Experiences of running a hydroponic system in a pilot scale for resource-efficient water reuse

Alexa Bliedung, Thomas Dockhorn, Jörn Germer, Claudia Mayerl and Marius Mohr

ABSTRACT

Within the research project HypoWave, a hydroponic system for plant production was investigated. The hydroponic system was fed with wastewater that had undergone specially adapted treatment. The principal aim was to develop a combined system for water treatment and hydroponic plant production, where water and nutrients were reused efficiently to produce marketable food products. Another goal was to find out whether the reuse of pre-treated wastewater for plant growth in a hydroponic system could also present an additional alternative wastewater treatment step for enhanced nutrient removal. A pilot plant, consisting of various treatment steps such as activated sludge process, ozonation and biological activated carbon filtration, was used to produce lettuce with irrigation water of different qualities. The hydroponic pilot plant was operated in two different modes – flow-through and feed & deplete. This paper focuses on the influence of the various modes of operation and accordingly varying nutrient concentrations (N, P, K) on plant growth. Furthermore, heavy metal content in the various types of treated wastewater and in the produced plants was investigated. In addition, the results of the different modes of operation were verified by mass balances for N, P and K.

Key words | heavy metals, hydroponic system, lettuce, macronutrients and micronutrients, mass balance, water reuse

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HIGHLIGHTS

- Regarding the processes of wastewater treatment as a very specific production of high-quality irrigation water, which is reused in a hydroponic system for growing lettuce.
- Investigation of the effects of various treatment processes (activated sludge process, expanded granular sludge bed reactor, ozonation, biological activated carbon filtration) on the quality and suitability of the produced waters for hydroponic lettuce production.
- Comparison of different modes of operation of the hydroponic system (flow-through and modified circulation system (feed & deplete)).
- Evaluation of nutrient and heavy metal content of the hydroponically cultivated lettuce.
- Verification of the achieved results in regard to the nutrient elimination and nutrient content of the lettuce by mass balances (N, P, K).

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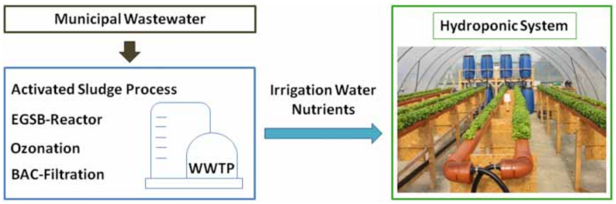
GRAPHICAL ABSTRACT



INTRODUCTION

Agriculture, which consumes 70% of the fresh water resources utilized by mankind, is the greatest water user of all (FAO 2017). Climate change, growing population and urbanization are expected to increase the pressure on water resources but also on the availability of arable land and nutrients (WHO 2006a, 2006b; Jiménez & Asano 2008). This makes it increasingly difficult to guarantee food security and to prevent the deterioration of ecosystems. Hence, the investigation of alternative water resources for agricultural irrigation and production is necessary. Countries suffering from significant water stress already use wastewater for irrigation purposes. A great advantage of this option is that not only the water itself is reused but also the nutrients contained in the treated water. However, even treated wastewater contains problematic substances, such as heavy metals, salts, micropollutants or human pathogens. Therefore, treated wastewater could cause salination of the soil and groundwater resulting in losses of plant productivity (WHO 2006b; Marcar *et al.* 2011; Jaramillo & Restrepo 2017). In addition, heavy metals could accumulate in plants if limit values of a certain pollutant are exceeded in the soil and when these plants are consumed by humans, an accumulation of heavy metals in the human body could occur (WHO 2006b). Once a critical concentration is reached in the human body, heavy metals become toxic.

Plant cultivation in hydroponic systems can be an alternative to conventional agriculture. In hydroponic systems, plants are cultivated without soil, where the plant roots either anchor in neutral substrates (e.g. rockwool) or simply extend into nutrient solutions without any substrate at all (Maucieri *et al.* 2019). Such cultivation systems allow very accurate nutrient management and avoid pollution of bodies of water (Jones 2005). The Hoagland solution, for example, is a nutrient solution containing all essential nutrients in balanced concentrations. Depending on the plant to be cultivated or the climatic conditions, the nutrient solution can be optimized. Furthermore, hydroponic systems are more efficient in regard to water and nutrient utilization than conventional agriculture (Sambo *et al.* 2019). In many countries, hydroponic systems already play an important role in agriculture and their contribution to vegetable and fruit production is expected to grow continuously. The most common types of substrateless hydroponic systems are the Nutrient Film Technique, the Deep Water Culture (DWC) and the Aeroponic System (Maucieri *et al.* 2019).

Considering growing water scarcity and the resulting pressure on finding alternative resources for irrigation water, the combination of hydroponic systems and water reuse constitutes a possible option for the solution of this challenge although this is not a new concept (Tzanakakis *et al.* 2014; Magwaza *et al.* 2020). Previous studies either focused on the decentralized treatment of domestic wastewater (Ayaz & Saygin 1996; Norsträm *et al.* 2003; Vaillant *et al.* 2004; Keller *et al.* 2008; da Silva Carvalho *et al.* 2018) or investigated the treatment of brackish water or industrial wastewater (e.g. brewery wastewater) (Soares *et al.* 2015; Gebeyehu *et al.* 2018; Soares da Silva *et al.* 2018). In addition, all these investigations were carried out in small-scale systems with the focus on treating wastewater with the production of plants as an inevitable byproduct. Therefore, the approach of regarding the processes of wastewater treatment as a very specific production of high-quality irrigation water, which is used in hydroponic systems for the production of products of high quality, is new.

