UNIVERSITY OF HOHENHEIM

Institute of Agricultural Sciences in the Tropics

Management of Crop Water Stress in the Tropics and Subtropics

Evaluation of a Low-Tech Approach to Mobilize Nutrients from Organic Residues for the Production of a Hydroponic Nutrient Solution

Master Thesis

Sebastian Heintze

Matriculation Number: 629973

E-mail: sebastian.heintze@uni-hohenheim.de

First Examiner: Prof. Dr. Folkard Asch

Second Examiner: M.Sc. Marc Beckett

4th July 2023

Fraunhofer IGB

Acknowledgments

First of all, I would like to thank Prof. Dr. Folkard Asch, Dr. Jörn Germer, and M.Sc. Marc Beckett for making this master thesis possible. All of them contributed with helpful and constructive advice and suggestions to the completion of this thesis. The great support and shared interest in the topic of this work were very encouraging. Furthermore, I would also like to thank Dr. Lukas Kriem, who was a great help to me, especially in the last and most tedious part of this work.

Great appreciation goes to all the supporting people at the University of Hohenheim and the IGB for their advice, help, and for putting up with some of the odors associated with the experimental part. In this context, I would like to make special mention of Julia Asch, Hedwig Pilgram, and Gabriele Bott.

Finally, I would like to thank my friends and family for their great emotional support during my master thesis and their shared enthusiasm for hydroponics.

Abstract

Hydroponic plant production has great potential as food can be produced without fertile soil, and resource use is more efficient than in traditional agriculture. Nonetheless, hydroponics rely on mineral fertilizers as source for plant nutrients. Mineral fertilizers are unsustainable due to the depletion of some fossil nutrient resources (P) and high energy requirements for production. This contributes to the cost of fertilizers and hydroponics and thus reduces accessibility and affordability for potentially benefitting stakeholder groups.

A possible option to render hydroponic plant production more sustainable is bioponics, where nutrient solutions are derived from nutrient-rich organic residues. However, the nutrient compositions of bioponic solutions are often unbalanced and, when used in hydroponic plant production, rarely result in yields comparable to those achieved with mineral fertilizers. This study aimed to produce residue-derived bioponic nutrient solutions rich in either nitrogen, phosphate, or potassium and subsequently mix the solutions to create a balanced nutrient solution. The production should be feasible with low technical effort and use organic residues available in dry lands. Food production in dry lands is challenged by high temperatures and low water availability, and hydroponics would be a way to increase food security in these regions.

Blood meal, bone meal, and potato peel were selected as N-, P-, and K-rich residues, respectively. Each residue was mixed with water and digested anaerobically. Bone meal and potato peel for 123 days, blood meal for 54 days. The NH_4^+-rich digestate derived from blood meal was subsequently aerated for 18 days to transform NH_4^+ into NO_3^-. Samples were regularly taken from each solution and analyzed for targeted nutrients. The three solutions were mixed based on the final NH_4^+-N, NO_3^--N, PO_4^{3-}-P, and K^+ concentrations. The resulting bioponic nutrient solution contained 58 mg/l NH_4^+-N, 43 mg/l NO_3^--N, 50 mg/l PO_4^{3-}-P, and 247 mg/l K^+. The bioponic solution was tested against a mineral solution for lettuce var. *Hawking* in a deep water culture system for 25 days. In regular intervals the nutrient solution was replaced, plant fresh mass was measured, and samples of the initial and replaced solution were taken. At harvest, plants grown in bioponics had produced 23 % of the lettuce yield of plants grown in mineral solution. Poor growth of the plants grown in bioponics was attributed to an unfavorable NH_4^+:NO_3^--ratio, changes in nutrient composition during the experiment, and a high microorganism load in the bioponic solution.

The approach of separately digesting organic residues with high N, P, or K concentrations and subsequent mixing did not result in a nutrient solution enabling good plant growth. However, important new insights into the production and use of bioponic nutrient solutions have been obtained, which may form the basis for further research and optimizations.

Table of Contents

List of Abbreviations

AE ..... *Aerobic*
AN ..... *Anaerobic*
ATP ..... *Adenosine Triphosphate*
B*NS* ..... *Bioponic Nutrient Solution*
CFA ..... *Continuous-Flow Analysis, Continuous-Flow Analysis*
DM ..... *Dry Mass*
DO ..... *Dissolved Oxygen*
DOE ..... *Day of Experiment*
EC ..... Electrical Conductivity
FM ..... *Fresh Mass*
ICP-OES ..... Inductively Coupled Plasma Optical Emission Spectroscopy
IGB ..... Institute for Interfacial Engineering and Biotechnology
MBBR ..... Moving Bed Biofilm Reactor
M*NS* ..... *Mineral Nutrient Solution*
MO ..... Microorganism
NFT ..... *Nutrient Film Technique*
*PG**B*** ..... *Plants Grown in Bioponic nutrient solution*
*PG**M*** ..... Plants Grown in Mineral nutrient solution
*PG**SB*** ..... Plants Grown in Spiked Bioponic nutrient solution
pH ..... *Hydrogen Potential*
SB*NS* ..... *Spiked Bioponic Nutrient Solution*
TOC ..... *Total Organic Carbon*

List of Figures

List of Tables

1. Introduction

Dry lands, consisting of hyperarid, arid, semi-arid, and dry subhumid areas, cover over 46 % of the Earth's total surface (Mirzabaev et al., 2019). They are characterized by water scarcity since the potential evapotranspiration is higher than the average annual precipitation (Middleton & Thomas, 1997). They are home to three billion people worldwide and are predominately located in countries of the global South (Mirzabaev et al., 2019). Due to the climatic conditions, agriculture is only possible to a limited extent and must be adapted accordingly. In arid areas, rainfall is inadequate for crop production and pastoral agriculture is the dominant form of land-use. In contrast, in semi-arid and subhumid areas, rainfall is sufficient for cultivating rainfed crops, with limitations regarding variety and yield (Arnon, 1992).

However, progressive land degradation in dry lands, known as desertification, threatens crop production in the semi-arid and dry subhumid areas and thus, millions of people's livelihood and food security (Mirzabaev et al., 2019). Desertification has accelerated in recent decades due to human activities interacting with climate change, such as unsustainable land use practices, overgrazing, cropland expansion, and population growth (Mirzabaev et al., 2019). Besides the reduction in agricultural productivity and the accompanied reduced incomes, desertification causes loss in biodiversity and ecosystem services and is an incentive for migration, thereby increasing pressure on fertile land and accelerating the loss of natural habitats.

To preserve the livelihood of many people, irrigation can be used to continue crop production in dry lands that are already degraded or at risk of degradation (Mirzabaev et al., 2019). However, depending on the method, irrigation of agricultural fields is often associated with high evaporation and run-off losses, and groundwater depletion (Mirzabaev et al., 2019; Poudyal & Cregg, 2019); thus intensifying the problems caused by desertification.

An alternative way to grow crops in areas unsuitable for traditional agriculture, thus diversifying diet and increasing food security, is hydroponics. The term is composed of the Greek words *hydro* (water) and *ponos* (labor) and is defined as “the cultivation of plants in nutrient-enriched water, with or without the mechanical support of an inert medium such as sand, gravel, or perlite” (Britannica, 2023a). Due to its independence from soil, hydroponics is suitable even in areas with nonarable soils. Furthermore, hydroponic plant production is more efficient in water use than conventional agriculture (Resh, 2013). Barbosa et al. (2015) reported a water saving of 92 % of hydroponically compared to conventionally (soil-based) cultivated lettuce in Arizona, USA. A major advantage, especially in arid regions. Further benefits of hydroponics compared to conventional agriculture are fertilizer savings of up to 85 %, an increase in yield

between 100 % and 250 % (AlShrouf, 2017), and a more efficient use of space, enabling year-round indoor plant production in regions with limited space, such as urban areas (Resh, 2013).

However, hydroponics also has its drawbacks. Compared to conventional agriculture, the initial costs are higher, and the energy demand is increased due to the use of pumps necessary to aerate or circulate the nutrient solution. The energy demand increases further when artificial lighting is used for indoor cultivation (Resh, 2013). Furthermore, hydroponics traditionally involves mineral fertilizers. On the one hand, they are unsustainable due to the depletion of some nutrient deposits (Cordell et al., 2009) and the high energy demand for production (Basosi et al., 2014). On the other hand, mineral fertilizers are not available to everyone and in every region due to the costly production process (Basosi et al., 2014) and the distance to markets. This is one reason for the limited use of hydroponics in drylands and generally in the global South (Mordor Intelligence, 2023). In the Sahel, for instance, a semiarid transition zone between the Sahara Desert in the North and the dry savannah in the South, 80 % of the population has less than 2 US $ per day for a living (Villalón, 2021). A cheaper and regionally available source of fertilizer is needed to enable the use of hydroponics in these areas, and thereby increasing food security. This source could be regionally available organic residues that contain many essential nutrients for plant growth.

Various attempts have been made to produce hydroponic fertilizers from organic residues - with varying success. The challenge is to mineralize the nutrients bound in the organic residues into soluble, plant-available forms. The main methods applied are anaerobic and aerobic digestion of the organic residues in an aqueous solution. Another obstacle to producing a bioponic nutrient solution is that the nitrogen must be mainly in nitrate form since ammonium is toxic to plants in high concentrations. However, only low nitrification rates can be obtained by aerobic digestion itself due to the missing and slow growth of the relevant microbial community (Garland et al., 1997; Mackowiak et al., 1996). A solution for this was provided by Shinohara et al. (2011), who added compost, rich in nitrifying bacteria, to the aerobic digestion process and achieved high nitrification rates. For their promising results, Shinohara et al. (2011) used fish-based soluble fertilizer, which is unavailable in dry lands. Another source frequently used for producing bioponic nutrient solutions is effluent from biodigesters of biogas plants (Bergstrand et al., 2020; Pelayo Lind et al., 2021), requiring a high input of organic material that is difficult to obtain in drylands (Jesus et al., 2021). Animal manures have also been used in different studies, but the results varied greatly (Kechasov et al., 2021; Liedl et al., 2004; Wongkiew et al., 2021).

All these studies had in common that, if the bioponic solutions were used in a hydroponic system, few produced comparable yields to those obtained with mineral nutrient solutions, and none exhibited a optimal balanced nutrient ratio, essential for good plant growth.

1.1 Research Objective, Question, and Hypotheses

The objective of the present study was to produce a nutrient solution for hydroponic plant production from organic residues available in arid areas. The solution should have a balanced nutrient concentration regarding the main nutrients nitrogen (N), phosphorus (P), and potassium (K) and thus be able to achieve comparable yields to mineral solutions. The production should be associated with as little technical effort as possible to enable it in resource- and financially limited regions.

One reason identified for the unbalanced nutrient solution of the studies mentioned in the introduction is that either only one organic residue was used (Kechasov et al., 2021; Liedl et al., 2004; Mowa, 2015; Tikasz et al., 2019; Wongkiew et al., 2021) or when more than one residue was used, the nutrient ratio of the residues was not adjusted to the needs of the plants (Bergstrand et al., 2020; Krishnasamy et al., 2012; Pelayo Lind et al., 2021).

To address the issue of unbalanced bioponic nutrient solutions, the present study used different organic residues depending on their N, P, and K concentrations to produce a balanced bioponic nutrient solution. In a preliminary experiment (Appendix I), knowledge was gained about the mineralization of the main nutrients, the most suitable methods, and suitable organic residues. The key findings of the preliminary experiment are presented in Chapter 3. Based on these findings, the approach of this study was developed. The preliminary experiment revealed that different conditions and methods are most suitable for the mineralization of each main nutrient. Therefore, organic residues were chosen rich in only one of the main nutrients. These residues were digested in separate reactors, with the respective optimum conditions for each nutrient as determined in the preliminary experiment. By this approach, solutions rich in N, P, or K should be produced and mixed in the best ratio to create a nutrient-balanced bioponic solution.

The following research question was formulated for this study:

Can a balanced hydroponic nutrient solution be produced by separate mineralization of N, P, and K-rich organic residues with low technical effort that produces comparable lettuce yields to a mineral nutrient solution?

The study is divided into two parts. The first part, the "mineralization experiment", addresses the production of a bioponic nutrient solution. The hypothesis (Hypothesis 1) for this part was formulated with consideration of the results of the preliminary experiment as follows.

Hypothesis 1: If the key messages learned in the preliminary experiment are implemented, an N mineralization rate into NH_4^+-N of 50 %, a conversion rate of NH_4^+-N into NO_3^--N of 50 %, a

P mineralization rate of 30 %, and a K mineralization rate of 80 % from organic residues can be achieved, and thus a balanced bioponic nutrient solution can be produced.

In the second part, the “hydroponic plant cultivation experiment”, the produced bioponic nutrient solution is tested against a commercial mineral solution in a hydroponic system on lettuce.

The hypothesis (Hypothesis 2) for this part is: If a bioponic nutrient solution with N, P, and K concentrations similar to recommended concentrations for hydroponic nutrient solutions is used in a hydroponic system on lettuce, comparable yields can be achieved as with a mineral nutrient solution.

2. Literature Review

The first chapter of this literature review (2.1) provides basic knowledge about hydroponics and the standard procedure of using mineral fertilizers. The second chapter (2.2) addresses the preparation of bioponic nutrient solutions.

2.1 Hydroponics

Hydroponics is defined as the cultivation of plants in water enriched with nutrients (Britannica, 2023a). Although the cultivation of crops in water had already been practiced in ancient times, it received increased attention in the 1920s and 1930s (Resh, 2013). The increased cultivation of food crops in greenhouses, where soils had to be replaced regularly due to fertility, soil structure, and soil-borne pests, stimulated interest in growing plants in nutrient solutions. In the early 1930s, William F. Gericke gave plant cultivation in nutrient solutions the name it bears today: Hydroponics (Resh, 2013). He also introduced the technique on a commercial scale by developing a practical system using gravel as support material for the plants (Morgan, 2021). Since these beginnings, numerous other hydroponic systems have been developed. The most used are summarized in Section 2.1.1.

Recently, hydroponic cultivation has gained increased attention. For 2023, the hydroponic sector is valued at US$ 2.78 billion and will grow further (Future Market Insights, 2023). From 2022 to 2027, a compound annual growth rate of the hydroponic market of 7.8 % is expected (Mordor Intelligence, 2023). Especially in indoor cultivation, hydroponics is very important. In 2019 hydroponics had a share of 72 % of all indoor farming methods worldwide (Shahbandeh, 2021). The largest hydroponics market is North America, whereas the fastest market growth occurs in the Asian Pacific region. Currently, hydroponic farming is more common in countries of the global North (Mordor Intelligence, 2023).

2.1.1 Hydroponic Systems

The six most commonly used hydroponic systems according to Lee and Lee (2015), are wick, drip, ebb and flow, deep water culture, nutrient film technique, and aeroponic systems, each with advantages and disadvantages. Most systems can be used with or without a substrate, supporting the plant and allowing the roots to grow to the nutrient solution. However, a substrate is necessary for the drip, ebb and flow, and wick system (Morgan, 2021). In addition, systems are divided into recirculating and flow-through systems, depending on whether the nutrient solution remains in the system after fertigating the plants or is disposed of directly (Resh, 2013).

The wick system is based on the capillary rise of water or nutrient solution in a wick, usually consisting of nylon. One end of the wick is immersed in the nutrient solution, whereas the other end reaches the root area of the plants (Figure 1). Through this, the plants are passively supplied with water and nutrients. Since only a relatively small amount of water can be transported in this manner at once, the system is not suitable for larger plants and has no major commercial significance (Lee & Lee, 2015; Resh, 2013).

The drip system, on the other hand, is a hydroponic system with great commercial importance (Lee & Lee, 2015). The system supplies the plants with nutrients and water quantities adapted to the plants' needs via drip nozzles. The nozzles are connected to a water pump via a hose system (Figure 1) (Morgan, 2021). The nutrient and water quantities can be adjusted precisely to the plants in the system, making it very effective. However, this requires some expertise, and the nozzles are susceptible to clogging by nutrient salts or organic matter if the solution is recirculated. To prevent the latter, filtration can be used (Morgan, 2021).

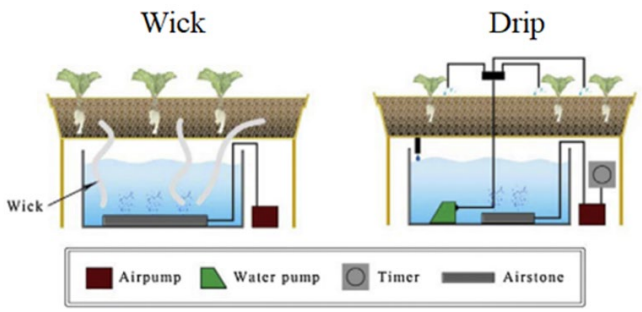


Figure 1: Wick (left) and drip irrigation (right) hydroponic systems. Adapted from Lee and Lee (2015).

In the ebb and flow system, the plant substrate is flooded with nutrient solution using a water pump in defined intervals. After the flooding, the nutrient solution drains into a reservoir (Figure 2). The fact that duration and frequency of flooding are freely selectable allows the cultivation of plants adapted to drier conditions (Morgan, 2021). However, this system also requires a certain amount of experience in order to select the flooding intervals correctly. Furthermore, it is susceptible to algae and mold growth (Lee & Lee, 2015). Although this system is particularly susceptible, mold, pathogens, and algae can also form in all other systems and cause problems, especially in recirculating systems. To prevent this, various filtration and disinfection methods can be applied (Dannehl et al., 2016).

The deep water culture is a relatively simple hydroponic system. Plants are placed above a nutrient solution reservoir in mounts on a floating raft or stationary board (Figure 2). The roots are continuously submerged in the nutrient solution, commonly aerated by an air pump. Too little oxygen supply can lead to root rot and yield loss. The simplicity and low initial investment costs are advantages of the deep water culture system (Lee & Lee, 2015).

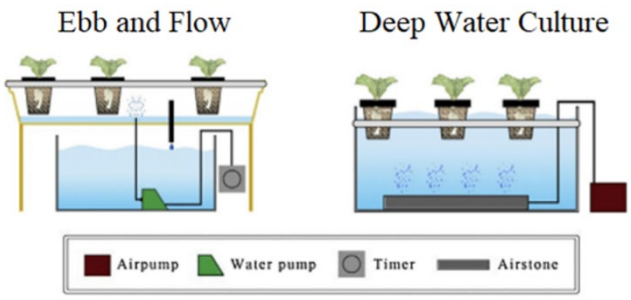


Figure 2: Ebb and flow (left) and deep water culture (right) hydroponic systems. Adapted from Lee and Lee (2015)(Lee & Lee, 2015).

