit DW). Experiment 2 was not included in the ANOVA. Fruit DW: df = 2, $p_{\text{treatment}} > 0.05$, $p_{\text{experiment}} < 0.05$. Fruit FW: df = 2, $p_{\text{treatment}} > 0.05$, $p_{\text{experiment}} < 0.05$. The results of the pairwise t test are given in the figure. *** = $p < 0.001$, ** = $p < 0.01$, n.s. = not significant.

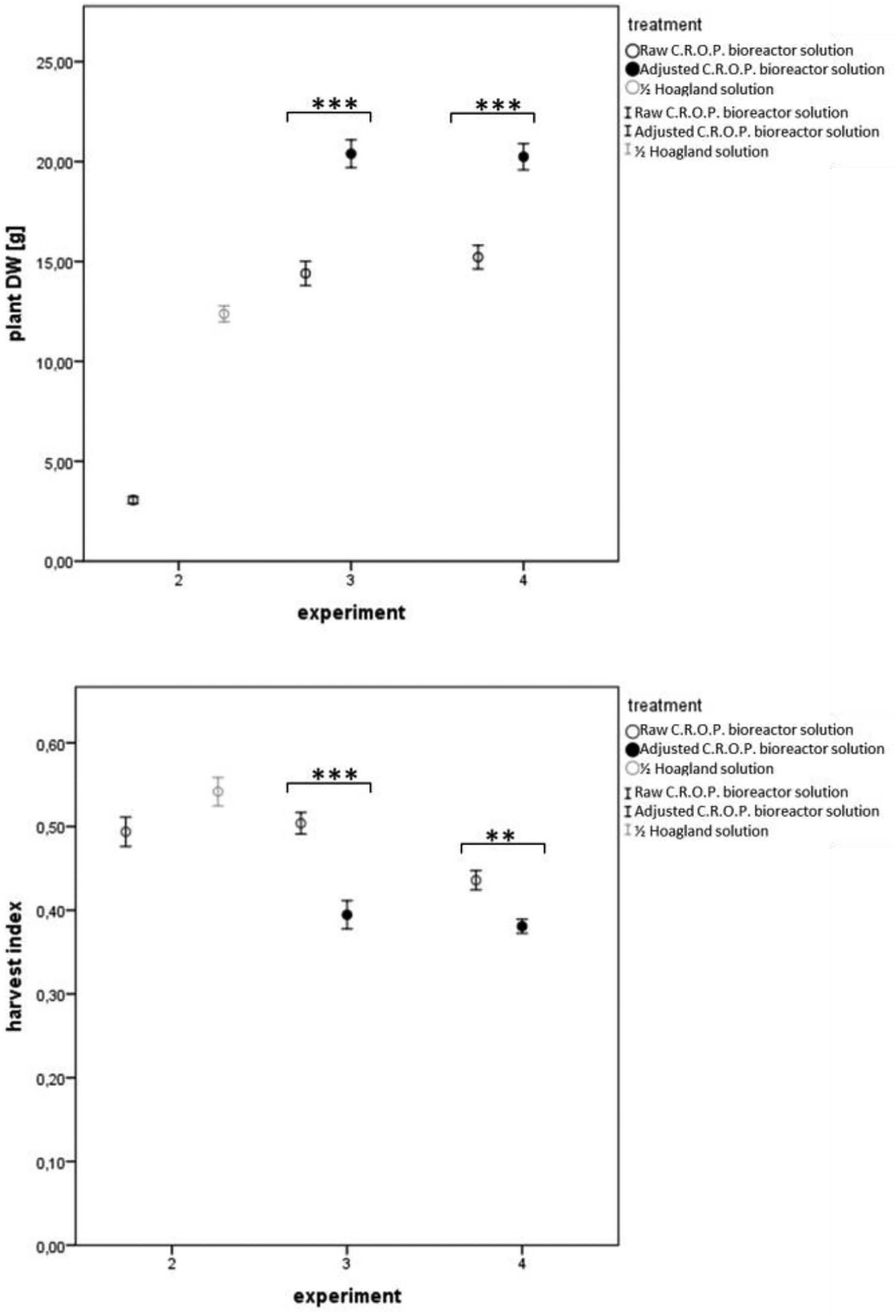

Fig. 7. Mean ± SE of plant dry weight (plant DW) and harvest index. Experiment 2 was not included in the ANOVA. Plant DW: df = 2, $p_{treatment} < 0.05$, $p_{experiment} > 0.05$. Harvest index: df = 2, $p_{treatment} < 0.05$, $p_{experiment} < 0.05$. The results of the pairwise *t* test are given in the figure. *** = $p < 0.001$, ** = $p < 0.01$, n.s. = not significant.

Hoagland solution produced more fruit than the plants grown with the other nutrient solutions. The half-strength Hoagland plants also produced more fruit FW and had a higher harvest index. When weighting the average fruit FW per plant over the growth cycle, which had a different length for all experiments, the difference between the plants grown with the Hoagland solution and the plants grown with the C.R.O.P. bioreactor solutions become even more visible.