This special focus of the research project, discussed in this paper, is already mirrored in its title 'HypoWave', a combination of the words 'hydroponic' and the official name of the research funding measure 'WavE' (WavE – Zukunftsfähige Technologien und Konzepte zur Erhöhung der Wasserverfügbarkeit durch Wasserwiederverwendung und Entsalzung/Future-oriented Technologies and Concepts to Increase Water Availability by Water Reuse and Desalination) of the Federal Ministry of Education and Research, Germany. The project investigated purpose-oriented treatment and utilization of municipal wastewater in hydroponic systems for efficient water and nutrient reuse. The central element of the project was a pilot plant located on a municipal wastewater treatment plant (WWTP). Technologies such as the activated sludge process (aerobic) or the Expanded Granular Sludge Bed Reactor (EGSB) (anaerobic) were used as basic treatment processes. biological activated carbon filtration (BACF) and ozonation were used as advanced treatment processes. Due to the different combinations of treatment processes, irrigation waters of different qualities for plant production could be produced. Further topics, such as possible operator models in regard to the associated legal framework, feasibility studies and an impact assessment formed a framework for the results of the piloting (Fischer *et al.* 2018; Mohr *et al.* 2018; Ebert *et al.* 2019a, 2019b; Winker *et al.* 2020; Zimmermann & Fischer 2020).

This paper will give an overview from the results of the pilot plant of the research project HypoWave and will focus on the data of the growing periods 2017 and 2018. First, a detailed description of the pilot plant is presented and second, the nutrient ratios of the differently treated wastewaters are described. In addition, the use of wastewater treated especially for the reuse in a hydroponic system for the cultivation of lettuce is discussed, taking into account the impact of heavy metal concentrations. Finally, the used hydroponic system is verified by mass balances together with a recommendation on which type of operation (flow-through, feed & deplete system) is suitable for which type of application, combining adapted wastewater treatment and hydroponic systems. Here, the first mode of operation was a flow-through system. It is a classic open hydroponic system without recycling of the nutrient solution (= treated wastewater) (Maucieri *et al.* 2019). In order to operate the hydroponic system as a recirculation system with treated wastewater (second mode of operation), a circulation system (feed & deplete system) especially optimized for the HypoWave project was used.

In addition to the investigations of the nutrients and heavy metals in the irrigation water and the plants, further investigations regarding pathogens, antibiotic resistances and organic micropollutants were carried out (Blau *et al.* in preparation; Kreuzig *et al.* in preparation; Mohr *et al.* 2020). Knowledge of these parameters is important to evaluate the quality of the products (irrigation water, plants) in regard to guidelines and regulations (DIN 19650:1999-02; WHO 2006b; EC 2018; Schramm *et al.* 2019). The integration of the results into an effective quality management system will be part of a follow-up project.

METHODS

In order to analyse the suitability of various types of treated wastewater for growing lettuce in a hydroponic system installed in a greenhouse, a modularly structured pilot plant was set up at the municipal WWTP Wolfsburg-Hattorf, Germany. The pilot plant consisted of three main modules: basic treatment, quality and hygiene and the hydroponic system (greenhouse). The modules and technologies used have been presented below in more detail as well as the various treatment combinations and modes of operation of the hydroponic system.

Basic treatment

The aim of this module was to reduce the amount of organic compounds of the mechanically pre-treated wastewater by using aerobic and anaerobic processes. Two treatment options were used for the reduction of organic compounds in this research project. Option one being the standard wastewater treatment of the WWTP Hattorf and option two, the combination of an EGSB and a sequencing batch reactor (SBR) within the pilot plant.

The WWTP Hattorf (6,200 PE; 396,000 $m^3\ a^{-1}$ (NMU 2017)) is located in a rural area and the wastewater is collected by a separate sewer system. As there are no industries in the area, only domestic wastewater is collected. Therefore, the incoming wastewater is especially suitable for reuse in hydroponic plant production systems. The treatment chain of the WWTP Hattorf consists of a mechanical treatment step, a compact treatment system for the joint removal of sand and screenings, an aerobic biological stage (activated sludge process) for the reduction of organic compounds and phosphorus and nitrogen removal consisting of biological phosphorus elimination and intermittent denitrification. Furthermore, advanced phosphorus removal is achieved by precipitation in the effluent of the aeration tank. Before the treated wastewater is discharged into the receiving water body, it passes through purification ponds.

For the pilot plant set-up, a pilot-scale EGSB was used. An EGSB is a type of anaerobic treatment, an upgraded upflow anaerobic sludge blanked reactor (UASB) (Meyer 2004). The reactor (ACS-Umwelttechnik GMBH & Co. KG, Rielasingen-Worbingen, Germany) had a volume of 200 L and was fed with the influent of the WWTP Hattorf (after mechanical pre-treatment) at a flow rate of 60 L h^{-1}. The advantage of this treatment combination is the reduction of organic compounds in terms of chemical oxygen demand (COD) without targeting the elimination of phosphorus and nitrogen.

In order to obtain a nutrient-rich effluent with low COD concentrations, an SBR was installed downstream of the EGSB. The exchange volume of the SBR was 240 L per cycle (40–60 L h^{-1}; equal to 1/3 of the volume). By combining the process steps of aeration, settling and decanting, the SBR makes it possible to convert ammonium into nitrate during the nitrification process. The conversion is necessary, since nitrate is more readily available for plants than ammonium (Tochobanoglous *et al.* 2014). At the same time, elimination of phosphorus and denitrification are avoided, thus preserving these nutrients in the irrigation water.

Quality and hygiene

The effluent of the conventional biological treatment process (secondary sedimentation tank (SST)) may still contain organic micropollutants or pathogens. These pollutants in treated wastewater could have a negative effect on the quality of the cultivated product. Therefore, further advanced treatment processes are required. In the Hypo-Wave pilot plant, ozonation and BACF were used as advanced treatment processes. Ozone is one of the principal disinfectants for treating wastewater and a strong oxidant (WHO 2006b; Tochobanoglous *et al.* 2014). Due to the direct oxidation of cell walls, pathogens are killed (Tochobanoglous *et al.* 2014). Additionally, organic micropollutants are eliminated by the oxidative process (Asano *et al.* 2007). BACF is not a common treatment process. One advantage of this technique is the synergetic effect between the adsorption properties of activated carbon, which serves as carrier material and an adapted biofilm (Simpson 2008; Rattier *et al.* 2012). At first, dissolved organic carbon is removed mainly by the physical adsorption process of the activated carbon filtration. Due to the increased retention time, an adapted biofilm grows on the activated carbon material. Formation of a mature biofilm and loading of the activated carbon material takes about three months. This process makes the degradation of hardly biodegradable carbon compounds possible (Simpson 2008; Aktaş & Çeçen 2012; Rattier *et al.* 2012). The operating lifetime of biological activated carbon filters can amount to 10 or even more years (Rattier *et al.* 2012).