In a nutrient film technique (NFT) system, a thin nutrient film flows along the roots. The plants are placed in line in a gutter with a certain inclination (Figure 3). The nutrient solution is pumped from the reservoir to the highest point of the gutter and flows back into the reservoir by gravity; by this, a continuous flow is achieved. The slope and the pumped volume can control the amount of water and oxygen available to the plants (Lee & Lee, 2015). NFT systems can be very space efficient since the gutters can be placed above each other due to their low weight. Furthermore, the plants are supplied optimally with nutrients and oxygen. However, a power failure, leakage, or a defect in the pump have drastic consequences and can lead to the total loss of the harvest (Morgan, 2021).

Probably the most technically advanced hydroponic system is aeroponics. The roots of the plants hang freely into a chamber where they are sprayed either permanently or at intervals with a fine mist of nutrient solution (Figure 3). By this, the roots are optimally supplied with oxygen (Lee & Lee, 2015). Since the system is complex and expensive in acquisition and maintenance, it is barely used commercially. However, it has some importance in growing high-quality medicinal plants as the roots can be harvested from the accessible spray chamber while the plant continues to grow (Hayden, 2006).

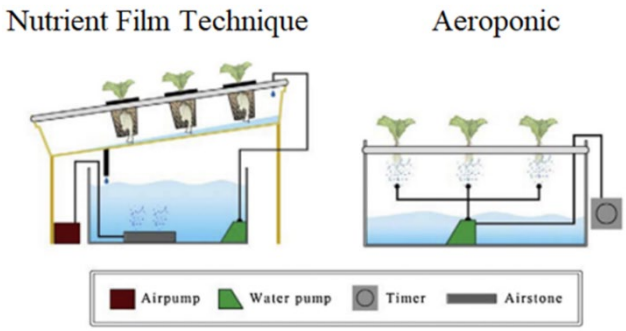


Figure 3: Nutrient film technique (left) and aeroponic (right) hydroponic systems. Adapted from Lee and Lee (2015).

The different hydroponic systems presented previously are best suited for specific purposes and have advantages and disadvantages. Generally, the advantages and limitations of

hydroponics compared to conventional soil-based agriculture can summarized as in the following table (Table 1).

Table 1: Advantages and limitations of hydroponic plant production compared to conventional agriculture (Lee & Lee, 2015; Resh, 2013; Velazquez-Gonzalez et al., 2022).

Advantages	Limitations
Independence of soil Soils can be unsuitable for crop cultivation (properties, contaminations, or unavailability) → Not present in hydroponics	**High initial costs** Costs of systems, measurement devices, and further equipment are relatively high
High space efficiency Depending on system and plant, vertical cultivation is possible → increased yield per area → plant production in urban areas	**Dependency on electricity** Depending on system and whether artificial lighting is used, electricity demand can be high → High electricity costs → Power supply failures can cause yield losses
Increased yields Optimal nutrition and water supply, eased root development Reduced impact of external factors (if environment is controlled) → More reliable yields	**Need for highly trained labor** Operation requires knowledge of agriculture, plant physiology, and chemistry
Consistent plant quality Equal amount of nutrients and water for each plant	**Not suitable for all plants** Root crops are not suitable for most systems
Increased sustainability More efficient water-, nutrient-, and space-use, no contact with soil If used in a controlled environment, no pesticides are necessary → Reduced pressure on resources and environment	**Potential environmental pollution** Inappropriate disposal of residual nutrient solution → can cause eutrophication of water bodies

2.1.2 Plant Essential Nutrients and Deficiency Symptoms

In addition to the three organic elements carbon (C), hydrogen (H), and oxygen (O), which are assimilated via air (O_2, CO_2) and water (H_2O), higher plants require 14 essential elements for healthy growth (Kadereit et al., 2014). They are divided into macro- and micronutrients, depending on the required quantities. Macronutrients are required in quantities of > 200 µg/g plant mass and are nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulfur (S), and magnesium (Mg). The micronutrients (< 2 µg/g) are iron (Fe), chlorine (Cl), manganese (Mn), boron (B), zinc (Zn), copper (Cu), molybdenum (Mo), and nickel (Ni) (Kadereit et al., 2014). In earlier work, Ni was not counted as an essential nutrient (Resh, 2013).

Each essential nutrient has specific functions in the plant, and an undersupply causes deficiency symptoms (Kadereit et al., 2014). The plant absorbs nutrients as ions, either positively charged cations or negatively charged anions, from the soil solution, or in hydroponics, from the nutrient solution. The ion forms of the essential nutrient absorbed by the plant are presented in Table 2.

Table 2: Ion forms of plant essential nutrients absorbed by plants. Adapted from Voroney (2019).

Nutrient	Forms absorbed by plant
Nitrogen	NH_4^+, NO_3^-
Phosphorus	$H_2PO_4^-$, HPO_4^{2-}
Potassium	K^+
Calcium	Ca^{2+}
Sulfur	SO_4^{3-}
Magnesium	Mg^{2+}
Iron	Fe^{2+}, Fe^{3+}
Chloride	Cl^-
Manganese	Mn^{2+}
Boron	H_3BO_3, $H_2BO_3^-$
Zinc	Zn^{2+}
Copper	Cu^{2+}
Molybdenum	MoO_4^{2-}
Nickel	Ni^{2+}

N is the nutrient the plant needs in highest quantities and is particularly important for vegetative growth (Kadereit et al., 2014). N is a component of Deoxyribonucleic acid (DNA), amino acids and proteins, adenosine triphosphate (ATP), vitamins, and hormones and is essential for metabolic activities (Resh, 2013). When N is absorbed as NO_3^-, it is first converted into NH_4^+ within the plant and consequently integrated into the amino acid glutamine (Morgan, 2021). Since N is mobile in the plant, it can be redistributed from older to younger parts of the plant, and deficiency symptoms are first visible in older leaves. A deficiency manifests in yellow discoloration (chlorosis) of the leaves and an overall reduced, stunted growth (Morgan, 2021). P is essential for cell division and growth and a component of ATP, thus necessary for energy supply. High amounts of P are necessary for seed and fruit formation (Morgan, 2021). As mobile nutrient P deficiency symptoms appear first in older leaves, which show a dull green color, followed by a brown discoloration. Photosynthesis is reduced, and sugar production and translocation are impeded, causing restricted root development. Shoot development is also restricted, and the leaf area is reduced (Morgan 2021).

Besides N, K is the most important nutrient in quantity. While K requirement for vegetative growth is almost the same as for N, it greatly increases during fruiting (Morgan, 2021). It is necessary to produce proteins, fats, and carbohydrates and the function of chlorophyll and specific enzymes. Furthermore, K is essential for maintaining the osmotic balance in cells and the opening and closing of stomata. Since K is also a mobile element, a deficiency is first noticeable in the older leaves. The most apparent symptom are scorched areas on the leaf edges. In addition, growth is reduced, and the plant is more susceptible to fungal diseases (Morgan, 2021).

Ca, the quantitively third most important nutrient, is required for the stability of cell membranes and the production of α-amylase (Resh, 2013). Once deposited, Ca is hardly mobile in the plant, and deficiency symptoms appear first in the younger leaves. Ca deficiency weakens cell membranes and walls, resulting in tip burn, blossom end rot, or a pale edge on young leaves (Morgan, 2021).

S is a component of the amino acids methionine and cysteine and certain coenzymes and proteins. It is essential for the vitamins biotin and thiamine. Due to the low mobility deficiency symptoms, a yellowing of the leaves, is first visible on younger leaves. However, S deficiency is rarely observed, as the plants' need for this element has a very flexible range (Morgan, 2021).

The last macronutrient, Mg, is essential for photosynthesis since it is the central ion of chlorophyll, thus indispensable for the light collection mechanism (Morgan, 2021). Furthermore, Mg is required for many enzymes and the structure of ribosomes (Resh, 2013). Due to its mobility, deficiency symptoms are first visible in older leaves as interveinal chlorosis (Morgan, 2021).

Of the micronutrients, Cl, Zn, Mo, and Ni are mobile, whereas Fe, Mn, B, and Cu are immobile (Kadereit et al., 2014). Cl is essential for photosynthesis, where it acts as an enzyme activator. It was the current penultimate micronutrient classified as essential for plants in 1954 (Brown et al., 1987). Mo is an electron carrier in the plant intern conversion of NO_3^- to NH_4^+. Fe is required for chlorophyll synthesis and is a compound of cytochrome, an electron carrier in photosynthesis. Mn activates enzymes necessary for DNA and ribonucleic acid (RNA) formation and is essential for O_2 production in photosynthesis. Cl, Mo, Fe, or Mn deficiency manifests mainly in chlorosis symptoms (Resh, 2013).

Zn is involved in the production of the hormone indoleacetic acid and is necessary to activate enzymes. A deficiency causes a reduced leaf area and internodes. B is required in the production of cell walls for cell division. Deficiency symptoms vary greatly depending on the species but are often visible in damages in root and stem meristems (Resh, 2013). Cu activates

enzymes necessary in photosynthesis. The most common deficiency symptom is a dark green discoloration of the young leaves with death tissue (necrotic) spots. Ni is an essential component of the urease enzyme. An undersupply is visible in necrotic leaf tips (Resh, 2013). Ni was the last micronutrient to date, classified as essential for plants in 1987 (Brown et al., 1987).

Not only an undersupply but also an oversupply of nutrients can cause adverse effects on plant growth. An N toxicity is manifested by dark green leaves and limited root growth. No toxicity symptoms are common for the other two main nutrients, P and K (Resh, 2013).

This chapter has shown that plants must be supplied with all the essential nutrients in the right amounts to grow optimally. In hydroponics, water is enriched with nutrient ions to provide the plant with all essential nutrients in sufficient concentration and the optimal ratio.

2.1.3 Nutrient Solution Composition in Hydroponics

From the early days of commercial hydroponics to the present, dissolving mineral nutrient salts has been the common practice for preparing nutrient solutions (Morgan, 2021). To prepare a nutrient solution, highly concentrated stock solutions are first prepared. Therefore, chemical salts containing the essential nutrients are dissolved in water at a particular concentration. In chemistry, a salt is a compound of positively charged cations and negatively charged anions (Britannica, 2023b). When the nutrient salts are dissolved, they dissociate and release the ions of which they are composed. Plants can absorb these ions (Morgan, 2021). At least two separate stock solutions are prepared because some nutrients, such as Ca, S, and P, form insoluble salts when mixed in high concentrations (Morgan, 2021). The stock solutions are further diluted to obtain the nutrient solution used to fertilize plants in hydroponic systems.

Compositions for nutrient solutions to supply plants with the optimum ratio of nutrients were developed by various researchers, for instance, by Hoagland and Arnon in the 1930s to 1950s. These nutrient solutions were further adapted by other researchers and are still in use today (Resh, 2013). Below, the chemical composition of a modified Hoagland solution is presented (Table 3).

Table 3: Composition of a modified Hoagland mineral nutrient solution. From Taiz and Zeiger (2006).

Compound	Molecular weight	Concentration of stock solution	Concentration of stock solution	Volume of stock solution per liter of final solution	Element	Final concentration of element	
	g mol^{-1}	mM	g L^{-1}	mL		µM	ppm
Macronutrients							
KNO_3	101.10	1,000	101.10	6.0	N	16,000	224
$Ca(NO_3)_2 \cdot 4H_2O$	236.16	1,000	236.16	4.0	K	6,000	235
$NH_4H_2PO_4$	115.08	1,000	115.08	2.0	Ca	4,000	160
$MgSO_4 \cdot 7H_2O$	246.48	1,000	246.49	1.0	P	2,000	62
					S	1,000	32
					Mg	1,000	24
Micronutrients							
KCl	74.55	25	1.864	2.0	Cl	50	1.77
H_3BO_3	61.83	12.5	0.773		B	25	0.27
$MnSO_4 \cdot H_2O$	169.01	1.0	0.169		Mn	2.0	0.11
$ZnSO_4 \cdot 7H_2O$	287.54	1.0	0.288		Zn	2.0	0.13
$CuSO_4 \cdot 5H_2O$	249.68	0.25	0.062		Cu	0.5	0.03
H_2MoO_4 (85% MoO_3)	161.97	0.25	0.040		Mo	0.5	0.05
NaFeDTPA (10% Fe)	468.20	64	30.0	0.3–1.0	Fe	16.1–53.7	1.00–3.00
Optional[a]							
$NiSO_4 \cdot 6H_2O$	262.86	0.25	0.066	2.0	Ni	0.5	0.03
$Na_2SiO_3 \cdot 9H_2O$	284.20	1,000	284.20	1.0	Si	1,000	28

Source: After Epstein 1972.

Note: The macronutrients are added separately from stock solutions to prevent precipitation during preparation of the nutrient solution. A combined stock solution is made up containing all micronutrients except iron. Iron is added as sodium ferric diethylenetriaminepentaacetate (NaFeDTPA, trade name Ciba-Geigy Sequestrene 330 Fe; see Figure 5.2); some plants, such as maize, require the higher level of iron shown in the table.

[a]Nickel is usually present as a contaminant of the other chemicals, so it may not need to be added explicitly. Silicon, if included, should be added first and the pH adjusted with HCl to prevent precipitation of the other nutrients.

As can be seen from the table above, N in hydroponics is provided mainly as NO_3^- and only in small amounts as NH_4^+. It is generally recommended that NH_4^+-N should not exceed 25 % of the total added N (Savvas et al., 2006) since the NH_4^+ ion is absorbed very quickly by the plant roots. This poses the risk that at high available concentrations, more NH_4^+ is absorbed than the plant can utilize. An oversupply of absorbed NH_4^+ leads to toxicity which causes physiological disorders in the plant (Morgan, 2021).

The nutrient concentrations of some mineral hydroponic solutions compiled by Resh (2013) are presented in Table 4. Since Ni and Cl are the two most recently discovered essential micronutrients, they are not included, especially in older nutrient solutions (Table 4) or noted as optional (Ni) (Table 3). They may be added as contaminants by the other chemicals or the water if no desalinated water is used.

Table 4: Nutrient concentrations of some mineral nutrient solutions, compiled by Resh (2013). All essential plant nutrients except Cl and Ni are listed.

References		NH_4^+ - N	NO_3^- - N	PO_4^{3-} - P	K	Ca	Mg	S	B	Fe	Zn	Cu	Mn	Mo
								[mg/l]						
Jones & Shive (1921)		39	204	65	102	292	172	227	/	0.8	/	/	/	/
Hoagland & Amon (1938)		14	196	31	234	160	48	64	0.5	0.6	0.05	0.02	0.5	0.01
Purdue (1948)	A	28	70	63	390	200	96	607	0.5	2.0	0.05	0.02	0.3	/
	B	28	140	63	390	200	96	447	0.5	1.0	0.05	0.02	0.3	/
	C	14	224	63	390	120	96	64	0.5	1.0	0.05	0.02	0.3	/
Schwartz (California)		15	196	31	234	160	48	64	/	/	/	/	/	/
Schwartz (New Jersey)		20	126	71	90	180	55	96	/	/	/	/	/	/
CDA		33	93	36.7	209	131	22	29.5	0.46	1.7	0.094	0.035	0.8	0.027
Saanichton		33	135	36.7	209	146	22	29.5	0.46	1.7	0.094	0.035	0.8	0.027
B.C. Canada		33	177	36.7	209	146	22	29.5	0.46	1.7	0.094	0.035	0.8	0.027
Range		14 - 39	70 - 224	31 - 71	90 - 390	120 - 292	22 - 172	29.5 - 607	0.46 - 0.5	0.6 - 2.0	0.05 - 0.094	0.02 - 0.035	0.3 - 0.8	0.01 - 0.027
Means		**26**	**156**	**50**	**246**	**174**	**68**	**166**	**0.483**	**1.313**	**0.069**	**0.026**	**0.543**	**0.023**

2.1.4 Nutrient Solution Management

In addition to providing a nutrient solution, other measures are required to enable optimal plant growth in hydroponics. Hydrogen potential (pH) management, nutrient concentration monitoring, and solution exchange are the most important ones.

The pH is defined as the reciprocal of the decadic logarithm of the H^+ ion activity and a measure of acidity. A pH between 0 and 6.9 is acidic, and between 7.1 and 14 is alkaline. A pH of 7.0 is neutral (Mesmel & Holmes, 1992). Just as in soil, nutrient availability in hydroponics is dependent on pH. Generally, a pH between 5.5 and 6.5 in the root zone is considered optimal in hydroponics. However, the exact optimum pH depends on the plant species (Morgan, 2021). The more the pH value deviates from the optimum range, the less readily available the nutrients are (Figure 4).

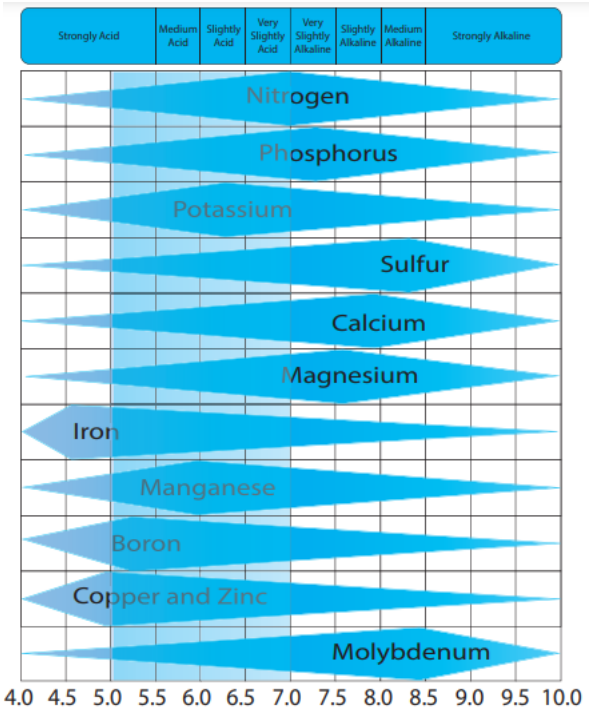


Figure 4: Availability of nutrients as a function of pH in hydroponics from Velazquez-Gonzalez et al. (2022).

In general, too high pH values reduce the availability of micronutrients in particular, while low values reduce the availability of macronutrients (Goddek et al., 2019). High pH values can cause the formation of insoluble Fe^{2+}, Mn^{2+}, PO_4^{3-}, Ca^{2+}, and Mg^{2+} salts (Resh, 2013).