The results of the two C.R.O.P.® bioreactor solutions show similarities in the average fruit FW per plant. There are, however differences in the average number of fruit and less significant in the average size of the fruit. The plants grown with the raw C.R.O.P.® bioreactor solution produced more fruit but with less average fruit FW than the plants cultivated with the adjusted C.R.O.P.® bioreactor nutrient solution.

The plant DW and harvest index values indicate that the adjusted C.R.O.P.® bioreactor solution plants produced more leaves and other inedible biomass than the plants cultivated with the other solutions. Consequently, these plants have the lowest harvest index. This is most likely caused by an imbalance in the nutrient solution, mainly the deficit in potassium which tomato plants require to grow fruit.

4. Discussion

The goal of the experiment was to determine whether a nutrient solution derived from recycled human urine (C.R.O.P.® bioreactor solution) can be used for plant cultivation in space greenhouses. When taking all three experiment runs into account, the following summary can be listed:

- The experiments took more than two years to be conducted.
- A total of 144 plants were grown and 143 reached maturity.
- Over 6600 tomato fruit were harvested.
- The fruit had a total fresh weight of over 15 kg.

The experiment results show that plants can be grown with the C.R.O.P.® bioreactor solution. The tomato plants reached maturity, developed flowers and ripe fruit.

The plants cultivated with the C.R.O.P.® bioreactor solution showed signs of imbalances and deficiencies of the nutrient solution. These were likely caused by the high concentrations of sodium and chloride ions, but also by high concentrations of nitrate and a lack of potassium, magnesium and calcium. These nutrient stresses affect crop growth in various ways (e.g. nutrient uptake), which put a burden on the development of the plant. As a consequence of these issues, the yield was lower for the plants fed with the urine-derived nutrient solution compared to those fed with the reference solution. The average number of fruit was higher than for the plants of the reference solution, but the average fruit size was smaller.

Attempts to adjust the C.R.O.P.® bioreactor solution by adding specific nutrients have not been completely successful. Although the leaves of the plants fed with the adjusted C.R.O.P.® bioreactor nutrient solution showed less signs of nutrient deficits and the plant mass was higher, the yield was not significantly improved compared to the raw C.R.O.P.® bioreactor solution.

The experiments conducted during this study show the general feasibility of cultivating plants with C.R.O.P. bioreactor solutions. Recycling urine using the C.R.O.P.® filter is therefore a potential element for future life support systems and terrestrial applications. The filter produces valuable nutrients from human metabolic waste products. The smaller yield of plants grown with the C.R.O.P. bioreactor solution can be acceptable for future life support system concepts, especially when loop closure outweighs optimal plant yields. When growing plants with recycled human waste products food safety needs to be considered. Urine, besides minerals and water, also contains organic components which should be removed during the recycling process. The C.R.O.P.® filters were only used to recycle artificial urine in the past years, because the laboratory was not yet outfitted to work with human urine. Consequently, the plant experiments described in this paper could only be performed with recycled artificial urine. Plant cultivation experiments with recycled human urine are planned for the next stage of the project.

The experiments have also shown certain weaknesses in the nutrient composition of the artificial urine-derived solutions. The high content of sodium and chloride ions is a general problem when working with urine. Means to reduce the amount of both ions in the resulting nutrient solutions require more research. Plant development would greatly benefit from a reduced amount of these ions. One way to reduce the amount of sodium and chloride ions in the final product of the C.R.O.P.® filters could be the coupling of the biofiltration process with the cultivation of *Salicornia europaea*. These halophytic plants can accumulate sodium and chloride and therefore reduce the amount of both elements in the nutrient solution (Tikhomirova et al., 2005, 2011). It is also possible to use a nutrient solution with high concentrations of sodium in subsequent cultivation beds containing different crops (Subbarao et al., 2000a). There have also been studies with red beet plants on how much sodium can substitute potassium in plant tissue (Subbarao et al., 1999, 2000b).

Another way is the reduction of both elements in the nutrition of the crew. This seems to be possible in future space missions, because the nutrition of astronauts is already controlled and measured today. Furthermore, the solution derived from urine could be mixed with other recycled waste streams, e.g., with the product stream of recycled plant material (also possible with the C.R.O.P.® bioreactor), which is naturally high in potassium.

Further experiments with other crop species are highly recommended to prove the acceptability of the urine-derived nutrient solution. Here plant species with a higher demand of nitrogen and lower demand of potassium should be investigated in particular, because these plants might require fewer adjustments to the C.R.O.P.® bioreactor solution

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Annex

Table 8.

Table 8
Composition of 1000 ml synthetic urine according to Feng and Wu (2006).

$CaCl_2 \cdot 2H_2O$	0.5 g
K_2HPO_4	4.12 g
$MgCl_2 \cdot H_2O$	0.47 g
KCl	0.29 g
NaCl	4.83
NH_4Cl	1.55 g
Na_2SO_4	2.37 g
Urea	13.34 g
Creatinine	1.0 g
Sodium citrate (pH 6.8)	0.65 g

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