Both the ozone reactor and the biological activated carbon filter were used in a pilot scale. The ozone reactor (Xylem Services GmbH, Herford, Germany) was operated at a flow rate of 1.5 m^3 h^{-1} and a dosage of 8 g of ozone m^{-3}. The biological activated carbon filter was filled with 100 L of granulated activated carbon (Epibon A 4×8, 2.36–4.74 mm; Donau Carbon GmbH, Frankfurt, Germany) and was operated at a flow rate of 30 L h^{-1} corresponding to an empty bed contact time of 3.3 h. In order to reduce the maturing time of the biofilm and ensure that biological degradation prevailed during the experiments, the activated carbon was pre-treated with the effluent of the SST at the WWTP Wolfsburg-Hattorf before the experiments were started.

Construction and operation of the wastewater treatment lines

According to the modules 'basic treatment' and 'hygiene and quality', four main treatment lines were developed and used during the experiments. The different treatment processes were combined in the following way:

- Treatment line A: effluent SST WWTP Wolfsburg-Hattorf
- Treatment line B: effluent SST WWTP Wolfsburg-Hattorf – ozone reactor
- Treatment line C: influent aeration tank (I AT) – EGSB – SBR (nitrification)
- Treatment line D: influent aeration tank (I AT) – EGSB – SBR (nitrification) – BACF

The effluent from the hydroponic system was returned to the WWTP (Figure 1).

Hydroponic system

Lettuce (*Lactuca sativa* L. var Hawking RZ; Salanova, Rijk Zwaan Welver GmbH, Welver, Germany) was cultivated hydroponically. The plants thrived in about 7 to 8 cm deep water that was kept in an intermittent circular flow. Due to the high water level maintained in the pipes, the set-up was resilient against water or power cuts. On the other side, the larger volume provided by a barrel in each line increased the necessary exchange intervals, thus facilitating the manual exchange procedure during the feed & deplete mode. The hydroponic set-up consisted of eight treatment lines. Each line was made up of a PVC tube with a diameter of 10 cm and a length of 8 m. The water level in the pipes depended on the length of the roots. Therefore, the air gap was between 20 and 30 mm during the experiments. Pipes were installed without slope and connected to storage tanks. Feeding took place by gravity (year 2017) and pumps (year 2018). Outer lines on each side (two on the south side, one on the north side) served as border lines, which were used to ensure that conditions (e.g. shading, neighbourhood) were identical for all lines/plants. Four lines were operated with the various treated wastewaters and one line was operated as a reference line. In the reference line, a nutrient solution was used with a nutrient concentration corresponding to a 50% Hoagland nutrient solution (Epstein & Bloom 2005). Preliminary tests had shown that 100% Hoagland nutrient solution was too highly concentrated and could damage the plants. Therefore, a diluted 50% solution was used to simulate conventional hydroponic lettuce cultivation as reference.

Modes of operation of the hydroponic system

The experiments were carried out in 2017 and 2018 from April to November. Every growing period lasted about 35 days. Within the project, two modes of operation were investigated. In 2017, the hydroponic system was operated as a flow-through system without any further addition of nutrients. The hydroponic system was operated at an average flow rate of 23.5 L h^{-1} and with 68 lettuce plants per line. During this test period, research investigations covered the analysis of the effluent from the treatment lines A to D.

In a second test period in 2018, a feed & deplete system was used. The treated wastewater ($V_{start} = 185$ L) circulated

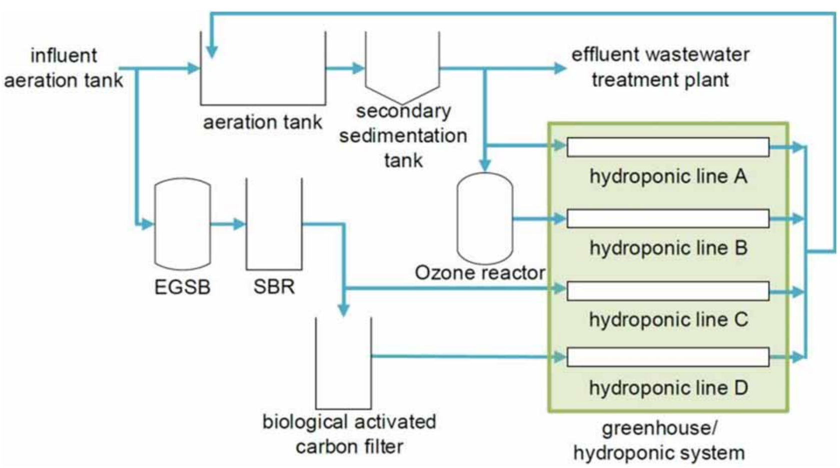


Figure 1 | Scheme of the pilot plant (Bliedung *et al.* 2019b).

in the system until the nutrient concentration of nitrate dropped below a defined threshold. Then the water was exchanged with freshly treated water. This process was repeated until the plants in line B to D reached a target weight of 275 g fresh mass (line A was not in operation in 2018). This target weight was defined, according to standard weight for lettuce sales in supermarkets. In both test periods, 20-day old seedlings were planted in the tubes after nursing and stayed in the system for approximately 35 days depending on the weight and size of the lettuce. In 2018, only 36 plants were inserted in each line, as a result of experiences made in 2017. Based on the nutrient composition of the treated wastewaters, eventually required nutrients were added.

Analyses of treated wastewater types and lettuce samples

The treated wastewater was regularly analysed in regard to its organic content (COD), macronutrients, micronutrients, as well as heavy metals. Forty-two random samples were taken at the effluent of the treatments and the hydroponic system in 2017 and 16 random samples in 2018. A limited number of samples was defined because concentrations are equalized by the different treatment steps of the pilot plant and sampling conditions in the greenhouse were difficult. In addition, in 2018, sampling only took place on those days when the water in the hydroponic lines was exchanged. In the case of the harvested lettuce, fresh and dry matter, the concentration of macronutrients and micronutrients, as well as heavy metals, was examined. Table 1 shows the methods used for analysis.