The pH is adjusted to the desired value, if necessary, after the stock solutions have been mixed to form the final solution. Usually, either sulfuric acid (H_2SO_4) or potassium hydroxide (KOH) are used for this purpose (Goddek et al., 2019). The pH must be regularly checked and adjusted, if necessary, since the pH changes due to nutrient absorption by the plant. The uptake of nutrients in the form of anions or cations by the roots leads to an efflux of hydroxyls

(OH^-) or protons (H^+) from the root into the solution. By this, the electric charge balance within the plant is maintained. However, depending on the charge of the absorbed ions, this changes the pH of the nutrient solution (Figure 5) (Goddek et al., 2019).

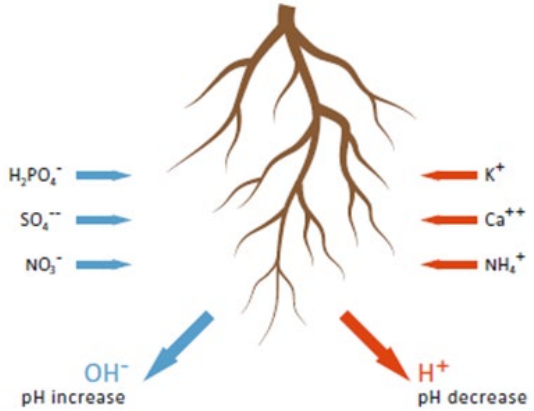


Figure 5: Alkalization or acidification of nutrient solution by ion uptake. Uptake of anions (-) causes efflux of OH^-, cations (+) of H^+ from the root. From Goddek et al. (2019).

In commercial hydroponics, the rule of thumb is to exchange the nutrient solution after one to three weeks if a recirculating system is used (Resh, 2013). The exchange is necessary because the nutrient concentration decreases, and the ratio becomes unbalanced over time. Responsible for this is the varying degree of nutrient uptake by the plant; thus, certain nutrients are depleted before others. In addition, the accumulation of non-essential or harmful salts for plant growth in the solution, such as Na, makes an exchange necessary (Resh, 2013).

The accurate determination of nutrient concentrations in the solution at any point in time would require expensive analysis methods (Resh, 2013). A simpler and cheaper method to obtain information about the nutrient concentration of a solution is to measure the electrical conductivity (EC). EC is a measure of the ability of a material to conduct electrical current measured in mS/cm or µS/cm. In an aqueous solution, the conductivity depends on the total number of electrically charged particles. Positively charged cations and negatively charged anions, present in the nutrient solution, conduct electricity and can be measured using an EC meter (Morgan, 2021). Thus, EC allows conclusions about the concentration of nutrients in solution. The recommended EC range differs depending on the crop (Table 5).

Table 5: Recommendations for Electrical Conductivity (EC) of nutrient solution for the cultivation of different crops in hydroponics. From Dunn and Singh (2016).

Crops	EC [mS/cm]
Asparagus	1.4 - 1.8
Basil	1.0 - 1.6
Broccoli	2.8 - 3.5
Cabbage	2.5 - 3.0
Cucumber	1.7 - 2.0
Lettuce	1.2 - 1.8
Pak Choi	1.5 - 2.0
Peppers	0.8 - 1.8
Spinach	1.8 - 2.3
Tomato	2.0 - 4.0

EC allows conclusions about the solution's nutrient concentration but not the nutrient composition. Therefore, the EC value is a suitable measure to monitor the development of nutrient concentrations over time in conventional hydroponic solutions. However, EC is not well-suitable for nutrient solutions that contain dissolved salts other than nutrient salts, which also influence EC. This can be the case for nutrient solutions produced by organic residues (Morgan, 2021). Furthermore, adding acids or bases, which dissociate into H^+ or OH^- in water, influences the EC (MacDonald & Boyack, 1969).

Additionally, to the aeration of nutrient solution, which is done in different ways depending on the system (2.1.1 Hydroponic Systems 2.1.1) and is essential for nutrient uptake and root respiration (Boru et al. 2003), disinfection and filtration of the nutrient solution are often performed in commercial hydroponics (Morgan 2021). This practice reduces or eliminates pathogens that may be introduced by the water used to prepare the nutrient solution or develop in recirculating nutrient solutions. Pathogens commonly detected in hydroponic systems are *Colletotrichum, Pythium, Phytophthora,* and *Fusarium* species (Constantino et al., 2013; Li et al., 2013; Nahalkova et al., 2008), which can cause substantial yield reductions. Sand and membrane filtration methods are used in hydroponics (Velazquez-Gonzalez et al., 2022). Disinfection methods that can be applied are ultraviolet, chlorine, hydrogen peroxide, ozone, or heat disinfection (Morgan, 2021).

2.2 Bioponic Nutrient Solutions

A bioponic nutrient solution is a solution in which nutrients are added via organic sources rather than the usual nutrient salts. In general, when preparing a bioponic nutrient solution, the aim is to mobilize the inorganic nutrients bound in organic materials by mineralization into plant available forms. In soil, this mineralization takes place through the existing soil microorganisms

(MOs); in a hydroponic system, these processes must be brought about by special methods and the addition of MOs.

Initial interest in hydroponic cultivation of plants in nutrient solutions derived from organic waste arose to reduce nutrients in wastewater (Law, 1969) or to grow plants in locations without access to mineral fertilizers. In a series of experiments, Garland and Mackowiak attempted to isolate the inorganic nutrients from organic waste materials for a life support system for NASA's long-term space habitation program (Garland & Mackowiak, 1990; Garland et al., 1997; Mackowiak et al., 1996). Today, more reasons have been added while the above mentioned still hold truth. In particular, replacing the unsustainable mineral fertilizer salts is of increased interest (Szekely & Jijakli, 2022). For N, P, and K, the high energy requirements for fertilizer production and the depletion of deposits pose a threat to the environment and future fertilizer production (Basosi et al., 2014; Moomen & Dewan, 2017; U.S. Geological Survey, 2021).

2.2.1 Unsustainability of Mineral Fertilizers

Ammonia (NH_3) is the feedstock for 97 % of N fertilizer produced worldwide (Basosi et al., 2014). Most NH_3 is produced by processes based on the Haber-Bosch process developed in the early 20th century. In the Haber-Bosch process, a mixture of three parts H to one part N is synthesized into NH_3 at high temperatures and pressure. Whereas all N used in the process is obtained from the air, H is produced by natural gas steam reforming, coal gasification, or partial oxidation of oils or coal (Basosi et al., 2014). In particular, the latter two methods have high energy requirements. In total, for producing one ton of NH_3, 53 GJ of energy is necessary (Basosi et al., 2014).

P and K fertilizer production mainly relies on mining of non-renewable rock phosphate and potash. Mining is an energy-intensive extraction method and causes land degradation and water pollution (Moomen & Dewan, 2017). Apart from the environmental impact, the rock phosphate and potash deposits are spread over a few countries, which poses the risk of political conflicts and supply bottlenecks. Morocco and the Western Sahara alone have 70 % of the world's rock phosphate reserves (U.S. Geological Survey, 2021). Conservative estimates suggest that worldwide rock phosphate reserves could be depleted in 50 to 100 years (Cordell et al., 2009). 75 % of the total recoverable potash is located in Canada, Belarus, Russia, and China (U.S. Geological Survey, 2021). The historic increase in fertilizer prices caused by sanctions imposed on Russia and Belarus by several economies as a result of Russia's attack on Ukraine in 2022 underscores the vulnerability of the fertilizer market to crises (Schnitkey et al., 2022).

These are all reasons why an alternative source of fertilizer for hydroponic systems that reduces dependence on mineral fertilizers is necessary. One possible source are organic materials.

2.2.2 Organic Materials for Nutrient Solution Production

The starting materials for producing bioponic nutrient solutions are mostly organic waste or residues with high plant-relevant nutrient concentrations without further use. Some organic materials and their N, P, and K content that can be used to produce bioponic nutrient solutions are shown in Table 6.

Table 6: N, P, and K content in percentage dry mass (DM) of different organic materials.

Organic Material		N	P	K	Reference
			[% DM]		
Manures					
	Beef	1.2	0.9	1.7	Rosen & Eliason (2005)
	Poultry	3.0	2.2	1.7	
	Swine	2.5	0.9	0.8	
	Bat guano	6.0	2.2	2.5	
	Goat manure	1.7	1.5	1.6	Cho et al. (2017)
Bone meal		3.0	9.6	0.0	Rosen & Eliason (2005)
Blood meal		13.0	0.9	0.8	
Fish meal		10.0	2.6	4.1	
Alfalfa hay		2.5	0.2	2.1	
Cotton seed meal		6.0	1.3	1.2	
Potato peel (ash)		5.0	1.4	21.6	Majee et al. (2021)
Banana peel		0.8	0.3	6.8	Anhwange et al. (2009) Archibald (1949) Jambhale & Gohatre (2019)

Various organic materials were used in previous studies to produce bioponic nutrient solutions (Table 7, p.22, Table 8, p.25). Animal manure was particularly common (El-shinawy et al., 1999; Kechasov et al., 2021; Krishnasamy et al., 2012; Liedl et al., 2004), but also household waste (Bergstrand et al., 2020), plant residues (Garland & Mackowiak, 1990; Phibunwatthanawong & Riddech, 2019) and other organic residues were used (Shinohara et al., 2011).

The most commonly applied methods to release the inorganic nutrients from the organic residues are aerobic (Mowa, 2015; Shinohara et al., 2011; Wongkiew et al., 2021) and anaerobic digestion (Krishnasamy et al., 2012; Liedl et al., 2004; Phibunwatthanawong &

Riddech, 2019) or a combination of both (Bergstrand et al., 2020; Kechasov et al., 2021; Pelayo Lind et al., 2021).

2.2.3 Anaerobic Digestion

Anaerobic digestion, also called anaerobic fermentation, is the decomposition of organic material in the absence of O_2. This process results in partial gasification, liquefication, and mineralization of the organic material (Wang et al., 2007). Gaseous CH_4 and CO_2 are released, leaving behind stable organic residues and a nutrient-rich effluent called digestate. Mainly, anaerobic digestion is used to produce biogas or stabilize organic waste in wastewater treatment plants (Wang et al., 2007). For optimal anaerobic digestion, temperatures should be between 35 - 70 °C, and the C:N ratio of the material should not be below 25:1 (Meegoda et al., 2018). The anaerobic digestion process consists of four successive steps: Hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 6). In each step, specialized MOs break down the organic material (Meegoda et al., 2018).

In the first step, the hydrolysis, complex organic polymers like polysaccharides, proteins, and lipids, inaccessible for the MOs of the successive steps, are broken down into sugars, amino acids, and long-chain fatty acids (Liu & Whitman, 2008). In anaerobic digestion, heterotrophic hydrolytic bacteria mainly perform this step through the excretion of enzymes. This step takes different amounts of time depending on the organic material used. For example, the presence of lignin or cellulose extraordinarily prolongs the hydrolysis time. The optimum pH for hydrolysis is between five and seven (Meegoda et al., 2018). The sugars, amino acids, and long-chain fatty acids, formed in the hydrolysis step, are absorbed by acidogenic MOs in the acidogenesis step. They are transformed into volatile fatty acids, such as propionate, and small amounts of ethanol and lactate (Meegoda et al., 2018). The formation of the volatile fatty acids causes a lowering of the pH. If this reduction is too extreme, it can cause the further anaerobic digestion process to stop.

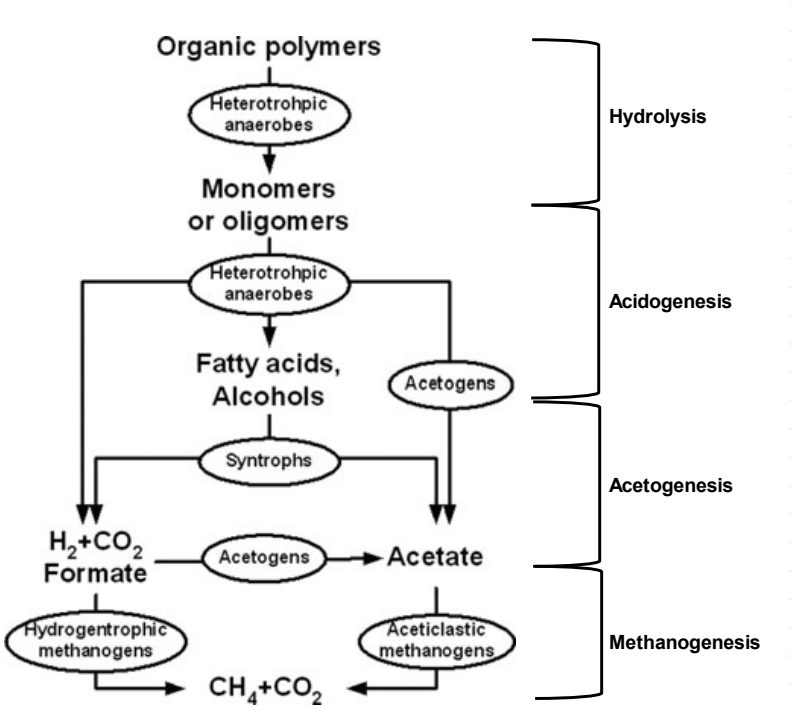


Figure 6: The four steps of anaerobic digestion, hydrolysis, acidogenesis, acetogenesis, methanogenesis. Involved microorganisms are shown in ellipses. Adapted from Liu and Whitman (2008).

Due to the interest in N for fertilizer production, amino acid degradation in the acidogenesis will be discussed in more detail. Amino acids consist of four active groups attached to a central C atom. These are an amino group ($–NH_2$), a carboxylic group (–COOH), a hydrogen atom (-H), and a functional group (-R) (Nazifa et al., 2021). Deamination initiated by acidogenic MOs, which include ammonifying bacteria, cleaves NH_3 and volatile fatty acids off (Equation 1).

Equation 1: Deamination of amino acid and release of ammonia (Nazifa et al., 2021).

$$H_2N - R - COOH + H_2O \rightarrow NH_3 + RCOOH$$

In aqueous solutions, NH_3 reacts with water to an alkaline solution of NH_4^+ and OH^- (Equation 2). The equilibrium between NH_3 and NH_4^+ is influenced by pH, temperature, and concentration. At a pH above seven, the ratio of the gaseous NH_3 increases and can cause N losses (Körner, 2009).

Equation 2: ammonium and ammonia equilibrium in aqueous solution (Körner, 2009).

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$

By the digestion of N-rich organic materials (low C:N ratio), high NH_3 concentrations can occur, hindering further anaerobic digestion (Meegoda et al., 2018).

The next step of anaerobic digestion, the acetogenesis, converts volatile fatty acids into acetate. Acetogenic MOs perform the conversion. Additionally, H is produced (Meegoda et al., 2018). Finally, methanogenic MOs absorb the intermediates and produce CH_4 and CO_2. Depending on the source they grow, methanogenic MOs are divided into acetoclastic methanogens (acetate) and hydrogenotrophic methanogens (CO_2 and H_2). As obligate anaerobic archaea, both are very sensitive to O_2 and prefer a neutral pH (Janesch et al., 2021; Meegoda et al., 2018). A digestate remains that contains the unused nutrients, for instance, P and K, from the organic materials used. When anaerobic digestion is completed, the digestate commonly has a pH between seven and nine (Nkoa, 2014).

Several studies have investigated the suitability of anaerobic digestate as a fertilizer in hydroponic systems, with varying results. Liedl et al. (2004) used 30-day-old digestate from an anaerobic digester fed with chicken manure to cultivate lettuce in an NFT system. In four trials, they compared different dilutions of the digestate, based on the total N concentration, against a commercial nutrient solution. The best results were obtained in all four trials as the digestate was diluted to 100 mg/l total N. In two trials, the harvested shoot fresh mass (FM) differed not significantly from the control after a cultivation period of five weeks. However, NO_3^-, NH_4^+, P, and K concentrations were only published for the fourth trial. (Table 7). The yield of trial number four was significantly lower than the mineral control. Nevertheless, 56 % of the control yield was achieved. At lower dilutions of the digestate, the plants showed a significant decrease in growth (Liedl et al., 2004). Krishnasamy et al. (2012) tested the effect of different dilutions of an anaerobic digestate derived from food and vegetable waste on the growth of silverbeet in a deep water culture. The most appropriate dilution of the bioponic solution yielded just 8 % of the mineral control. The best results were obtained when the digestate concentration was 20 %. A 50 % digestate concentration caused the death of the plants within two weeks.

Liedl et al. (2004) and Krishnasamy et al. (2012) are some of few who tested anaerobic digestate as bioponic nutrient solution in hydroponics and published NH_4^+, NO_3^-, P, and K concentrations of the solutions (Table 7). In both studies, reduced growth of plants growing in bioponic solutions was associated with high NH_4^+ and low NO_3^- as well as dissolved oxygen (DO) concentrations. Thus, the need for adequate dilution was emphasized (Krishnasamy et al., 2012; Liedl et al., 2004). Other studies, which published less comprehensive nutrient concentrations of the bioponic solutions used, came to similar conclusions. Mupambwa et al. (2019) showed that anaerobic digestate from cow manure is not a suitable fertilizer for hydroponic cultivation of tomatoes. At 10 % digestate concentration lowest phytotoxicity was detected; however, nutrient concentrations were likewise low. Good results were reported by Phibunwatthanawong and Riddech (2019), who cultivated lettuce in digestate derived from a

mix of molasses, distillery slop, and sugarcane leaves. A 1 % digestate concentration achieved comparable yields to mineral fertilizers.

Table 7: Anaerobic digestate used as nutrient solution in hydroponics. Only studies were selected where NO_3^-, NH_4^+, P, and K concentrations were published. Digestate concentration refers to the digestate concentration added to the hydroponic system. Yield in % of mineral control solution. AN: Anaerobic. DWC: Deep water culture. NFT: Nutrient film technique.

Starting material added to AN Digestion	Digestate concentration	Main Nutrients				Cultivated Plant	Hydroponic System	Yield	Reference
		NO_3^--N [mg/l]	NH_4^+-N [mg/l]	P [mg/l]	K [mg/l]			[%]	
Food and vegetable waste	20%	14	95	6	112	Siverbeet (*Beta vulgaris*)	DWC	8	Krishnasamy et al. 2012
Poultry manure	100 mg/l N_{total}	0	44	411	216	Lettuce (*Lactuca sativa*)	NFT	56	Liedl et al. 2004

Despite some promising results, the use of anaerobic digestate as a hydroponic fertilizer is fraught with many uncertainties and obstacles to plant growth. In particular, the high NH_4^+ concentration in anaerobic digestate is an obstacle to hydroponics. Under anaerobic conditions, no conversion of NH_4^+ to NO_3^-, the preferred N form in hydroponics, can occur. Furthermore, low DO, phytotoxic compounds, and a high pH are commonly reported and hinder plant growth in hydroponics (Krishnasamy et al., 2012; Liedl et al., 2004; Mupambwa et al., 2019; Phibunwatthanawong & Riddech, 2019). In addition, high total organic carbon (TOC) concentrations can be present in anaerobic digestate, especially if the digestion is incomplete (Botheju et al., 2010; Mackowiak et al., 1996). TOC is a measure that captures MOs and C, which serves as food for MOs. A heavy MO load can adversely affect hydroponic systems (Botheju et al., 2010; Mackowiak et al., 1996).

Aerobic digestion is the other method commonly used to produce bioponic nutrient solutions, which can reduce some of the adverse effects found in anaerobic digestate.

2.2.4 Aerobic Digestion

Aerobic digestion is the oxidization of organic material driven by heterotrophic MOs in the presence of O_2. It is applied in wastewater treatment to stabilize waste (Arvanitoyannis, 2008), where air-blowers supply O_2 (Goddek et al., 2019). The end product, CO_2, is released in solution. Furthermore, the nutrients bound in the organic material are released in the aerobic digestate – this process is called aerobic mineralization (Goddek et al., 2019). Therefore, aerobic digestion is an interesting method to produce nutrient solutions from organic residues. The fact that nitrification can only occur under aerobic conditions (Goddek et al., 2019) makes aerobic digestion a necessary measure for the production of a nutrient-balanced bioponic solution. Nitrification is the oxidation of NH_4^+ to NO_2^- and finally to NO_3^-, which can be performed by auto- and heterotrophic MOs (nitrifying bacteria). Nitrification is a two-step process; first, NH_4^+ is oxidized to NO_2^-, in the second step, the NO_2^- is converted to NO_3^-

(Equation 3). In the autotrophic nitrification, the first step is carried out by *Nitroso*-bacteria of the genus *Nitrosomonas, -coccus, -spira, -lobus,* and *-vibrio. Nitro*-bacteria performs the second step, for instance, *Nitrobacter, -spira, -coccus,* and *-spina.* Several MOs carry out heterotrophic nitrification, including the fungi species *Aspergillus* and *Penicillium* spp. and bacteria such as *Pseudomonas*, *Bacillus,* and *Streptomyces* spp. (Körner, 2009).

Equation 3: Two-step nitrification (Körner, 2009).

$$NH_4^+ + 1.5\,O_2 \rightarrow NO_2^- + H_2O + 2H^+ + 318\,kJ$$

$$NO_2^- + 0.5\,O_2 \rightarrow NO_3^- + 75\,kJ$$

An optimal nitrification process depends on several factors. Besides the MOs, which must be present in sufficient quantity and, depending on the species, grow slowly, DO concentrations higher than 3 mg/l, a pH between 7.5 – 7.8, and temperatures of 25 - 35 °C are optimal for nitrification (Stefanakis et al., 2014).

In addition to nitrification, reduction of phytotoxic properties is also possible under aerobic conditions. Garland and Mackowiak (1990), who isolated inorganic nutrients from the inedible portion of wheat by simple leaching, noted that high TOC and phytotoxic effects in the leachate hindered hydroponic plant growth. In subsequent studies they showed that these harmful compounds can be reduced by aeration and microbial activity (Garland et al., 1997; Mackowiak et al., 1996). A major advance was made in producing bioponic nutrient solutions by aerobic digestion through the research of Shinohara et al. (2011). They developed the method of adding an inoculum to an aerated aqueous solution containing organic residues. The inoculum contained nitrifying bacteria; thus, Shinohara et al. (2011) achieved a conversion rate of added organic N into NO_3^--N of 97.6 %. In their experiments, they used 5 g/l bark compost as inoculum and added 0.5 g/l fish-based soluble fertilizer daily for seven days. The 0.5 g/l fertilizer contained 31.5 mg/l N, higher fertilizer dosages produced no NO_3^-. These findings were the basis for further research.

Wongkiew et al. (2021) added 400 g of dried chicken manure in a biofilter directly into an NFT system with a water capacity of 35 l. As inoculum 20 ml/l liquid compost was used. The system was operated for 20 days prior to the transplantation of 18 romaine lettuce (*Lactuca sativa longifolia* cv. Jericho) plants which were cultivated for 35 days. A maximum concentration of 21 mg/l NO_3^--N, 8 mg/l NH_4^+-N, and 68 mg/l PO_4^{3-}-P were measured in the bioponic nutrient solution during the cultivation period. No K measurements and no mineral control were conducted. The bioponic system yielded, on average, 113 g FM per lettuce head (Wongkiew

et al., 2021). In contrast, Rader and Karlsson (2006) yielded an average of 478 g per romaine lettuce head with conventional soil-based cultivation.

The inoculation method developed by Shinohara et al. (2011) can solve one of the problems of anaerobic digestate, namely high NH_4^+ and low NO_3^- concentrations. Thus, both methods were combined in several studies. Anaerobic digestate, rich in NH_4^+ and other nutrients, was aerobically digested in a second step.

Bergstrand et al. (2020) used anaerobic digestate from a commercial biogas digester fed mainly organic household waste, manure, and slaughter residues. Microorganism carriers (MO-carriers) were added to the digestate and aerated for nitrification for two weeks. The obtained solution was diluted to EC values of 1, 2, and 3 mS/cm, respectively, and tested on Pak Choi (*Brassica rapa* var. *chinensis*) in an NFT system. EC of the bioponic solutions was maintained by regularly adding undiluted solution. The best results were obtained for the solution with 1 mS/cm. However, the harvested FM was significantly lower than the yield of the plants grown in the mineral control solution (Bergstrand et al., 2020).

Pelayo Lind et al. (2021) tested diluted anaerobic digestate from a biogas reactor fed with plant material, nitrified using a moving bed biofilm reactor (MBBR). The MBBR was inoculated with activated sewage sludge and either integrated into the NFT system used or operated externally. Pak Choi was cultivated for 28 days before being harvested. No significant difference in shoot FM was measured between the system with extern MBBR and integrated MBBR. However, both produced significantly lower shoot FM than the mineral control solution (Pelayo Lind et al., 2021). Even though the nutrient concentrations of the bioponic solution were comparatively close to that of a mineral one (Table 8). However, the NO_3^--N concentration was significantly lower than that of a mineral solution, and the NO_2^--N concentration was high with 78 mg/l. Bergstrand et al. (2020) showed that as little as 30 mg/l NO_2^--N can reduce Pak Choi yield by half if bioponic nutrient solutions are used. Like Pelayo Lind et al. (2021), Kechasov et al. (2021) used an MBBR integrated into a hydroponic system to compare a bioponic versus a mineral nutrient solution. They used an aeroponic system to cultivate tomatoes. Anaerobically digested pig manure 20 times diluted and autoclaved was added to the system and was aerobically nitrified by the MBBR. Despite the comparatively low nutrient concentrations of the bioponic solution (Table 8), the harvested yield per plant differed not significantly from the control. Only the produced biomass of the tomato plants grown in bioponic solution was significantly lower (Kechasov et al., 2021).

Table 8: Aerobic digestate used as nutrient solution in hydroponics. Only studies were selected where NO_3^-, NH_4^+, P, and K concentrations were published, exception: Wongkiew et al. 2021. Digestate concentration refers to the digestate/material concentration added to the hydroponic system. Yield in % of mineral control solution. AE: Aerobic AN-D: Anaerobic digestate was used as feedstock for AE digestion. NFT: Nutrient film technique.

Starting material added to AE Digestion	Digestate concentration	Main Nutrients				Cultivated Plant	Hydroponic System	Yield	Reference
		NO_3^--N	NH_4^+-N	P	K				
		[mg/l]	[mg/l]	[mg/l]	[mg/l]			[%]	
Chicken manure	400 g chicken manure / 35 l water	21	8	68	/	Romaine lettuce (*Lactuca sativa longifolia*)	NFT	23 *	Wongkiew et al. 2021
AN-D (organic household waste, manure, slaughter residues)	1 EC/cm	45	38	2	60	Pak Choi (*Brassica rapa* var. *Chinensis*)	NFT	53	Bergstrand et al. 2020
AN-D - diluted to 200 mg/l NH_4^+-N (plant material)	/	90	14	41	250	Pak Choi (*Brassica rapa* var. *Chinensis*)	NFT	48	Pelayo Lind et al. 2021
AN-D - diluted 20 times (Pig manure)	/	21	3	26	31	Tomato (*Solanum lycopersicum*)	Aeroponic	79	Kechasov et al. 2021

**in % of yield achieved with coventional methods by Rader and Karlsson (2006).*

By enabling nitrification, aerobic digestion is an essential method for producing bioponic nutrient solutions. Especially the combination of anaerobic digestate treated aerobically showed some promising results. However, problems such as low and unbalanced nutrient concentrations (Table 8), phytotoxicities, and high pH values also occur in aerobically produced bioponic nutrient solutions (Bergstrand et al., 2020; Kechasov et al., 2021; Pelayo Lind et al., 2021).

3. Preliminary Experiment's Key Messages and Chosen Approach

The present study used different organic residues depending on their N, P, and K concentrations to produce a bioponic nutrient solution. Thereby a nutrient-balanced solution should be achieved.

A preliminary experiment was conducted in pursuit of this goal. It aimed to determine to what extent the main nutrients are mineralized into soluble forms if several organic residues are mixed in a particular ratio, depending on their N, P, and K concentrations, and if this approach can yield a nutrient-balanced hydroponic solution. For this purpose, two recipes were prepared, one containing exclusively the animal residues bone meal and goat manure (R1), available in arid regions. The other recipe was based on R1 but adjusted with potato- and banana peel (R2). After mixing, both recipes were digested anaerobically (AN) or aerobically (AE) for 31 days, with either a controlled pH of 6.5 or an uncontrolled pH.

The preliminary experiment's detailed material and methods and results can be found in the appendix. The evaluation of the experiment showed that a balanced nutrient solution is hard to achieve with this approach since different modalities are most suitable for mineralizing the different main nutrients and transforming NH_4^+ into NO_3^-. Furthermore, the mineralization time and rates were different for each nutrient. The mineralization rates of nutrients contained in the organic residues into soluble forms were 12.5 % for N into NH_4^+-N, 22.8 % for P into PO_4^{3-}-P, and 100 % for K. The key messages of the preliminary experiment for each main nutrient are given below.

The mineralization of N bound in organic residues into NH_4^+ was higher with AN than with AE digestion. In treatments with a pH above eight most of the time, lowest NH_4^+ was measured, probably due to gaseous NH_3 losses. The highest mineralization rate achieved in the preliminary experiment of the added N into NH_4^+-N was 12.5 %. To increase the N mineralization rate, only one organic N source with a low C:N ratio could be advantageous. Despite the better mineralization of N in AN conditions, the transformation of NH_4^+ into NO_3^- requires O_2. Therefore, a two-step process is advantageous, where N of the organic residues is mineralized into NH_4^+ in an AN process and subsequently transformed into NO_3^- in an AE process. For the AE nitrification process, pH control is even more critical than for the AN process.

With AN digestion, higher P mineralization rates of the P inherent in organic residues into solution were achieved. Nonetheless, the maximum mineralization rate of the added P into PO_4^{3-}-P was only 22.8. The importance of the pH must be emphasized for the mineralization

and the solubility of P. For the mineralization process, a lower pH is beneficial. If P, inherent in bone meal, should be mineralized, the pH must be below 5.5 (Epple & Enax, 2018).

Of the three main nutrients, K was most efficiently mineralized into solution since it exists as a soluble ion (K^+) in organic residues, whereas N and P are bound in complex molecules (Ako et al., 2003; Sardans & Peñuelas, 2015). To distinguish, potassium contained in organic residues is designated K, while potassium in nutrient solutions is designated K^+. The K release was independent of pH, and high concentrations of K^+ were measured already after one day in solution. Large portions of K from organic residues can be solved by simple leaching. However, AN digestion resulted in significantly higher K^+ concentrations. The analyses indicated that all K contained in the organic residues was mineralized within the experimental period of 31 days.

Based on these findings, mixing organic residues in a specific ratio with subsequent digestion was discarded since the chances of achieving a nutrient-balanced solution are low. A different approach was chosen to produce larger quantities of a bioponic nutrient solution (***B****NS*) for the hydroponic cultivation experiment.

For this new approach, organic residues were selected, rich in only one of the main nutrients. These residues were digested in separate reactors, with the respective optimum conditions determined in the preliminary experiment. By this, “bioponic stock solutions” rich in N, P, or K should be produced and mixed in the best ratio to create a nutrient-balanced solution.

4. Material and Methods

The first part (4.1) of the following chapter presents the used materials and methods of the mineralization experiment to produce nutrient solutions rich in either N, P, or K from organic residues. In the second part (4.2), the materials and methods of the plant cultivation experiment are presented, in which the prepared ***B****NS* was tested on lettuce compared to a mineral nutrient solution (***M****NS*).

4.1 Production of a Bioponic Nutrient Solution by Separate Mineralization of the Main Nutrients

Materials and methods used in the mineralization experiment for the production of a ***B****NS* are presented in the following.

4.1.1 Reference Crop

Lettuce (*Lactuca sativa*), the most cultivated plant in hydroponics (Jan et al., 2020), was selected as reference crop. Lettuce requires the same nutrient balance throughout the growing phase; this simplifies cultivation compared to fruit-bearing crops, such as tomatoes. Sapkota et al. (2019) stated the optimum NPK composition of a hydroponic nutrient solution for buttercrunch lettuce with 250 mg/l N_{total}, 56 mg/l P, and 300 mg/l K. These concentrations were the reference for the mass of organic residues used to achieve nutrient concentrations high enough for good plant growth. Within the scope of this master thesis, the focus was on the main nutrients, N, P, and K.

4.1.2 Organic Residues

After literature research, blood meal, bone meal, and potato peel (Table 6) were selected as N-, P-, and K-rich organic residues, respectively. Blood meal and bone meal, both derived from a mixture of different animals, were purchased from Beckmann & Brehm GmbH, 27243 Beckeln. Potato peel was supplied by the restaurant "Speisekammer West" in 70193 Stuttgart.

First, each organic residue's dry mass (DM) content was determined. Four samples of each residue were taken, weighed to determine the samples' FM, dried at 105 °C for 24 h, and weighed again to determine the DM. The average DM content in percent was calculated from these masses (Table 9).

The organic residues of interest were analyzed on their N, P, and K concentrations to obtain the exact values of these residues. Four repetitions of dried samples of the residues were used for each analysis method, dried as described above.

N concentration was analyzed using Kjeldahl method as developed by Kjeldahl (1883). As digestion apparatus, the K20-Behrotest, for distillation, the Gerhard Vapodest 45 s was used. P of the organic residues was extracted by microwave extraction adapted as described by Wu et al. (1997) using the ETHOS.lab (mws microwave laboratory systems) microwave. The microwave extract was analyzed for its PO_4^{3-}-P concentration using a Continuous-Flow Analysis (CFA) system from Alliance instruments. K was extracted using a hot water extraction as described by Matsushita and Matoh (1991). The extract was analyzed on its K^+ concentration using the Jenway™ PFP7 flame photometer. A more detailed explanation of the analysis methods is found in Chapter 4.3. The obtained N, P, and K concentrations (Table 9) refer to the organic residues' DM.

Table 9: Analyzed N, P, and K of used organic residues in % of the dry mass (DM) and DM in % of the fresh mass (FM).

Organic Residue	N	P	K	DM
	[% DM]			[% FM]
Blood meal	15.5 ± 0.0	0.2 ± 0.0	0.6 ± 0.1	92.1 ± 0.2
Bone meal	7.0 ± 0.0	20.1 ± 0.3	0.0 ± 0.0	95.2 ± 0.1
Potato peel	2.5 ± 0.1	0.3 ± 0.0	1.7 ± 0.1	16.5 ± 0.7

The FM of blood meal and bone meal was used in the mineralization experiment. The nutrient concentrations of the FM were calculated using the respective DM content and are presented in Table 10.

Table 10: Analyzed N, P, and K of blood and bone meal in % of the fresh mass (FM).

Organic Residue	N	P	K
		[% FM]	
Blood meal	14.28	0.18	0.55
Bone meal	6.66	19.14	0.00

All potato peels provided by the restaurant "Speisekammer West", on which the NPK analyses were based, were used for the preliminary experiment (Appendix I). For the main experiment, much higher amounts of potato peel were necessary than could be supplied by the restaurant. Therefore, a different supplier was chosen. The necessary amount was provided by Sautter Potato processing, 71149 Bondorf, as a mix of potato peel and pulp (potato mix), as it accrues during the mechanical peeling of potatoes. The potato mix was dried at 110 °C for 48 h and ground to a coarse powder (Figure 7) using the Retsch Grindomix GM 200 before being used as K source. This preparation reduced the necessary volume and should reduce mold formation.

Figure 7: Dried and ground potato peel and pulp.

Due to the different origin and composition of the potato mix, it differed in N, P, and K concentrations from the analyzed potato peel; thus, the concentrations in Table 9 were not applicable.

Based on the preliminary experiment results, it can be assumed that a high amount of K inherent in potato peels and other organic residues is released in solution after 24 h. Since the hot water extraction in the preliminary experiment showed somewhat inaccurate results (Appendix I), a slightly different approach was chosen this time. 5.7 g of dried and ground potato mix and 100 ml of distilled water were added into glass bottles and sealed. This was done in triplicates. The bottles were placed in the mt-multitron incubation shaker for 24 h at 300 rpm and 60 °C. After 24 h, one sample of each bottle was taken and analyzed on K^+ concentration using the Jenway PFP7 flame photometer. The K content of the dried potato mix was based on the concentration obtained. Since especially the K in the potato mix was of interest, only this and no N and P were analyzed.

4.1.3 Digestion Parameters of the Reactors

The selected organic residues were digested in separate reactors to obtain solutions rich in either N, P, or K. For each reactor, the parameters found to be most suitable for mineralization of N, P, or K based on literature research and preliminary experiment were selected. Bone meal and potato mix were digested anaerobically in the "*P-Reactor*" and "*K-Reactor*", respectively. Blood meal was anaerobically digested first to accumulate NH_4^+ ("*NH_4^+-Reactor*"); afterward, the NH_4^+-rich solution was transferred to an aerobic reactor to allow transformation into NO_3^- ("*NO_3^--Reactor*") by nitrification. pH control was carried out in the *P*-, *NH_4^+*-, and *NO_3^--Reactor*. Due to the large volumes, four 60 l barrels were used as reactors, no repetitions

were made. Figure 8 gives a graphical overview of the reactors and the procedure. The following section (4.1.4) presents the setup and operation of each reactor in detail.