After the roots were cut off, the fresh weight of the lettuce was measured on the day of the harvest. After weighing, the lettuce was slowly dried at a temperature of 70 °C, to make sure that the material was not destroyed by the heat. The drying process took 3 days. The subsequent analyses were carried out either by the Abwasserverband Braunschweig (Braunschweig, Germany), the Wolfsburger Entwässerungsbetriebe (Wolfsburg, Germany), the TU Braunschweig (Institute of Sanitary and Environmental Engineering TU Braunschweig, Braunschweig, Germany) or the University of Hohenheim (Stuttgart, Germany).

Mass balances

Mass balances were used to check the validity of the system and the plausibility of achieved results. Mass balances were drawn up for the greenhouse covering the load of each parameter in the influent of the hydroponic lines, the effluent of the hydroponic lines and in the harvested lettuce. Loads were calculated on the basis of medians (concentration, mass).

$$F_{\text{Input}}(\text{g}) = F_{\text{Output}}(\text{g})$$

$$F_{\text{IN,water}}(\text{g}) = F_{\text{OUT,water}}(\text{g}) + F_{\sum \text{lettuce}}(\text{g})$$

$$\text{Delta}(\%) = \frac{(F_{\text{Input}}(\text{g}) - F_{\text{Output}}(\text{g}))}{F_{\text{Input}}(\text{g})} \cdot 100$$

Table 1 | Overview of the methods of analysis

Sample	Parameter	Analysis
Water	P, Ca, Mg, K, S, Fe, Mn, Pb, Cd, Cr, Cu, Ni, Zn, Hg	ICP-OES (DIN EN ISO 11885 E22:2009-09)
Water	COD, N_{tot}, NO_3-N, NO_2-N	Cuvette test (Hach-Lange, Düsseldorf, Germany)
Water	NH_4-N	ISE-Sensor NH 500/2; pH/Ion 7310 (Xylem Analytics Germany Sales GmbH & Co. KG., Weilheim, Germany)
Lettuce (shoot)	As, Cd, Co, Cr, Cu, Ni, Pb	ICP-MS (VDLUFA 2011 (vol. VII, cap. 2.2.3.1 (2014)))
Lettuce (shoot)	K, P	ICP-OES (VDLUFA 1995 (vol. II.1, cap. 8.10 (2007)))
Lettuce (shoot)	N	EA (VDLUFA 1995 (vol. II.1, cap. 3.5.2.7 (2000)))
Lettuce (shoot)	Hg	KD-AAS (VDLUFA 2011(vol. VII, cap. 2.2.2.9))

Notation: N_{tot} was analysed in the influent of the aeration tank. For the determination in the irrigation water, the sum of NO_3-N, NO_2-N und NH_4-N was calculated as N_{tot} at the other sampling points.

Nitrogen losses could occur in a hydroponic system on account of microbial nitrification, denitrification or ammonia volatilization (Zerche & Kuchenbuch 1995). During the test periods, it was not possible to measure the loss of nitrogen as gas. Therefore, phosphorus and potassium were balanced as additional control parameters. The growing periods of the lettuce in the hydroponic lines were used as the balancing period and defined system boundaries. The nursing time of 20 days was not considered. Mass balancing in this study was limited to hydroponic treatment line D. Two different runs in two years were chosen for mass balancing, RUN I (14 August to 21 September 2017) and RUN II (19 June to 24 July 2018). For balancing, 24 random water samples of the influent and the effluent of the hydroponic treatment line D were taken during a period of 38 days and 21 lettuce plants per line were considered for RUN I in 2017. For RUN II, five random water samples of the influent and effluent during a period of 35 days were taken and 15 lettuce plants were harvested. Nutrients added before irrigation were also taken into account for mass balancing.

Former research has shown that mass balances of wastewater treatment systems are considered to be closed, if the balance gap is less than or equal to 10% (Mieske 2018). Due to additional sources of error, in this research project, mass balances for hydroponic systems were considered to be closed if the balance gap was less than or equal to 20%. Reasons for this assumption were that the period of nursing (20 days) and the nutrient composition of the roots after harvesting were not considered in the mass balancing. Further, the structure of lettuce is not homogeneous and random samples were taken instead of composite samples on account of the equalization of the treated waters during the various treatment processes.

RESULTS AND DISCUSSION

Treated wastewater

In order to be able to use a larger database, the mean average concentrations of measured values of 2017 and 2018 are presented in the following. To quantify the elimination of organic compounds, the parameter COD was used. In the existing WWTP (processes A and B), COD could be decreased by 96%. This is contrasted by treatment process C, where due to the operation of the SBR, the elimination of the COD only amounted to $\eta = 89\%$. The best performance for COD elimination could be observed for the treatment process D with $\eta = 97\%$. Moreover, approximately 54% of nitrogen could be retained in the treated wastewater by the treatment processes C and D, while the effluent of treatment lines A and B showed a nitrogen content of only 12% compared to the influent of the WWTP. A similar tendency could be observed for phosphorus. Figure 2 shows the concentration of macronutrients in the treated wastewater.

Interestingly, no significant difference in the concentration of potassium, calcium, magnesium and sulphur could be observed in all lines. These findings support the idea that nitrogen and phosphorus are retained in the treated wastewater by treatment processes C and D. This is caused by the higher concentrations of nitrogen and phosphorus in the influent of the aeration tank.

For illustrating data shown in Figure 2 and for emphasizing their significance for the cultivation of lettuce, ratios of nitrogen, phosphorus and potassium were formed (covering both years) and compared to the reference solution. The reference solution had a N:P:K ratio of 10:2.8:12.3. The treated wastewater from line A had a ratio of 10:21:151 and was very similar to the ratio of water from line B (10:20:137). The lack of nitrogen and the considerable surplus of phosphorus and potassium in comparison to the reference solution thus became more obvious and addition of nitrogen and phosphorus is recommended for waters A and B. A close look at the ratios of water from lines C (10:2.3:6) and D (10:2.1:6) shows that there is a much better balance between nitrogen and phosphorus. The concentration of nitrogen in the waters of lines C and D corresponds to 37% of the nitrogen concentration of the reference solution, phosphorus to 29% and potassium to 18%. Potassium, calcium and magnesium were not reduced by any of the wastewater treatment combinations. These findings support the idea that if no specific salt removal process is used, no accidental salt reduction occurs (Marcar *et al.* 2011). However, potassium concentration (24 mg L^{-1}) was low in the treated wastewater, since influent concentrations were already low. In contrast to this, calcium (71 mg L^{-1}) and magnesium (11.5 mg L^{-1}) met the

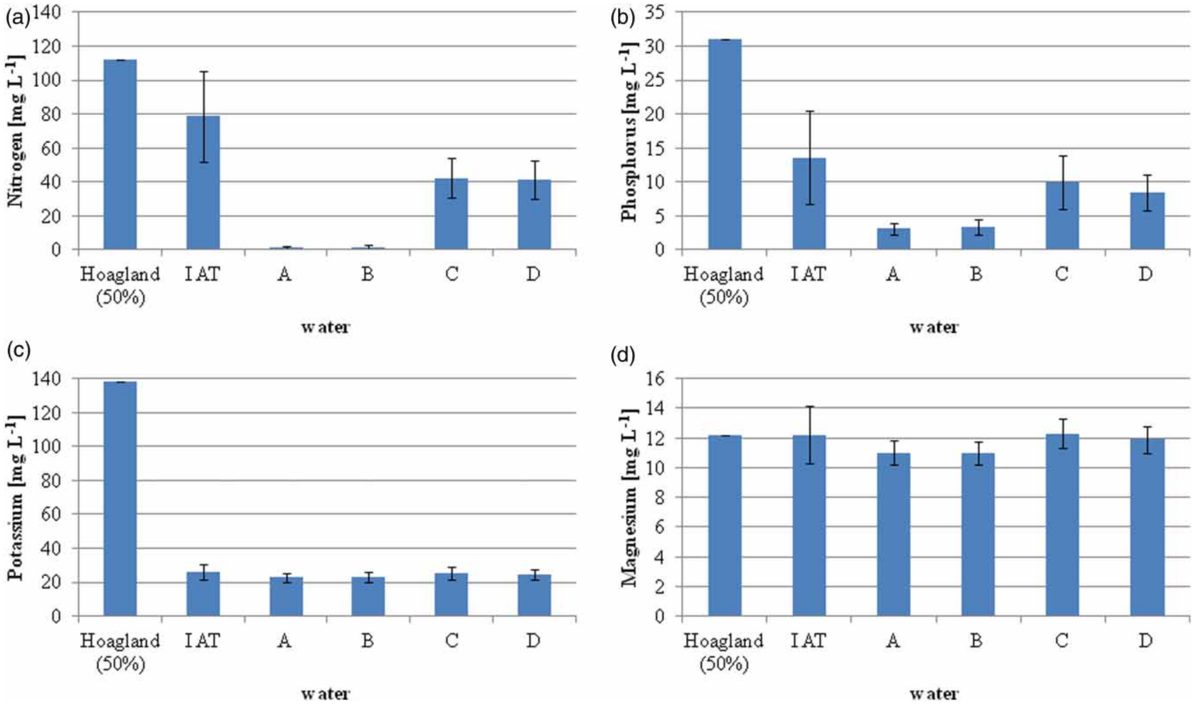


Figure 2 | Average concentrations of N, P, K and Mg in the inflow of the aeration tank (IAT) and the effluent of the differently treated wastewaters (a, b, c and d) in comparison to the reference nutrient solution (2017–2018).

requirements ($C_{Ca} = 80\ mg\ L^{-1}$, $C_{Mg} = 12\ mg\ L^{-1}$). Like, nitrogen and phosphorus, other nutrients, such as iron ($<0.5\ mg\ L^{-1}$) and manganese ($<0.05\ mg\ L^{-1}$) were not available in sufficient quantities in the influent of the aeration tank and accordingly in the treated wastewater. Copper, manganese and zinc were reduced by the different wastewater treatment processes. It could be observed that the treatment process D eliminated metals that are important for plant growth. These findings are in agreement with literature where the reduction of heavy metals by adsorption to activated carbon is described (Asano *et al.* 2007).

These results help to understand the composition of nutrients of the treated wastewater and its impact on plant growth. In all treated wastewater, the nutrient composition was not well balanced. A balanced nutrient composition is important, because plant growth is limited by the scarcest resource (Finck 1989). It seems as if nitrogen had the main impact in water from treatment lines A and B, whereas in water from treatment lines C and D, one of the micronutrients seemed to be the critical factor. Hence, to operate the feed & deplete system, nutrients were added to the treated wastewater depending on the results of the water analyses. Nutrient addition included potassium, zinc, sulphur, iron, manganese, copper and molybdenum.

Due to the low concentrations of nitrogen and phosphorus, water from lines A and B was not suitable for a feed & deplete mode of operation. Therefore, water from lines C and D was used for the feed & deplete system. In addition, the effluent of treatment line B was used as well after adding nitrogen and phosphorus to the storage tank in the greenhouse. This altered the average nutrient ratio of water from line B to 10:2.1:37 (N:P:K). Due to the addition of nitrogen in the form of potassium nitrate, the amount of potassium was noticeably higher.

Heavy metals were analysed in the treated wastewater as well. Some of these metals, like iron and zinc, are essential for plant growth. As already mentioned above, these were present in insufficient concentrations. Water from treatment line C contained only 25% of the iron concentration of the reference solution, water from lines A and B 13% and water from treatment line D contained only 3%. A similar tendency could be observed for zinc. The zinc concentration in the water of treatment line C corresponded to

85% of the concentration of the reference solution, for waters from treatment lines A and B to approximately 37% and for water from treatment line D to only 17%. Since iron and zinc concentrations are below values of the reference solution, toxicity is not a problem. Heavy metals, such as mercury, cadmium and chromium, are not needed for plant metabolism. Depending on availability, heavy metals accumulate in plants (FAO 1985; Asano *et al.* 2007). Table 2 presents an overview of the average concentrations of heavy metals in the treated wastewater during the 2 years of investigation. The concentration of mercury in the influent of the aeration tank was below the limit of quantification (0.2 μg L^{-1}), thus mercury could not be detected in any of the treated wastewater samples. The same accounts for cadmium, where the measured values were also below the limit of quantification (0.4 μg L^{-1}).