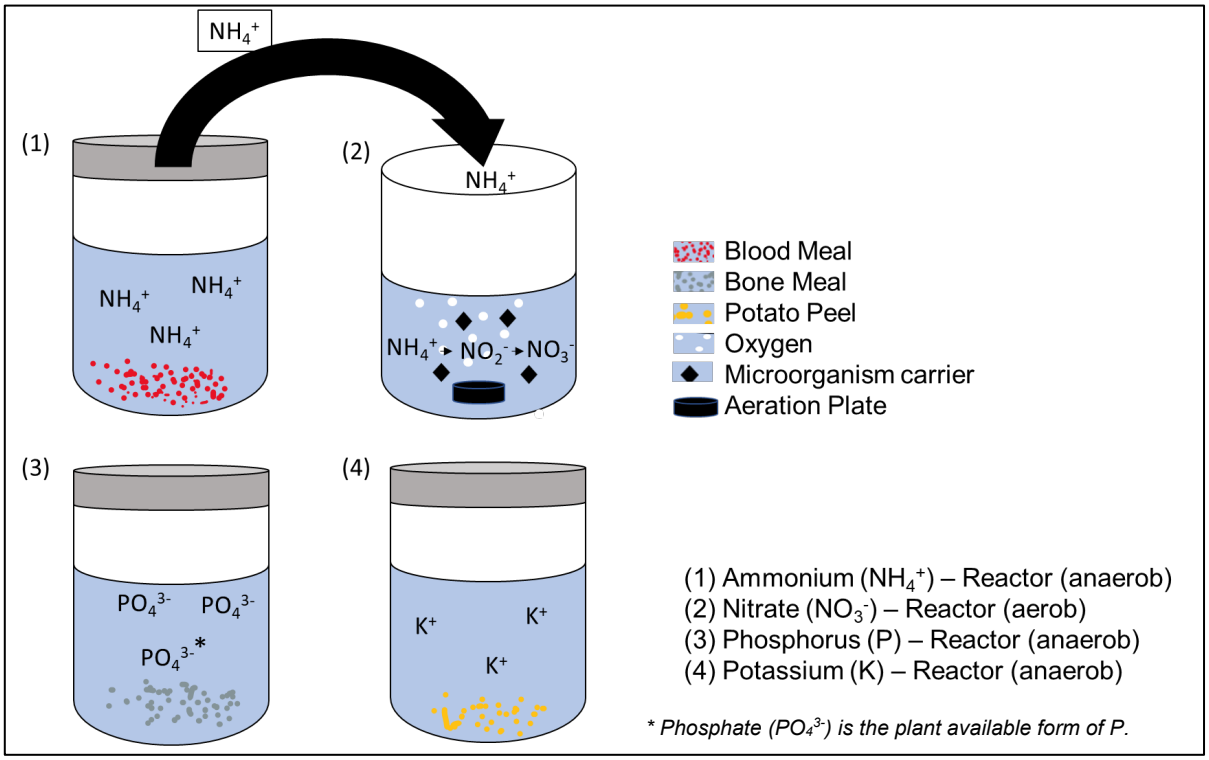


Figure 8: Graphical illustration of the four reactors and the process of ammonium-rich solution transfer into the aerated nitrate reactor for nitrification.

4.1.4 Reactors and Experimental Procedure

The experiment was conducted at the Fraunhofer IGB Stuttgart in a large, unheated hall integrated into a building complex. The reactors were placed in two separate acrylic glass enclosures, each equipped with a fume hood. The experimental period of the mineralization experiment lasted from 14/04/2022, day of experiment (DOE) 0, to 15/08/2022 (DOE 123).

4.1.4.1 NH_4^+ -Reactor

Purpose: Production of an NH_4^+-rich solution as the feedstock for nitrification in the *NO_3^--Reactor*. The digestate from the *NH_4^+-Reactor* should not be used as nutrient solution in the hydroponic cultivation experiment, only the solution of the *NO_3^--Reactor*.

Organic source: Blood meal

Reactor set-up: Two time-shifted *NH_4^+-Reactors* were used. (*NH_4^+-Reactor I, NH_4^+-Reactor II).* As *NH_4^+-Reactor*, a 60 l barrel filled with 45 l desalinated water was used.

NH_4^+-Reactor I was started on 14/04/22 (DOE 0). 370 g FM blood meal and 225 g of not fully composted compost (Figure 9) as a source of heterotrophic MOs for the anaerobic digestion

were added in four 145-micron Baven mash bags, weighted with disinfected stones. The compost was obtained from the compost plant Kirchheim in 73230 Kirchheim unter Teck.

Figure 9: Ammonium-Reactor and Compost. Ammonium-Reactor after addition of blood meal on day of experiment zero (left). Compost used as microorganism source (right).

The added blood meal contained 52836 mg N, the added compost 3465 mg N, according to the information supplied by the compost plant (RAL-Gütesicherung Kompost, 2022). Thus, a maximum N concentration of 1251 mg/l could be achieved for the used 45 l of water. This corresponds to approximately five times the N concentration that Sapkota et al. (2019) recommended for hydroponic solutions.

A 300 W aquaristics heating rod from NICREW was added to the reactor to increase the temperature to 30 °C, the maximum achievable by this method. The reactor was closed with a lid, which was equipped with a self-made gas wash bottle to allow forming gas to escape (Figure 10). If the pH exceeded 7, it was manually lowered to 6.5 to reduce NH_3 losses by adding acetic acid. Every third day 67.5 g of glucose was added to supply 0.5 g of glucose per liter and day as readily available feed for heterotrophic MOs performing anaerobic digestion.

Figure 10: Gas-wash bottle installed to create anaerobic conditions.

Since the NH_4^+-rich solution of the *NH_4^+-Reactor* was gradually transferred into the *NO_3^--Reactor,* the solution volume of the *NH_4^+-Reactor* decreased. The experimental period of *NH_4^+-Reactor I* ended on 22/06/2022 (DOE 69). The same day the second *NH_4^+-Reactor* (*NH_4^+-Reactor II*) was started. The barrel and mash bags of the first run (*NH_4^+-Reactor I)* were used again. No new compost was added; otherwise, the same procedure was followed as for the first run. The only expectation was that if the pH exceeded 7, it was more drastically lowered to 6.0. As for *NH_4^+-Reactor I,* 52836 mg FM blood meal was added to 45 l of water. A maximum N concentration of 1174 mg/l could be achieved.

4.1.4.2 NO_3^--Reactor

Purpose: Create a reactor with optimum conditions for nitrification, where the NH_4^+ of the *NH_4^+-Reactor* solution is transformed into NO_3^-.

Organic source: As feedstock, the NH_4^+-rich solution of the *NH_4^+-Reactor* derived from blood meal served.

Reactor set-up: The *NO_3^--Reactor* was designed according to the operation of an MBBR as invented by Ødegaard et al. (1994). Two temporal shifted *NO_3^--Reactor* runs were conducted (*NO_3^--Reactor I, NO_3^--Reactor II). NO_3^--Reactor I* was started on 14/04/2022 (DOE 0). 20 l of desalinated water, 140 g (7 g/l) of not fully composted compost (Figure 9) from the compost plant Kirchheim as a source of nitrifying MOs, 2 g/l more than recommended by Shinohara et al. (2011), and 1400 g MO-carriers “Kaldes K1” (Figure 11) were added to a 60 l barrel. For aeration, four 13 cm Ø Pondlife aeration plates were evenly placed on the bottom of the barrel and connected to a compressed air supply. Thereby, a DO concentration minimum of 3.5 mg/l should be provided, monitored using the Hach Lange LDO HQ-10 O_2 oxygen meter. Stefanakis

et al. (2014) stated that the optimum DO concentration for nitrification is between 3 and 4 mg/l. A 100 W NICREW heating rod was added to increase the temperature to 30 °C.

Before addition to *NO_3^--Reactor I*, the MO-carriers were inoculated in a separate barrel in 20 l of aerated activated sludge (Figure 11) from the Institute of Sanitary Engineering, Water Quality, and Waste Management at the University of Stuttgart for two weeks (start phase). Also, in the start phase the temperature was adjusted to 30 °C using the 100 W NICREW heating rod. Within the start phase, a nitrifying bacteria community should establish on the MO-carrier surfaces.

Every third day 30 g glucose (0.5 g/l and day) was added to the *NO_3^--Reactor I* to feed the MOs as it is done in wastewater treatment research to cultivate MOs (Sun et al., 2019). The same daily mass of glucose was already supplied in the start phase. To compensate for evaporation losses, caused by aeration, the *NO_3^--Reactor I* volume was regularly readjusted to 20 l by adding desalinated water. To keep the pH in a favorable range for nitrification and prevent high NH_3 losses by outgassing the Endress+Hauser CLM253-ID0010 conductivity transmitter LIQUISYS-M lowered the pH to 7.3 by adding acetic acid if the pH exceeded 7.5.

Figure 11: Microorganism carriers: During the start phase for inoculation (left). Inoculated Microorganism carriers (right), as transferred into the NO_3^--Reactor.

On 05/05/2022 (DOE 21), the stepwise transfer of 1 l of NH_4^+-rich solution from *NH_4^+-Reactor I* to *NO_3^--Reactor I* was started. Henceforth, 1 l of NH_4^+-rich solution was transferred to *NO_3^--Reactor I* every day until 22/06/2022 (DOE 69), except on weekends. A total of 35 l of the NH_4^+-rich solution was added to *NO_3^--Reactor I*. On DOE 69, *NO_3^--Reactor I* was stopped.

Figure 12: Nitrate-Reactor. Visible aeration hoses connected to aeration plates.

The second *NO_3^--Reactor* (*NO_3^--Reactor II*) was started on 07/07/2022 (DOE 84) by mixing 10 l of desalinated water, 1400 g of fresh, non-inoculated MO-carriers, and 10 l of sewage sludge in an empty 60 l barrel. Thus, a slightly different approach was used as in the first run, where the MO carriers were inoculated in a separate barrel, and only the inoculated MO-carriers, not the sewage sludge, were used. pH, temperature, and aeration were identical as in the first run; only the mass of glucose was doubled to 60 g every third day, to 1 g/l and day. After three weeks, on 28/07/2022 (DOE 105), the transfer process of the NH_4^+-rich solution from *NH_4^+-Reactor II* was started. As in the first run, 1 l per day was transferred. The second run lasted until 15/08/2022 (DOE 123). In total, 16 l of the NH_4^+-rich solution were transferred.

4.1.4.3 P-Reactor

Purpose: Production of a nutrient solution rich in PO_4^{3-}.

Organic source: Bone meal

Reactor set-up: The mineralization period of the *P-Reactor* lasted from 14/04/2022 (DOE 0) to 15/08/2022 (DOE 123). A 60 l barrel was filled with 45 l of desalinated water and 195 g FM bone meal, filled in three 145-micron Baven mash bags. A maximum concentration of 829 mg/l PO_4^{3-}-P could be reached regarding the concentration of the used bone meal, about 15 times the recommended concentration by Sapkota et al. (2019). The high concentration was chosen since only low mineralization rates of P inherent in organic residues into PO_4^{3-}-P were achieved in the preliminary experiment (Appendix I). As for the *NH_4^+-Reactor*, a 300 W NICREW heating rod increased the temperature to 30 °C.

Figure 13: P-Reactor on day of experiment zero. After the addition of bone meal in mash bags.

The pH was manually lowered to 4.5 when it exceeded a value of 5.5 by adding acetic acid as needed. The main component of bones, hydroxyapatite $Ca_5(PO_4)_3(OH)$, is only dissolved at pH values below 5.5 (Epple & Enax, 2018).

Every third day the solution of the *P-Reactor* was stirred for about one minute. The barrel was closed with a lid, and a gas wash bottle, as for the *NH_4^+-Reactor* (Figure 10), was installed to create anaerobic conditions.

4.1.4.4 K-Reactor

Purpose: Production of a nutrient solution with high K^+ concentration.

Organic source: Dried potato mix from mechanical peeling of potatoes.

Reactor set-up: The mineralization period of the *K-Reactor* lasted from 14/04/2022 (DOE 0) to 15/08/2022 (DOE 123). Again a 60 l barrel was used as reactor. 30 l desalinated water was added on DOE 0. 3642 g dried and ground potato mix was added, spread over three points in time. 1992 g on DOE 0 (Figure 14), 910 g on DOE 24 and 740 g on DOE 84. Not all dried potato mix was added at once due to the considerable time requirements of the drying process. An anaerobic environment was created the same way as in the *P-* and *NH_4^+-Reactors*. No pH control took place.

In the following, the bioponic solution obtained from the *NH_4^+-Reactor* is called "*NH_4^+-solution*", from the *NO_3^--Reactor* "*NO_3^--solution*", from the *P-Reactor* "*P-solution*", and from the *K-Reactor* "*K-solution*". An overview of the time course of the mineralization experiment with the different reactors can be found in Figure 18, p.43.

Figure 14: K-Reactor on day of experiment zero. After adding 1992 g of dried and ground potato peel and pulp.

4.1.5 Storage of the Produced Bioponic Solutions

On DOE 123, the last day of the mineralization experiment, the organic residues were removed from the *NO_3^--Reactor II*, and the *P-* and *K-Reactors*. The produced solutions were centrifuged at 7500 RPM for ten minutes, using the Beckman-coulter avanti j-26 xp, and filtered using 185 mm pleated filters. By these steps, further mineralization should be stopped or decelerated, and solid particles should be removed. The *NO_3^--, P-*, and *K-solutions* were stored in clean barrels in the acrylic glass enclosure until used in the hydroponic experiment. The *NO_3^--solution* was further aerated to prevent denitrification, the barrels storing the *P-*, and *K-solutions* were sealed. It was decided not to store the solutions refrigerated, as this would be difficult in the conditions prevailing in arid regions.

4.1.6 Sampling and Measurements

All sampling and analysis were performed in triplicates. For the first twelve days of each run, samples were taken from the *NH_4^+-Reactor I, NH_4^+-Reactor II,* the *P-* and *K-Reactor* every three days. After DOE 12, samples were taken every 12th day. For the two runs of the *NO_3^--Reactor*, samples were taken every sixth day. If the last day of a reactor did not fall within these time intervals, which was the case for all reactors except the two *NO_3^--Reactors*, an irregular sample was taken on the last day of the reactor.

The samples were analyzed for the nutrients in question (NH_4^+-N in the *NH_4^+-Reactors*, PO_4^{3-}-P in the *P-Reactor*, and K^+ in the *K-Reactor*) using Hach-Lange LCK cuvette tests (Section 4.3.5). *NO_3^--Reactor I* samples were analyzed only for NO_3^-, whereas *NO_3^--Reactor II* samples were additionally analyzed for NH_4^+ and NO_2^-. Also, the *NO_3^--Reactor* samples were analyzed using Hach-Lange LCK cuvette tests.

In the final analysis on DOE 123, each solution produced by the different reactors was analyzed for NH_4^+, NO_3^- and PO_4^{3-} using the CFA system from Alliance Instruments and for K^+ and Na^+ using the Jenway™ PFP7 flame photometer. Furthermore, DOE 123 samples of each solution were sent to the Core Facility Hohenheim in 70599 Stuttgart to analyze the micronutrients mangan, copper, zinc, molybdenum, and boron and the macronutrients magnesium, sulfate, and iron. The Core Facility Hohenheim used the analytical method of inductively coupled plasma optical emission spectroscopy (ICP-OES).

Throughout the experimental period, pH, using the wtw pH/ion 340i, EC with the Welikera EC measurement device, and temperature were measured every third day in each reactor. DO (Hach Lange LDO HQ-10 O_2 oxygen meter) was measured in the *NO_3^--Reactors* every third day, whereas in the anaerobic reactors, DO was measured only every sixth day. Measurements were made before pH was adjusted, if necessary, in the pH-controlled reactors.

4.1.7 Compositions of the Nutrient Solutions Used in the Hydroponic Experiment

The ***B****NS* was mixed from the solutions prepared in the reactors based on the final NH_4^+-N, NO_3^--N, PO_4^{3-}-P, and K^+ concentrations. The mean values of mineral nutrient solutions listed in Table 4 served as reference for the mixing ratio. Beside the ***B****NS,* a spiked bioponic nutrient solution (***SB****NS*) was mixed, spiked with $CaNO_3$. A modified Hoagland mineral nutrient solution (***M****NS*) was used as control. The ***B****NS* and ***SB****NS* mixing ratios are already presented in this chapter for overview purposes. Additionally, they are presented in the results (Chapter 5.1.6). As a control, the ***M****NS* by Taiz and Zeiger presented in Table 3 with some adaptations was used. NaFeDTPA was replaced with FeNaEDTA, and the concentrations of the chemicals were adjusted so that only one or two ml/l of stock solutions were required (Table 11).

Table 11: Adapted modified Hoagland mineral nutrient solution by Taiz and Zeiger (2006). Adapted by replacing NaFeDTPA with FeNaEDTA and changing the concentrations of the chemicals.

Modified Hoagland Culture Solution with FeEDTA adapted from Taiz Zeiger				
Label	Element	Chemical	Stock [g/L]	Stock / final [mL/L]
A	N, K	KNO_3	303.30	2
B	Ca	$Ca(NO_3)_2 * 4H_2O$	944.64	1
C	P	$NH_4H_2PO_4$	230.16	1
D	Mg, S	$MgSO_4 * 7H_2O$	246.49	1
E	Cl	KCl	3.73	1
	B	H_3BO_3	1.55	
	Mn	$MnSO_4 * H_2O$	0.34	
	Zn	$ZnSO_4 * 7H_2O$	0.58	
	Cu	$CuSO_4 * 5 H_2O$	0.12	
	Mo	H_2MoO_4	0.08	
F	Fe	FeNa - EDTA	9.27	1

The produced *NO_3^--*, *P-*, and *K-solutions* were mixed in the ratio presented in Table 12 to create one liter ***B****NS*.

Table 12: Mixing ratio for one liter bioponic nutrient solution (BNS).

Mixing Ratio BNS		
NO_3^--solution	621.30	ml
P-solution	297.00	ml
K-solution	81.70	ml

The bioponic *P-* and *K-solutions* were used for the ***SB****NS*, but no bioponic *NO_3^--solution* (Table 13). Instead, label B stock solution prepared for the ***M****NS* containing NO_3^- and Ca was used. 1.75 ml/l of B stock solution was used, adding a NO_3^--N concentration of 196.0 mg/l, the same NO_3^--N concentration as the ***M****NS* control.

Table 13: Mixing ratio for one liter spiked bioponic nutrient solution (SBNS). Spiked with NO_3^- using the B stock solution prepared for the adapted modified Hoagland solution.