Since no specific treatment processes for heavy metal reduction were used, water in treatment lines A and B showed nearly the same heavy metal concentrations. Although the treatment combination C eliminates heavy metals, concentrations were still higher than concentrations in lines A and B. It is possible that suspended solids, which could be contained in the effluent of treatment line C, had an impact on this (Hass *et al.* 2011). Due to the activated carbon treatment, water from line D had the lowest heavy metal concentrations. Table 2 shows that the average concentrations of heavy metals varied in 2018. The reason could be that the heavy metal concentrations in the influent of the aeration tank were higher due to less dilution.

Comparing the concentrations of heavy metals in all treated wastewaters to the German regulations of the Drinking Water Ordinance (TrinkwV), it can be seen that overall contaminations are very low and far below all limit values. Nevertheless, it is important to bear in mind that the main influencing factor of the composition of nutrients and heavy metals in the treated wastewater is the composition of the influent of the WWTP. It should be mentioned that results presented above and in the table below support and confirm previous findings of our research work focusing on the results of 2017 (Bliedung *et al.* 2019a, 2019b).

Table 2 | Average concentration and deviation of heavy metals given in μg L^{-1}

		Water line A	Water line B	Water line C	Water line D	TrinkwV (2001)
2017	Cd	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	3
2018		0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	
2017	Co	1.0 ± 1.0	1.0 ± 1.0	1.0 ± 1.0	1.0 ± 1.0	–
2018		2.0 ± 0.8	1.3 ± 0.9	1.0 ± 1.0	1.0 ± 1.0	
2017	Cr	1.0 ± 1.0	1.0 ± 1.0	1.2 ± 1.5	1.0 ± 1.0	50
2018		1.0 ± 1.0	1.4 ± 0.9	1.9 ± 3.4	1.0 ± 1.0	
2017	Cu	8.1 ± 4.5	8.1 ± 6.0	26 ± 24	3.7 ± 9.3	2000
2018		11 ± 4.2	12 ± 5.0	56 ± 67	9.3 ± 8.8	
2017	Hg	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	1
2018		–	–	–	–	
2017	Ni	1.0 ± 1.0	1.0 ± 1.0	1.2 ± 1.5	1.0 ± 1.0	20
2018		1.0 ± 1.0	1.3 ± 0.9	3.0 ± 3.1	1.0 ± 1.0	
2017	Pb	5.1 ± 2.7	5.1 ± 3.0	5.1 ± 3.6	4.6 ± 2.4	10
2018		8.0 ± 1.6	6.1 ± 2.7	7.2 ± 3.2	6.5 ± 2.8	

Fresh mass

For both RUN I and RUN II, the fresh mass of the shoots was measured after harvesting along the hydroponic line. Figure 3 shows the fresh mass (shoot) of RUN I (a) and RUN II (b) depending on the position in the hydroponic line. It can be seen in Figure 3 that the lettuce cultivated with the reference solution grew evenly over the entire hydroponic pipe length in both runs. Due to the constantly sufficient nutrient supply, lettuce growth met expectations. The lettuce from the reference line in RUN I had an average weight of 161 g and in RUN II of 553 g, although the duration of the growing period was nearly the same. There are several possible explanations for this effect. Because space per lettuce per line proved to be insufficient in 2017 (68 lettuce plants per line) and limited plant growth, the number of lettuce plants per line was reduced (36 lettuce plants per line) for further investigations. In addition, plant growth is subject to exponential growth; therefore, a small difference in growing period duration could have a large impact on the yield.

The results of the lettuce growth of RUN I are shown in Figure 3(a). The average weight of lettuce grown in line A was 81 g and in line B 103 g. Furthermore, the gradient of the weight along the lines was remarkable. These findings were unexpected, since the plants grew despite the fact that the concentration of nitrogen in wastewaters from line A and B corresponded to only 1.4% of the concentration

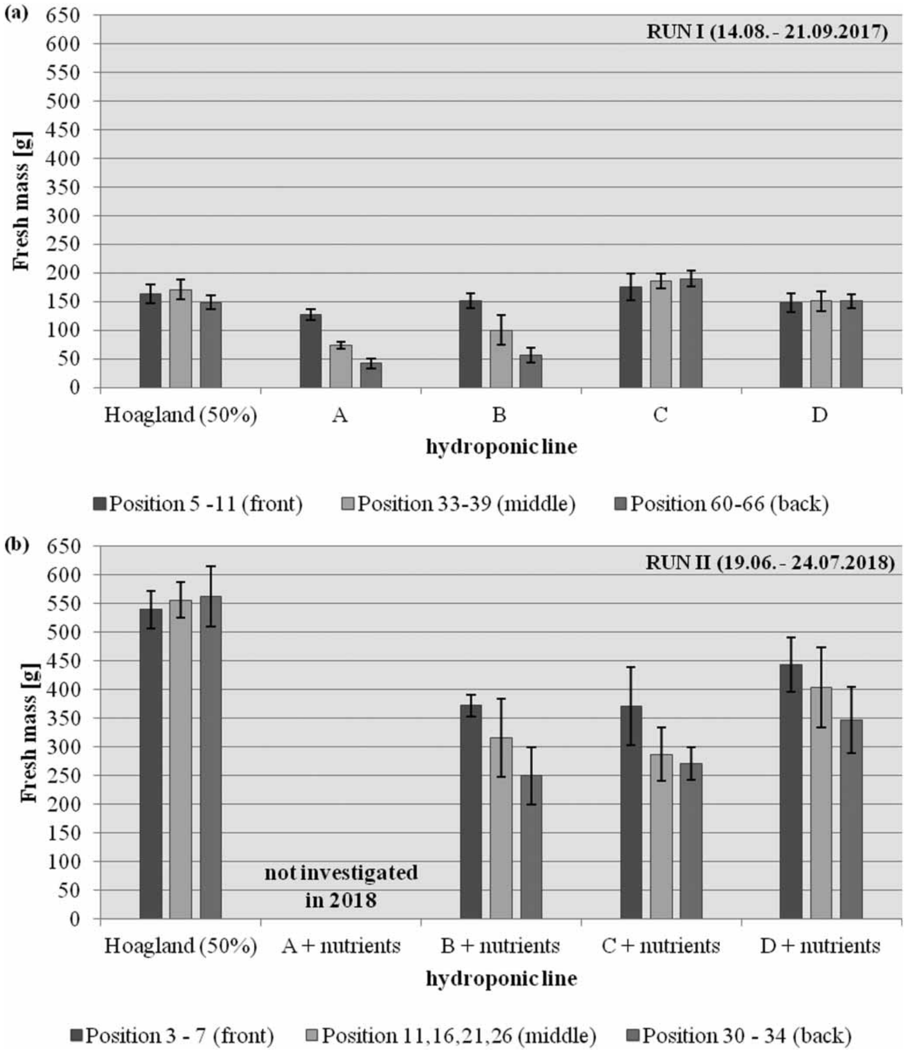


Figure 3 | Fresh mass of lettuce (shoot) for RUN I 2017 (a) and RUN II 2018 (b).

of the reference solution. A possible explanation for this might be the flow-through operation and the related load consistency (45 mg N h^{-1}) during the RUN I. The gradient seems to occur because of an unbalanced nutrient distribution along the lines. The difference in the average weight between A and B could be caused by the additional ozone treatment in line B. The ozone treatment resulted in an increased concentration of dissolved oxygen. Under certain environmental conditions, supersaturated dissolved oxygen has been observed to improve lettuce production (Suyantohadi *et al.* 2010).

In contrast to these results, the weight of the lettuce grown in lines C and D was similar along the entire line. It is noticeable that the lettuce plants in line D (150 g) were about 20% lighter than those in line C (183 g). This difference was attributed to the nutrient composition of the water in lines C and D. Treatment line C and D differ only in the biological activated carbon filter. As already discussed above, the biological activated carbon filter reduced some metals required for plant growth. Lower yield seems to be a consequence of these micronutrient deficiencies. The weight of the lettuce plants from lines D and C corresponded to the lettuce weight in the reference line. Thus, it can be deduced from these investigations that water used in treatment lines D and C is suitable as irrigation water in a hydroponic system operated as a flow-through system.

The results of RUN II are presented in Figure 3(b). The average weight of the lettuce plants grown in line B, C and D was 313, 312 and 398 g. Because of the theoretical calculation of the day of harvest, the lettuce plants were heavier than the defined target weight (275 g). The lettuce plants grown in treated wastewater were 38% lighter on average than those grown in the reference line (553 g). All lettuce plants were harvested at the same time, but the lettuce cultivated with treated wastewater with nutrient addition needed more time to reach the target weight. The most surprising aspect of the data in Figure 3(b) is the gradient of the lettuce weight along the line in all lines. According to the reference line, which was operated as a feed & deplete system, the weight was expected to be similar over the entire length of the lines. A possible explanation might be that the approach flow in the pipes might have varied at the end of the pipe, which could have had an impact on plant growth.

Overall, these results indicate that if the system is optimized (e.g. higher flow rate), it is possible to grow plants with the low concentrations of nutrients of the effluent of a WWTP in a hydroponic system operated as a flow-through system. For optimization, and to run a feed & deplete system, higher nutrient concentrations and a balanced nutrient composition are necessary. Nevertheless, it could be shown that an average concentration of 42 mg nitrogen L^{-1} is sufficient for achieving the required yield (275 g).

Quality of the lettuce

The N content of the shoot biomass corresponded to the biomass production presented above within and between the lines over the two experimental years. In the first year, the shoot N content was between 40 and 50 g kg^{-1} dry matter in the front of all lines and fell to 20 g kg^{-1} towards the end of lines A and B. This suggests that in RUN I, the depletion of N towards the end of these lines A and B limited plant growth. A small, but significant N decrease (less than 5 g kg^{-1}) in the shoot biomass was also observed in the reference line and line D. Accordingly, the shoot P content decreased from 6 to 4 g kg^{-1} along lines A and B. K was higher in the front section of the reference line and lines A and C. However, the K content of all shoots was in the magnitude of 60 g kg^{-1}. In RUN II, the reference shoot biomass contained 65 g N kg^{-1} in the front and 50 g N kg^{-1} in the end of the line, thus distinctly more than in 2017. In all lines fed with treated wastewater, the N content was between 37 and 48 g kg^{-1} without any remarkable difference between the front and end of the lines. The highest P content was measured in the plants from the front of the reference line (10.4 g kg^{-1}) and the lowest in the end of line D (5.4 g kg^{-1}). In all other samples, the P content was between 7.0 and 9.0 g kg^{-1}. Shoot K was 72 g kg^{-1} in the front of the reference line and between 50 and 60 g kg^{-1} in all wastewater lines. The K content fell about 10 to 20 g kg^{-1} in all lines. The shoot biomass nutrient contents are supported by the concentrations in the used water of the hydroponic system (effluent). At the exchange of the water in all lines, the average concentration of nitrogen was <1 mg L^{-1}. In lines C and D, potassium was <1 mg L^{-1} as well. The concentration of phosphorus in the used water of line C and D was between 3 and 2 mg L^{-1}. Since the first change of the irrigation water of RUN II had been scheduled too early, the concentrations (N, P, K) were higher in the effluent (e.g. line C and D 35 mg N L^{-1}).

Mercury was not detected in any of the plant samples of RUN I. As, Cd and Co were occasionally detected with maximum values below 120 μg kg^{-1}, Cr and Pb were detected in all samples with values below 2,100 and 260 μg kg^{-1} respectively. In RUN II, the maximum As value detected was 90 μg kg^{-1}, Co 190 μg kg^{-1} and Cd was below the detection limit in all cases. The highest Cr content was 3,200 and that of Pb 110 μg kg^{-1}.

Mass balances

In order to validate the system, mass balances were carried out exemplarily on the hydroponic line, operated with the effluent of treatment process D. The results of the mass balances are presented in Table 3. It is apparent that the nitrogen, phosphorus and potassium load in the influent, effluent and the lettuce in RUN I are distinctly higher than in RUN II, although the concentrations were similar (see Figure 2).