Mixing Ratio SBNS		
B stock solution	1.75	ml
P-solution	297.00	ml
K-solution	93.50	ml
Desalinated water	607.75	ml

4.2 Test of the Bioponic Nutrient Solution on Lettuce in a Deep Water Culture System

This chapter presents the materials and methods of the “hydroponic plant cultivation experiment”. The ***B****NS* and ***SB****NS* were tested against the ***M****NS* for lettuce var. *Hawking* in a hydroponic deep water culture system for 25 days. All nutrient solutions were mixed according to the ratios in Table 11, Table 12, and Table 13. A new timeline was started for the “hydroponic plant cultivation experiment” in distinction to the “mineralization experiment”, starting with the sowing of the lettuce seeds on DOE 0 (Timeline Figure 18, p.43).

4.2.1 Deep Water Culture System

The hydroponic system used was a simple deep-water culture. The system consisted of 15 white 1 l plastic buckets, sealed with a detachable lid. The buckets were wrapped in aluminum

foil to reduce light incidence and by this algae growth. A hole was drilled in the center of the lid, and a polyurethane sponge (Figure 15) holding the plant was inserted into it (Figure 16). The buckets were filled with nutrient solution and aerated via flexible hoses connected to an air pump for 45 min/h. Three air distributors in series connection were used to allow the aeration of all 15 buckets. A pipette tip was attached at the end of each hose to prevent clogging. The system was set up in a climate chamber (CLF PlantClimatics E-75L1) at the University of Hohenheim. During the cultivation trial, a diurnal modus was chosen in the climate chamber, with 12 h light, 22 °C, relative humidity (RH) of 55 %, and night temperatures of 20 °C and RH of 65 %. Temperature and RH were recorded every 15 minutes using a tinytag tv-4505.

4.2.2 Lettuce Propagation

The lettuce seeds of the variety Salanova Hawking were obtained from Rijk Zwaan Welver GmbH, Vegetable Breeding & Seeds, 5914 Welver. The seeds were sown in moist quartz sand on 11/07/2022 (DOE 0). For propagation, the seedlings were kept in the regularly flooded and drained sand (Figure 15) until 02/08/22 (DOE 22), as 15 plants were transplanted into the hydroponic system. To ensure that all plants tolerated the hydroponic cultivation and transplantation and were at the same developmental stage, they were cultivated in ***M****NS* for the first 3.5 weeks (initial phase). The plants were subdivided into pre-mineral-, pre-bioponic, and pre-spiked-bioponic groups in the initial phase. After the 3.5 weeks, they were fertilized with ***M****NS*, ***B****NS*, or ***SB****NS*, respectively (comparison phase).

For the first seven days of the initial phase, all plants were supplied with 25 % ***M****NS*, the following seven days with 75 %, and finally, for ten days with 100 % ***M****NS* until 25/08/2022 (DOE 46). In the initial phase, the ***M****NS* was renewed every seven days. From the exchanged solutions, 15 ml samples were taken from each container and stored at 4 °C for further analysis.

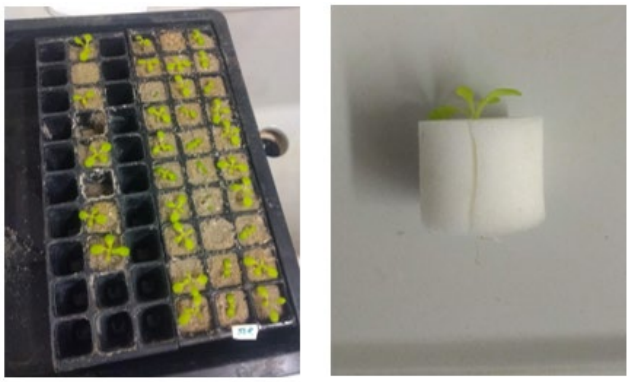


Figure 15: Lettuce seedlings and polyurethane sponge. Lettuce seedlings in quartz sand before transplantation into the hydroponic system on day of experiement 22 (left). Polyurethane sponge (right), holding the plant in the hydroponic system.

Figure 16: Deep-water culture containers. Left: The five deep-water culture containers during the pre-mineral phase on DOE 22, where the mineral control solution was further used after DOE 46. Right: Deep-water culture containers in the pre-bioponic phase on DOE 22, the bioponic solution was used from DOE 46.

4.2.3 Preparation of Nutrient Solutions

Before the bioponic solutions, which were stored in barrels in an acrylic glass enclosure, were used to mix the final bioponic nutrient solution, the pH in each solution was measured. It was adjusted to the pH during the mineralization experiment of the respective solution if it had changed by more than one unit. Every time the nutrient solutions in the deep-water culture containers were replaced, they were prepared as described in Section 4.1.7 by mixing them afresh. The three different nutrient solutions were mixed in separated barrels, the pH was
adjusted to 6.0 using acetic acid or potassium hydroxide and added to the containers.

Figure 17: Bioponic nutrient solution in a deep-water culture container.

4.2.4 Experimental Procedure

On Thursday, 25/08/2022 (DOE 46), the containers were filled with 1 l of either 100 % mineral, bioponic- or spiked bioponic-pH-adjusted nutrient solution. Each nutrient solution was tested in five containers. The nutrient solution was renewed every Monday and Thursday. This resulted in intervals of three and four days, respectively, during which the nutrient solution remained in the containers. The containers were rinsed with desalinated water before new nutrient solution was added.

Every week (DOE 53, 60, 67), 15 ml samples were taken from the newly added ***B****NS* and ***SB****NS* for nutrient analysis and stored at 4 °C. The starting concentration (DOE 46) was assumed according to the mixing ratios. The table below provides an overview of the addition of new and the sampling of remaining nutrient solutions (Table 14). For the added solutions, pH was measured in the barrels the solutions were mixed and, if required, pH was adjusted to 6.0. Afterward, the EC of each solution was measured. The solution mass added to each container was determined to calculate evapotranspiration.

Table 14: Sampling and addition of nutrient solution during the hydroponic experiment. Initial phase: All lettuce plants fertilized with mineral solution. Comparison phase: Plants fertilized with either mineral, bioponic (BNS), or spiked bioponic (SBNS) nutrient solution.

Phase	Day of Experiment	Addition of new nutrient solution	Sampling of new BNS and SBNS	Sampling of remaining nutrient solution
Initial phase	22	×		
	29	×		×
	36	×		×
	43	×		×
Comparison phase	46	×		×
	50	×		×
	53	×	×	×
	57	×		×
	60	×	×	×
	64	×		×
	67	×	×	×
	71			×

The exchanged solution's pH, EC, and mass were measured, and a 15 ml sample was taken for further analysis of each of the 15 containers. The samples were stored at 4 °C. Furthermore, every time the nutrient solution was replaced, the FM of the plants consisting of shoot and roots, was determined by subtracting the container, lid, hose, and moist sponge mass from the total mass.

The comparison phase lasted 25 days until 19/09/2022 (DOE 71). The plants were harvested, and the shoots and roots' FM was determined. Afterward, roots and shoots were dried at 60 °C for 48 h and weighed again to determine the DM. The dried shoot samples were ground and sent to the Core Facility Hohenheim for analysis on N, P, K, Ca, Mg, S, B, Zn, Mn, Fe, and Cu using ICP-OES.

Of the remaining bioponic *NO_3^--*, *P-*, and *K-solutions* samples were taken in triplicates on the day the cultivation ended and were analyzed with the samples taken during the cultivation trial for NO_3^-, NH_4^+, PO_4^{3-}, and K^+ using the CFA system and the Jenway PFP7 flame photometer. The analysis of the remaining bioponic *NO_3^--*, *P-*, and *K-solutions* is called *post-experiment reactor analysis*.

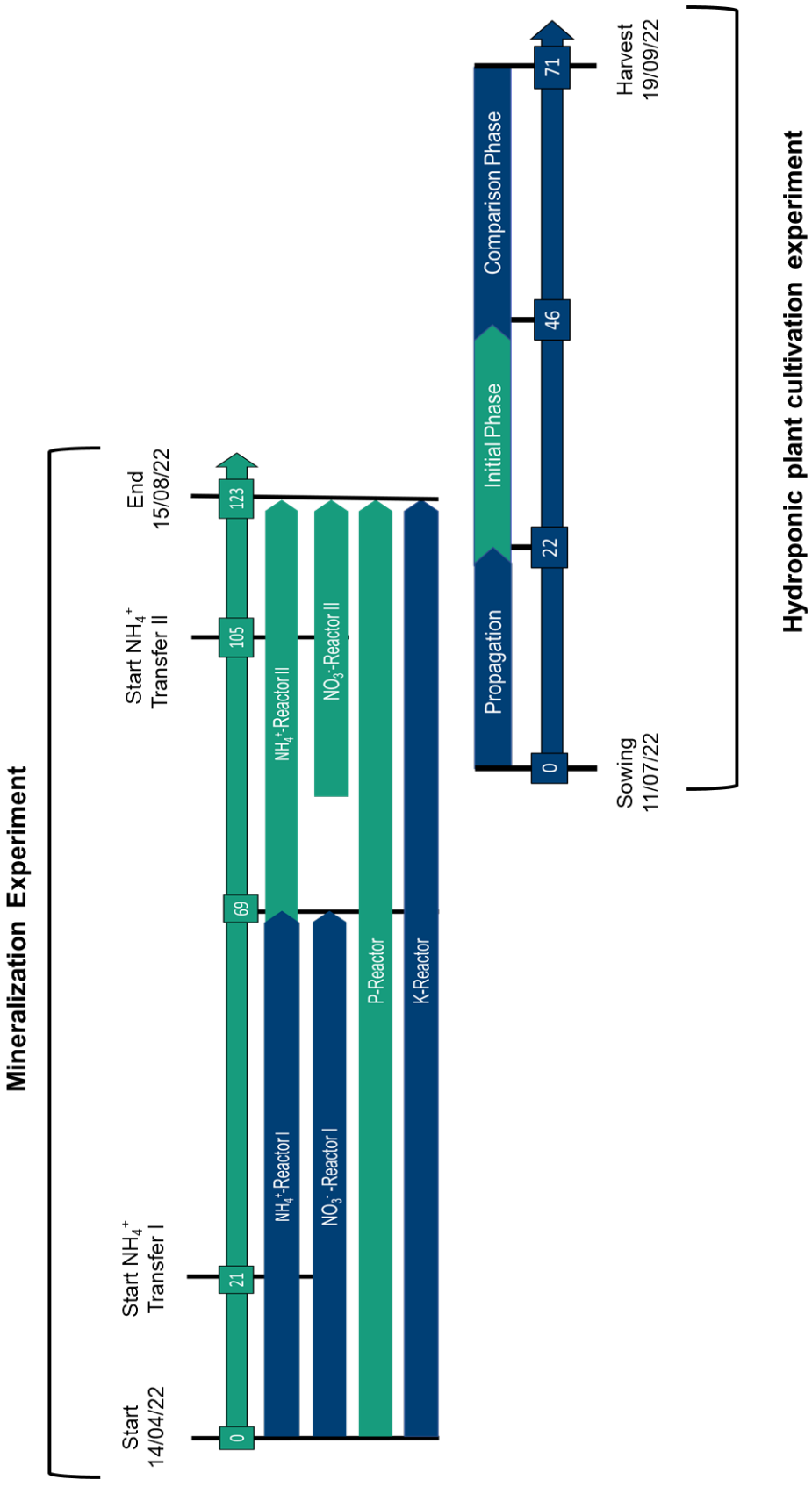


Figure 18: Graphical overview of the time course of mineralization and hydroponic experiment. The respective start and end days and the start of the NH_4^+-rich solution transfer into NO_3^--Reactor I (Start NH_4^+ Transfer I) and II (Start NH_4^+ Transfer II) are marked.

4.3 Extraction and Analysis Methods

This section presents the used analysis and extraction methods in more detail. Except for the Hach-Lange LCK Tests, conducted at the IGB Fraunhofer Stuttgart, all following extraction and analysis methods were conducted at the University of Hohenheim.

For the Kjeldahl method, microwave extraction and hot water extraction dried and ground samples were used. The samples were ground using the laboratory mill IKA A 10.

4.3.1 Kjeldahl Method

The Kjeldahl method (Kjeldahl, 1883) was used to determine the N inherent in the organic residues. 0.2 – 0.3 g of the sample (DM) was weighed on N-free paper and added into a digestion glass. KJELCAT Cu as catalysator and H_2SO_4 were added and were digested in the K-20 Behrotest for about 120 minutes. The digestate was diluted with deionized water. By the Vapodest 45 s, NaOH was added to release NH_3. NH_3 was separated by steam distillation and absorbed in $H_2BO_3^-$. Subsequently, N was quantitively determined by titration with 0.1 M H_2SO_4 and pH measurement, and the N content was calculated.

4.3.2 Microwave Extraction

To extract the P inherent in the organic residues, microwave digestion was conducted, adapted from Wu et al. (1997). About 0.2 g of the sample (DM) was weighed into a microwave extraction tube, and 2.5 ml HNO_3 and 2 ml H_2O_2 were added. After a soaking period of 60 minutes, the tube was inserted into a microwave digestion cartridge and was digested using the ETHOS.lab (mws Microwave Laboratory Systems) microwave. After digestion, the solution was filled up to 20 ml and filtered. Before analyzing PO_4^{3-}-P using the CFA system from Alliance instruments, the solution was diluted 1:5.

4.3.3 Hot Water Extraction

To extract the K from the respective organic residue, approximately 0.15 g of the dried and ground sample was added to 100 ml deionized water and was extracted in a 90 °C water bath for 60 minutes. After cooling down, the extract was filtered and analyzed on K^+ using a standard row with the Jenway™ PFP7 flame photometer.

4.3.4 Continuous Flow Analysis

For the analysis of the microwave extract for P as PO_4^{3-}, the final analysis of the produced bioponic stock solutions and the samples taken during the deep-water culture experiment for

NH_4^+, NO_3^-, and PO_4^{3-}, the CFA from Alliance instruments was used. The basic principles and solutions used are briefly presented in the following.

NH_4^+ was determined according to the standards ISO 11732 (1997) and DIN 38406-E23-2 (1993). The chemical compositions of the solutions used for NH_4^+ analysis are listed in Table 15. The principle is that citrate and tartrate avoid precipitation by forming complexes in the alkaline solution. The NH_4^+-ion reacts with salicylate and chlorite; by this, a blue indolphenol tint emerges that is measured photometrically at 650 nm.

Table 15: Chemical composition of the solutions used for the analysis of NH_4^+ in the continuous flow analysis system.

Solution name		Composition
Buffer	20 g	Sodium citrate $C_6H_5Na_3O_7 \times 2H_2O$
	7.5 g	Sodium Potassium Tartrate $NaKC_4H_4O_6 \times 4H_2O$
		filled up to 1000 ml with deionized water
Salic	34 g	Sodiumsalicylat $NaC_7H_5O_3$
	0.4 g	Nitroprusside- Sodium $Na_2Fe(CH)_5NO \times 2H_2O$
		Nitroprusside-Sodium dissolved in 500 ml deion. Water,
		addition of Sodiumsalicylat and filled up to 1000 ml
Hypochl	25 ml	Sodiumhypochlorite 13 % NaOCl
	10 g	NaOH
		NaOH dissolved in 50 ml deion. water, NaOCl addition,
		filled up to 1000 ml

NO_3^- was determined according to the norm ISO 13395 (1996). The hydrazine sulfate contained in the solution for NO_3^- determination (Table 16) reduces a NO_3^--ion to NO_2^- using Cu-II-Sulfate in alkaline conditions. The NO_2^--ion reacts with Sulfanilic acid and N-1-Naphthylethylenediamine di-hydrochloride in acidic conditions and forms a red diazo-dye. The extinction is measured at 520 nm.

Table 16: Chemical composition of the solutions used for the analysis of NO_3^- in the continuous flow analysis system.

Solution name		Composition
NaOHCIT	6 g	NaOH
	5 g	Sodium citrate $C_6H_5Na_3O_7 \times 2H_2O$
		before use addition of 1 ml/l Triton X 100
$NaOHPO_4$	8 g	NaOH
	1 g	Potassiumdihydrogen- phosphate KH_2PO_4
		filled up to 1000 ml with deion. water
		before use 1 ml/l Triton X 100 addition
Stock A	1.2 g	Copper (II) sulfate $CuSO_4 \times 5\ H_2O$
		filled up to 100 ml with deionized water
HYDRAZ	1.5 g	Hydrazine sulfate $H_6N_2O_4S$
	1 ml	Stock A
		Hydrazine sulfate dissolved in hot deion. Water, Stock A addition
		filled up to 500 ml with deion. water
COLOR	150 ml	concentrated phosphoric acid
	1 g	N-1-Naphthylethylenediamine di-hydrochloride (NED)
	10 g	Sulfanilamide
		Phosphoric acid dilued in 300 ml deion. water
		addition of other ingredients and filled up to 1000 ml with deion. water

PO_4^{3-} was analyzed according to the norm ISO DIN EN ISO 15681-2 (2001). PO_4^{3-} reacts with molybdate in the analysis solution (Table 17) in acid environments. Molybdenum blue is formed and measured at an extinction of 660 nm.

Table 17: Chemical composition of the solutions used for the analysis of PO_4^{3-} in the continuous flow analysis system.

Solution name		Composition
SDS	4 ml	20 % Sodium dodecyl sulfate filled up to 1000 ml with deion. water
MOLYBD	40 ml	concentrated sulfuric acid H_2SO_4
	4.8 g	Ammoniumheptamolybdat Tetrahydrat $(NH_4)_6MO_7O_{24}$ x 4 H_2O
		Dilution of sulfuric acid in 700 ml deion. water, additon of ammoniumheptamolybdat and filled-up to 1000 ml
ASCORB	18 g	Ascorbic acid $C_6H_8O_6$
	100 ml	Acetone
		Ascorbic acid dissolved in 700 ml deion. Water, addition of acetone filled up to 1000 ml with deion. water

4.3.5 Hach-Lange LCK Tests

The spectrophotometer DR3900 Hach Lange and the respective test kits (Table 18) were used to analyze nutrient concentrations during the mineralization experiment.

Table 18: Hach Lange LCK Tests used for the analysis of the respective nutrient.

Nutrient	LCK test kit
NH_4^+-N	LCK303
NO_3^--N	LCK340
NO_2^--N	LCK343
PO_4^{3-}-P	LCK350
K^+	LCK228

4.3.6 Jenway PFP7 Flame Photometer

The functioning of a flame photometer is based on the thermal dissociation of alkali and alkaline earth metals into their compounds by a flame. Some of the produced atoms are brought to a higher energy level. If they return to the ground state, they emit radiation. The wavelength depends on the element. The emission wavelength of K^+ is at 766 nm, of Na^+ 589 nm.