This difference between the loads is caused by the used amount of treated wastewater. With a flow rate of 23.5 L h^{-1} in total, 21.5 m^3 treated wastewater was used in RUN I. During RUN II, the treated wastewater in the hydroponic line needed to be changed five times. This resulted in an

Table 3 | Mass balances of hydroponic lines operated with treated wastewater from line D

		RUN I (14 August to 21 September 2017)			RUN II (19 June to 24 July 2018)		
		N	P	K	N	P	K
$F_{\mathrm{IN,water}}$	(g)	938	137	498	36	7.8	44
$F_{\mathrm{OUT,water}}$	(g)	946	131	459	5.5	2.8	3.4
F_{lettuce}	(g)	25	3.8	33	33	5.6	34
Delta	(g)	−33	2.2	6	−2.5	−0.6	6.6
Delta	(%)	−3.6	1.6	1.2	−6.9	−7.7	15

applied water volume of 0.93 m^3. Interestingly, the deviation in the balances of RUN I is below ±4%. It is possible therefore that the high flow rate and the comparatively low load in the lettuce plants minimized the error. Compared with this, the deviation of RUN II is higher. However, with a deviation of −6.9, −7.7 and 15%, the balances could still be considered closed. A possible reason for this deviation could be the inhomogeneous structure of the lettuce plants, which makes a homogenous sample difficult. However, altogether the mass balance results validate the research results. The presented data thus can be considered plausible and reliable.

CONCLUSION

This research focused on the question of whether treated wastewater is suitable for growing lettuce in a hydroponic system. In order to determine how plant growth can be optimized, various types of treated wastewaters were used and the system was operated in two different modes: as a flow-through system and as a feed & deplete system. For flow-through operation in 2017, it could be observed that the ratio of nutrients of the various types of treated wastewater differed from the nutrient ratio of the reference solution according to Hoagland. This was especially true for treated wastewater from treatment lines A and B, since a conventional treatment for nitrogen and phosphorus removal was used. Furthermore, these concentrations from line A and B were significantly below the concentration of wastewater used in previous investigations (Ayaz & Saygin 1996; Al-Karaki 2011; Keeratiurai 2013; da Silva Cuba Carvalho *et al.* 2018). Nevertheless, it could be observed that due to the constant flow rate of water in a flow-through system and the subsequently constant fresh supply of nutrients, it was possible to grow lettuce in these lines (A and B) despite the low nutrient concentrations. If the gradient of plant weight in lines A and B is compared to the gradient of plant weight in lines C and D, it can be observed that the system needs to be optimized. Either the number of plants per hydroponic line should be reduced or the flow rate increased to ensure a sufficient nutrient supply when using waters with low nutrient concentration such as waters from treatment lines A and B.

If the hydroponic system is operated as a feed & deplete system, treated wastewater with a nitrogen concentration of >40 mg L^{-1} (e.g. treated wastewater from lines C and D) is suitable. It was shown that this nutrient concentration is sufficient for good plant growth. The target weight (possible sales weight) of 275 g was achieved by all hydroponic lines operated as a feed & deplete system. As da Silva Cuba Carvalho *et al.* (2018) achieved similar weights, the weights of the investigations by Keller *et al.* (2008) were lower. Furthermore, nitrogen, phosphorus and potassium were reduced by the plant uptake (feed & deplete system) by 100, 67–78 and 100%. Therefore, a higher elimination could be achieved than by the studies compiled by Magwaza *et al.* (2020).

Nutrients in all samples, except for N content in the samples from the end of the A and B line in 2017, were within the ranges for soil grown lettuce developed by Hartz *et al.* (2007) according to the Diagnosis and Recommendation Integrated System (Walworth & Sumner 1987). In addition, the nitrogen and phosphorus content of the plants corresponded to the content in lettuce cultivated hydroponically by Bugbee (2004) with a nutrient solution and by da Silva Cuba Carvalho *et al.* (2018) with wastewater.

The detected heavy metal contents do not exceed any current threshold value and are comparable to or lower than reference values in literature. The highest contents of Cd and Pb detected were only a tenth or less of the maximum permissible levels in the EU (Commission Regulation (EC) 1881/2006) and internationally (WHO/FAO 2015). No permissible maximum levels for As, Cr and Co are established, but recommendations on and assessment of daily intake of these elements (EC 2003; Lison 2007; WHO 2010) suggest that these heavy metal contents are of no concern.

Previous studies did not validate the hydroponic system run with wastewater as irrigation water by mass balances. Therefore, to complete the investigations mass balances were performed for two runs (RUN I flow-through and RUN II feed & deplete) of the hydroponic lines operated with treated wastewater from line D in 2017 and 2018. The mass balances were calculated for irrigation water and grown biomass and verified the plausibility of achieved results, thus validating the hydroponic system used.

As a conclusion, it can be stated that it is generally possible to operate hydroponic systems using treated wastewater. Our research showed that wastewaters with high and with low nutrient concentrations are suitable, if the mode of operation is accordingly modified. Both modes of operation – flow-through and feed & deplete – produced satisfying results in regard to plant weight. The advantage of the feed & deplete system lies in the additional water treatment effect. High nutrient concentrations of the influent were reduced due to nutrient consumption by the plants (e.g. <1 mg N L^{-1}). However, the utilization of a flow-through system is possible if a complete WWTP already exists. Then a small amount of the treated wastewater and nutrients could be reused for plant production. Integration of a feed & deplete system is feasible, if the wastewater and the included nutrients are not only utilized for plant production but also used as a treatment step of wastewater. A significant reduction of the nutrients is possible in this process and requirements according to the heavy metals in the irrigation water and the lettuce could be met. A final conclusion in regard to a legal use of the irrigation water could not yet be done. Further parameters such as pathogens, antibiotic resistances, organic micropollutants, boundary conditions, operator models, etc. must be taken into account. Therefore, Winker *et al.* (2020) have investigated this question for Germany and have determined that under certain conditions an implementation of a hydroponic system operated with treated wastewater is possible.

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DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

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