4.4 Statistics and Software

Graphs were created using SigmaPlot 12.5 2011 Systat Software Inc. Also, for the linear regression conducted for EC vs. the nutrient concentrations SigmaPlot was used; by this, it should be figured out whether EC is a useful measure for the respective nutrient concentration. Tables were created with Microsoft Excel 365 MSO (Version 2304). Excel was also used for the calculation of standard deviations. All error bars shown in the present study are standard deviations. Microsoft Powerpoint 365 MSO (Version 2304) was used for graphical illustrations. A three-way ANOVA was conducted with Greenhouse-Geiser correction with subsequent post hoc Tuckey analyses for the plant masses produced in the plant cultivation experiment using R-Studio 2022.12.0 Build 353, Posit Software, PBC. A significance level of p=0.05 was chosen.

5. Results

The results of this study are presented in two parts, first, the results of the mineralization experiment (5.1), then the results of the hydroponic cultivation experiment (5.2).

5.1 Mineralization Experiment to Produce a Bioponic Nutrient Solution

This section presents the outcomes of the mineralization experiment to produce bioponic nutrient solutions rich in either N, P, or K, which were subsequently mixed. Each reactor has its own section where temperature, DO, and pH measurements are presented first, followed by the specific nutrient and EC concentrations.

5.1.1 NH_4^+-Reactors

The two *NH_4^+-Reactors* differed in length of time until they reached the intended temperature of 30 °C. It took 30 days until *NH_4^+-Reactor I* reached 30 °C, while *NH_4^+-Reactor II* reached the same temperature within 12 days (Figure 19). Only minor fluctuations in temperature were measured after the threshold was reached. Throughout the experimental period, DO remained low in both *NH_4^+-Reactors*. Only the first measurement of each reactor on DOE 0 exceeded 0.1 mg/l (Figure 19).

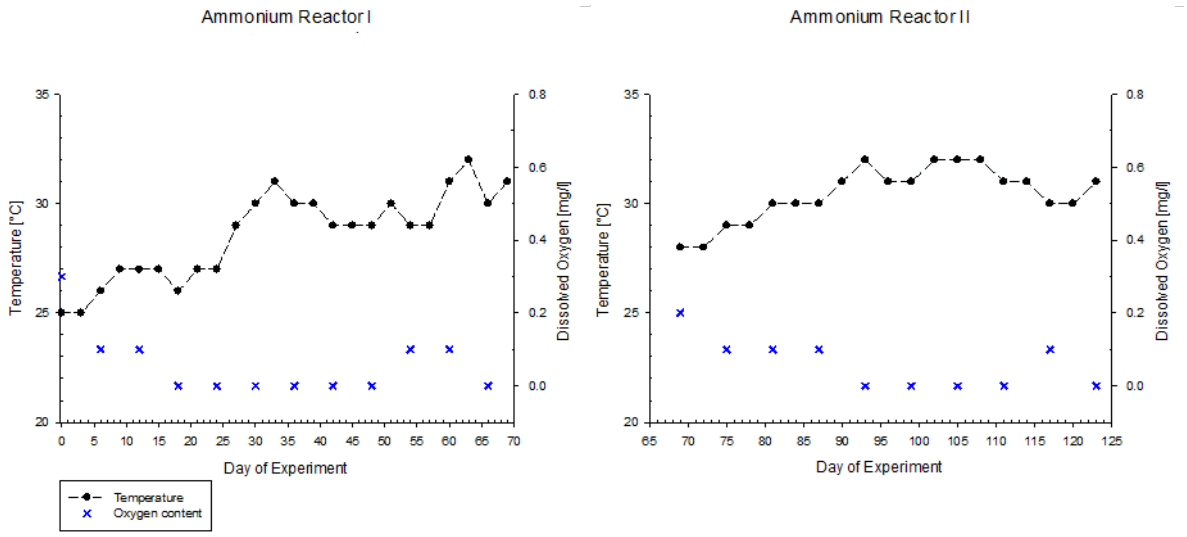


Figure 19: Ammonium-Reactors: Temperature and dissolved oxygen concentration development of the two time-shifted ammonium reactors. Ammonium Reactor I (left) and Ammonium Reactor II (right).

For both reactors, the pH increased over time (Figure 20). The increase was more substantial for *Reactor I*; despite the manual pH adjustment, the values exceeded 7.0 from DOE 24 onwards. Especially notable was the increase from DOE 21 to 33. Overall, *Reactor II* showed lower pH values. Fewer values exceeded 7, and if so, only marginally. The highest measured pH in *Reactor II* was 7.25 on DOE 120.

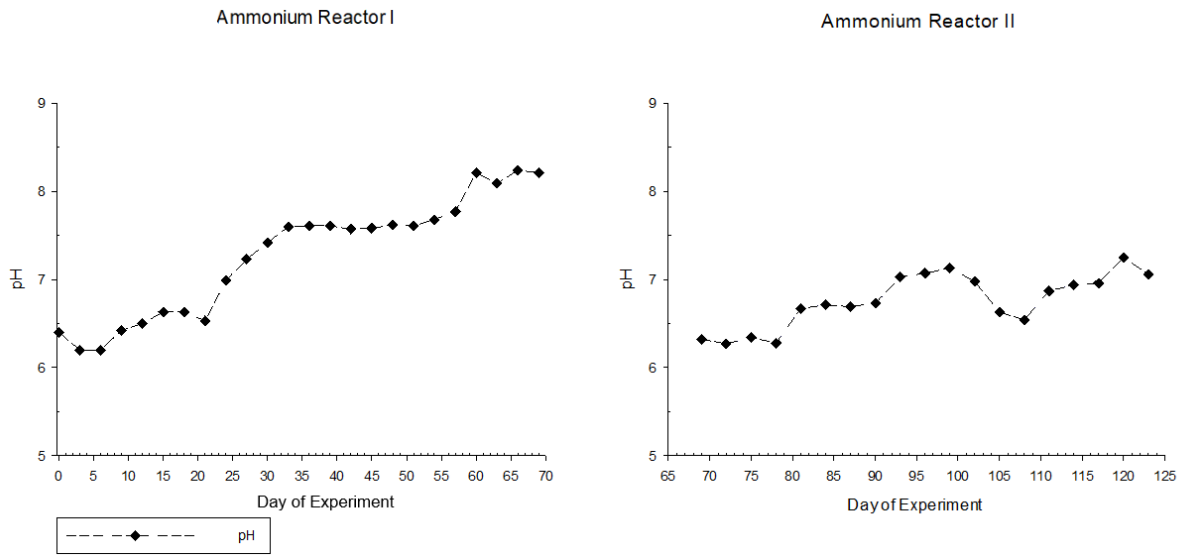


Figure 20: pH development of the two time-shifted ammonium reactors. Ammonium Reactor I (left) and Ammonium Reactor II (right).

Both *NH_4^+-Reactors* reached NH_4^+-N concentrations of over 1000 mg/l (Figure 21). However, the time to reach that concentration differed. The first time a concentration above 1000 mg/l was measured for *Reactor I* was after 48 days (DOE 48), while for *Reactor II,* an NH_4^+-N concentration of over 1000 mg/l was measured after 24 days (DOE 93) (Figure 21). In the following days, no considerable changes in concentration were observed in both reactors. The highest concentration of *Reactor I* was 1126 ± 7.8 mg/l NH_4^+-N, measured on DOE 48. This corresponds to a mineralization rate of 90 % of the added N by the organic residues into NH_4^+-N. For *Reactor II* the highest NH_4^+-N concentration of 1156 ± 3.3 mg/l was measured on DOE 123. 98 % of the added N by blood meal was mineralized into NH_4^+-N.

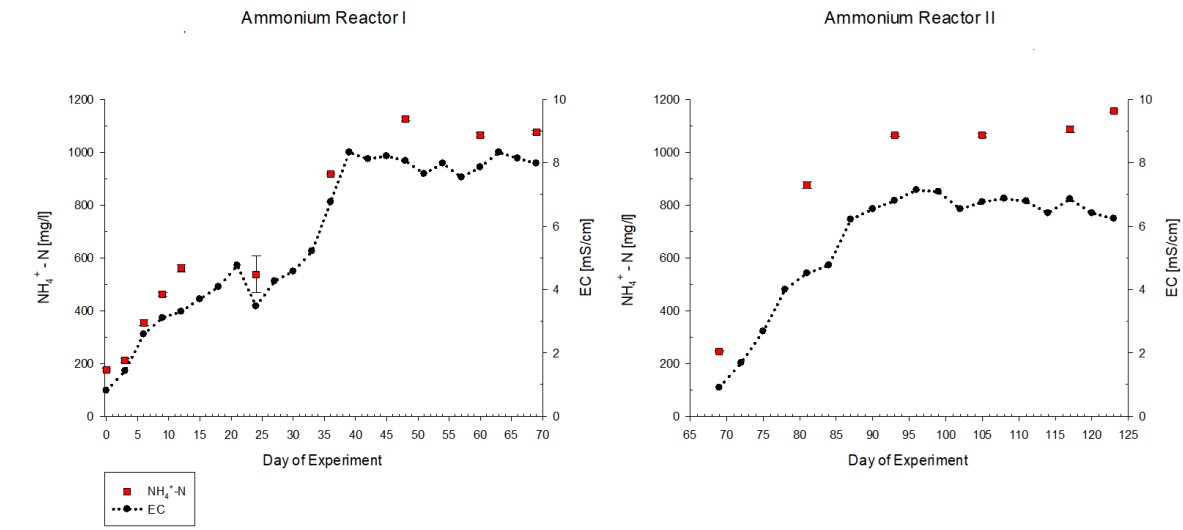


Figure 21: Ammonium-Reactors: Ammonium nitrogen (NH_4^+-N) and electrical conductivity (EC) concentration development of the two time-shifted ammonium reactors. Ammonium Reactor I (left) and Ammonium Reactor II (right).

The NH_4^+-N concentration and the EC showed a strong correlation with a coefficient of determination (R^2) of 0.99 for *Reactor I* and 0.95 for *Reactor II*, respectively. A linear regression line was fitted to describe the correlation between NH_4^+-N concentration as dependent and EC as explanatory variable (Figure 22). Therefore, EC and NH_4^+-N concentrations of both reactors were used. The regression line formula is presented in Figure 22. With the used model, 93 % of the variance of NH_4^+-N can be explained by the variable EC.

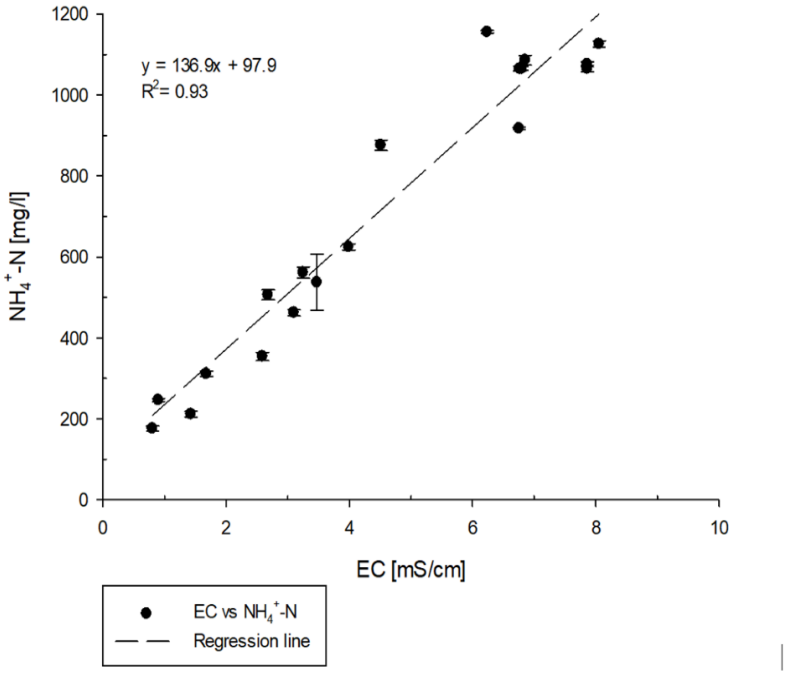


Figure 22: Electrical conductivity (EC) vs. ammonium nitrogen (NH_4^+-N): Scatter plot and fitted linear regression line with formula for NH_4^+-N as dependent and EC as explanatory variable.

During the experimental period, a thin film developed on the surface of the *NH_4^+-solution* of both temporally shifted reactors (Figure 23). Neither of the *NH_4^+-Reactors* showed visible mold formation.

Figure 23: Pictures of Ammonium-Reactor I on day of experiment 7 (left) and day 36 (right), with visible film on the surface.

5.1.2 NO_3^--Reactors

Following the results of the two temporally shifted *NO_3^--Reactors* are presented.

No larger temperature, DO, and pH differences were observed between *NO_3^--Reactor I* and *II* (Figure 24, Figure 25). On average, *Reactor II* was closer to the intended 30 °C than *Reactor I*, which had higher maximum temperatures. The DO concentration of both reactors was always above the set minimum threshold of 3.5 mg/l (Figure 24).

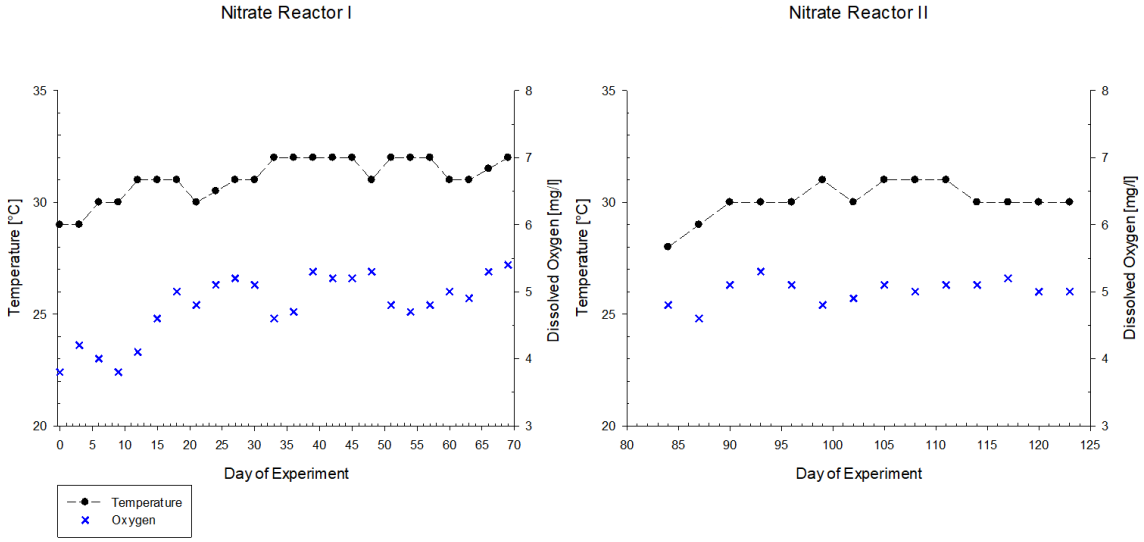


Figure 24: Nitrate-Reactors: Temperature and dissolved oxygen concentration development of the two time-shifted nitrate reactors. Nitrate Reactor I (left) and Nitrate Reactor II (right).

The pH ranged between 6.49 and 7.34 for *Reactor I* and between 6.74 and 7.55 for *Reactor II* (Figure 25).

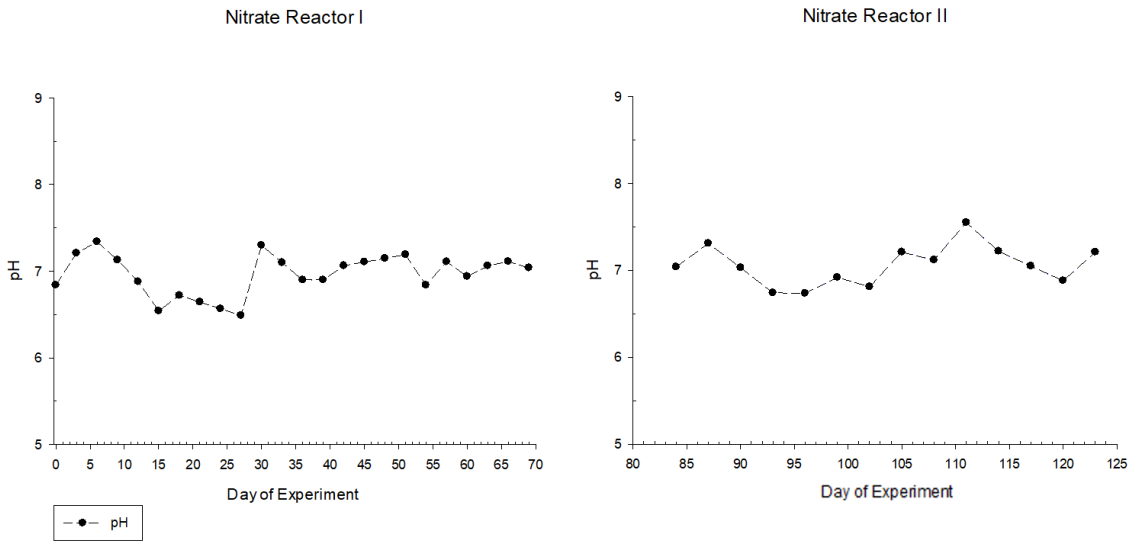


Figure 25: pH development of the two time-shifted nitrate reactors. Nitrate Reactor I (left) and Nitrate Reactor II (right).

Both temporal shifted *NO_3^--Reactors* showed a substantial increase in EC after the daily transfer of *NH_4^+-solution* started on DOE 21 for *Reactor I* (Figure 26) and DOE 105 for *Reactor II* (Figure 27). After that, the EC remained at a higher concentration but showed some fluctuations.

The highest NO_3^- concentration of *Reactor I* was measured 36 days after the start of the *NH_4^+-solution* transfer on DOE 57 (Figure 26). The measured concentration was 12.1 ± 0.3 mg/l NO_3^--N, an increase of 10.9 mg/l compared to the concentration measured on the start day of the transfer (DOE 21). After this day, the NO_3^- concentration decreased. Since no NH_4^+ measurements were conducted for *NO_3^--Reactor I,* the NH_4^+ concentration on the start day of transfer (DOE 21) was calculated by the temporal nearest NH_4^+ concentration of *NH_4^+-Reactor I* (DOE 24) (Figure 21), and the water volume of the *NO_3^--Reactor I,* 20 l. By this, an NH_4^+-N concentration of 26.9 ± 3.4 mg/l was calculated for the *NO_3^--Reactor I* on DOE 21.

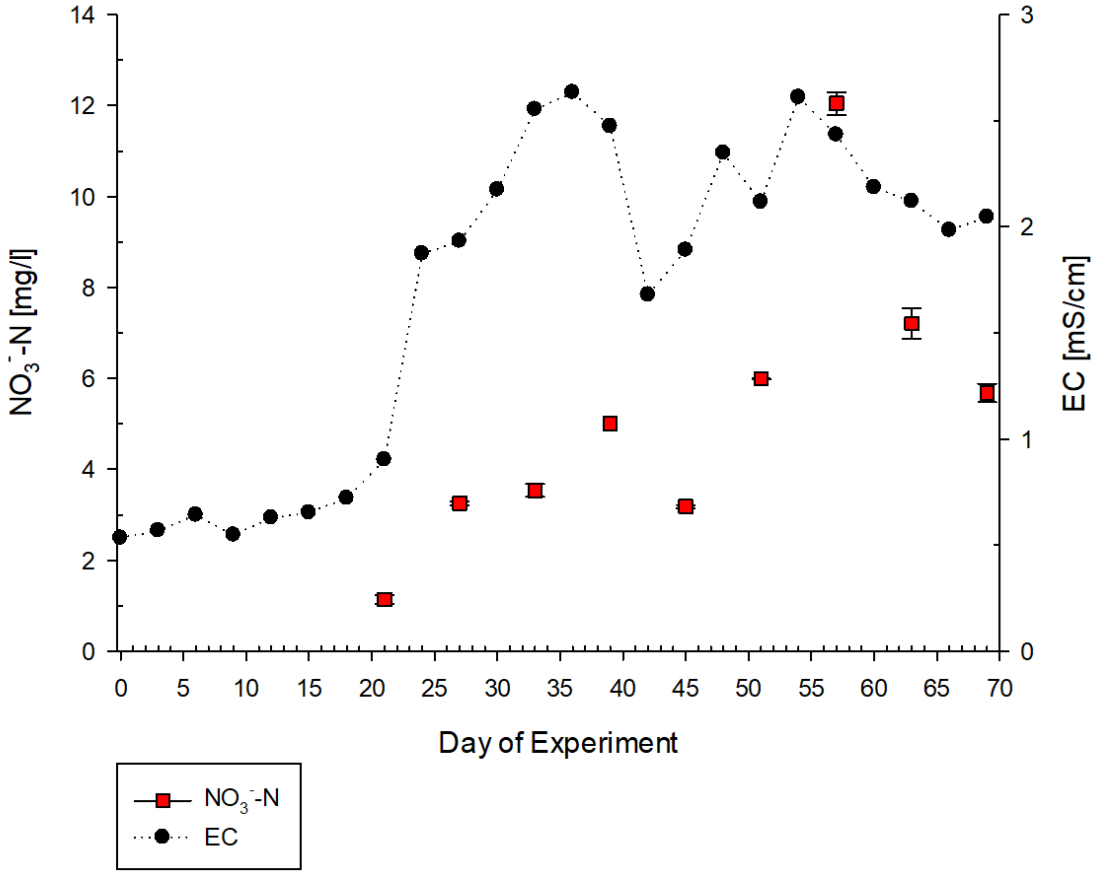


Figure 26: Nitrate Reactor I: Nitrate nitrogen (NO_3^--N) and electrical conductivity (EC) concentration development.

For *Reactor II*, were additionally to NO_3^- and EC, NH_4^+ and NO_2^- were analyzed, the highest NO_3^--N concentration of 68.9 ± 0.3 mg/l was measured on the last day of the experiment (DOE 123) (Figure 27). Compared to the start concentration on DOE 105, this was an increase of 43.8 mg/l or 876 mg in total for the volume of 20 l.

Based on the NH_4^+ concentrations measured for *NH_4^+-Reactor II* on DOE 105, 117, and 123 (Figure 21), an average of 1102 ± 39 mg NH_4^+-N was added to the *NO_3^--Reactor II* daily. In 16 days, a total of 17632 mg NH_4^+-N was added. Thus only 5 % of the added NH_4^+-N was converted into NO_3^--N.

Reactor II's NO_3^- and NH_4^+ concentrations followed contrary exponential curves, whereas NO_2^- concentration showed a bell-shaped curve (Figure 27).

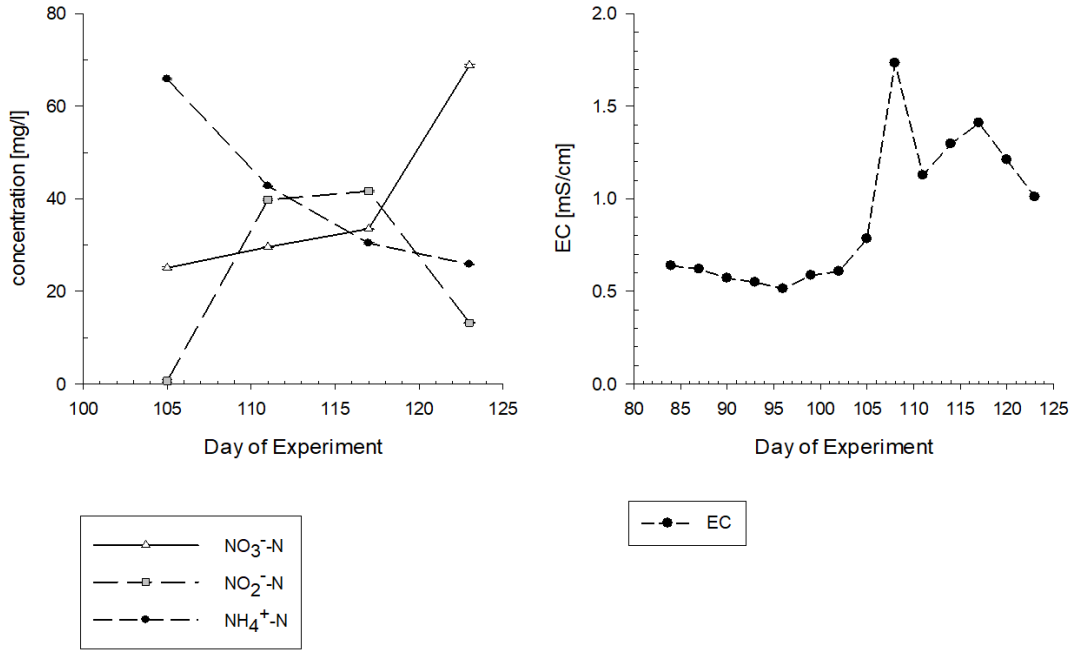


Figure 27: Nutrient and electrical conductivity (EC) concentration development of Nitrate Reactor II. Ammonium nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N), and nitrate nitrogen (NO_3^--N) concentrations (left) from day of experiment 105, as the transfer of NH_4^+ rich solution started and EC (right), measured from the starting day of the reactor.

5.1.3 P - Reactor

The temperature of the *P-Reactor* was below the intended 30 °C at the beginning but increased during the experiment. The strongest, almost continuous, increase was recorded between DOE 24 and 36. From DOE 84, the temperature was never below 30 °C (Figure 28).

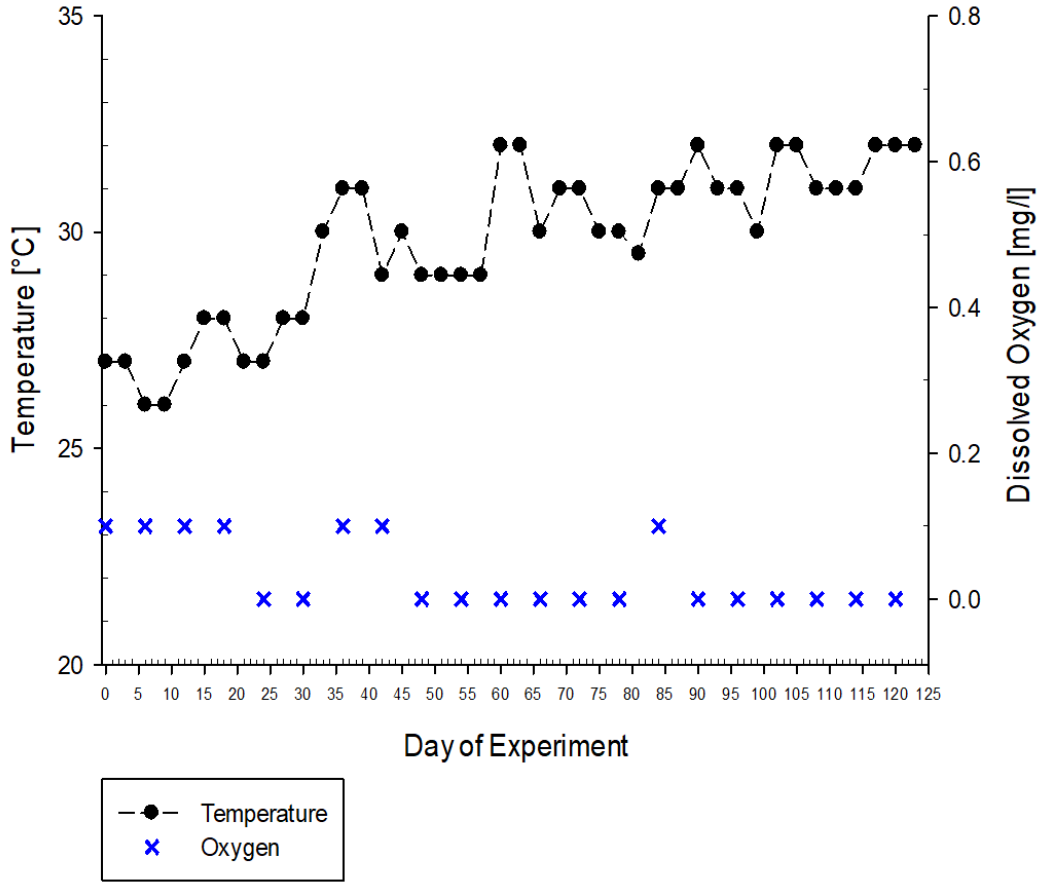


Figure 28: Phosphorus Reactor: Temperature and dissolved oxygen concentration development.

At the start of the experiment the pH of the *P-Reactor* was above 5.5 (Figure 29), and manual lowering was carried out. From DOE 27 onwards, the pH was consistently below 5.5, and no pH adjustment was necessary.

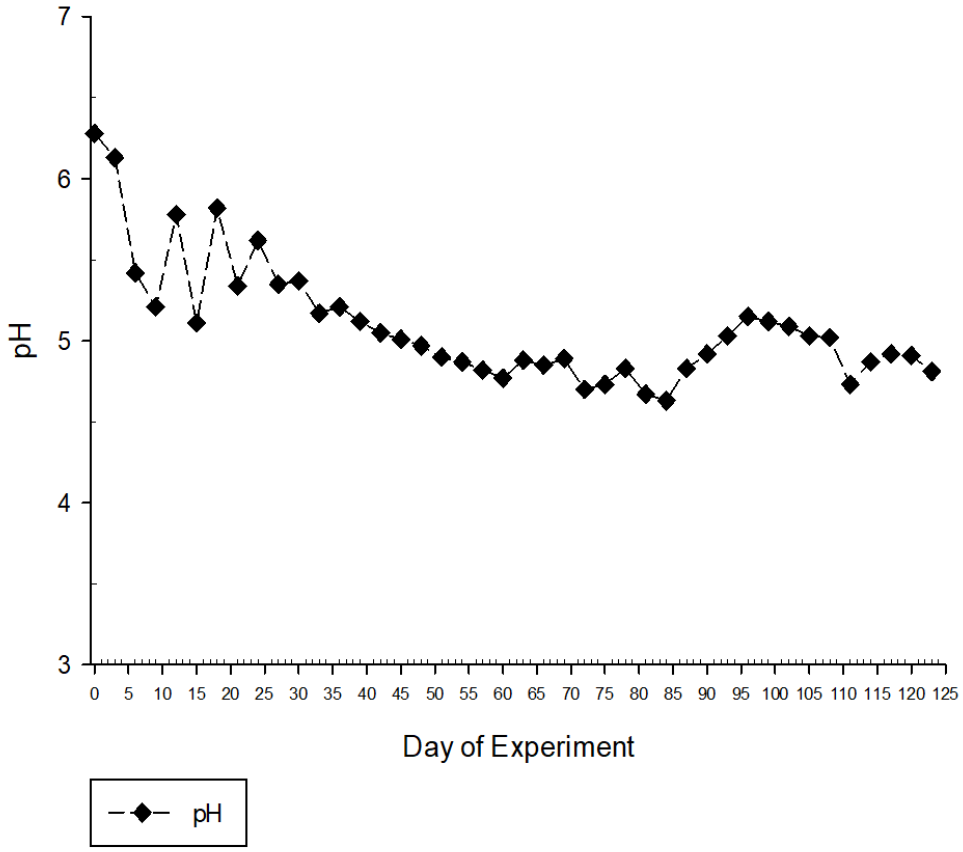


Figure 29: pH development of the Phosphorus Reactor.

The PO_4^{3-} concentration increased throughout the experiment (Figure 30). The sharpest increase between two consecutive measurements was between DOE 24 and DOE 36, as the concentration rose by 46.6 mg/l. The highest concentration of 166.0 ± 2.9 mg/l was measured on the last day of the experiment (DOE 123). Thus, 19.7 % of the added P, inherent in bone meal, was mineralized into plant-available PO_4^{3-}-P.

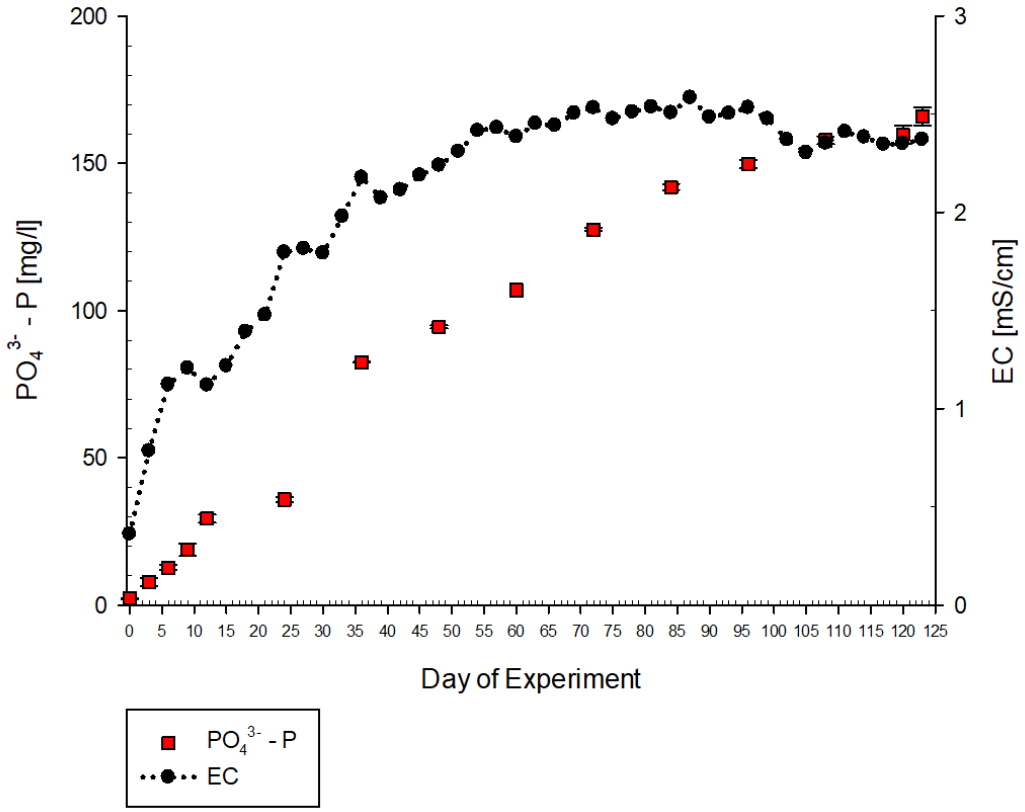


Figure 30: Phosphorus Reactor: Phosphate phosphorus (PO_4^{3-}-P) and electrical conductivity (EC) concentration development.

A nonlinear regression line was fitted with PO_4^{3-}-P as dependent and EC as the explanatory variable (Figure 31). By the used model, 83 % of the variance of PO_4^{3-}-P is explained by EC. The R^2 was lower than for the *NH_4^+-Reactor*s (Figure 22) and the *K-Reactor* (Figure 36).

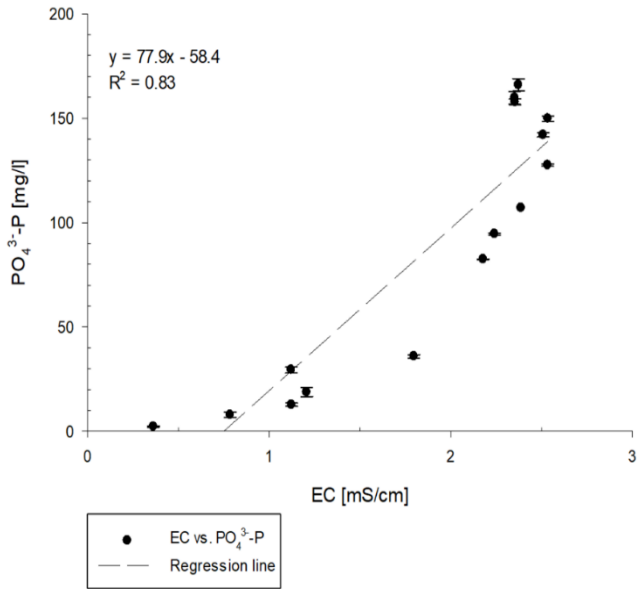


Figure 31: Electrical conductivity (EC) vs. phosphate phosphorus (PO_4^{3-}-P): Scatter plot and fitted linear regression line with formula for PO_4^{3-}-P as dependent and EC as explanatory variable.

In the course of the experiment, mold formation was observed on the surface of the *P-solution* from DOE 24 onwards. Initially, it was removed using a sieve. However, the mold quickly formed again (Figure 32), and removal was stopped.

Figure 32: Phosphorus Reactor on day of experiment 7 (left) and 75 (right) with visible mold formation.

5.1.4 K – Reactor

The pre-test to determine the K concentration in the used potato mix revealed that after 24 h, 1 g dried and ground potato mix released 23.8 ± 0.4 mg/l K^+, corresponding to 2.38 % K in the DM. This was assumed to be the maximum achievable concentration. Therewith, the total 3642 g dried potato mix added to the 30 l water of the *K-Reactor* could yield 2889.3 mg/l K^+.

The temperature of the *K-Reactor* increased during the experimental period (Figure 33). Initially, the measured DO was higher than in the other two anaerobic reactors (NH_4^+- and *P-Reactor*). However, it never exceeded 0.2 mg/l from DOE 12 onwards (Figure 33).

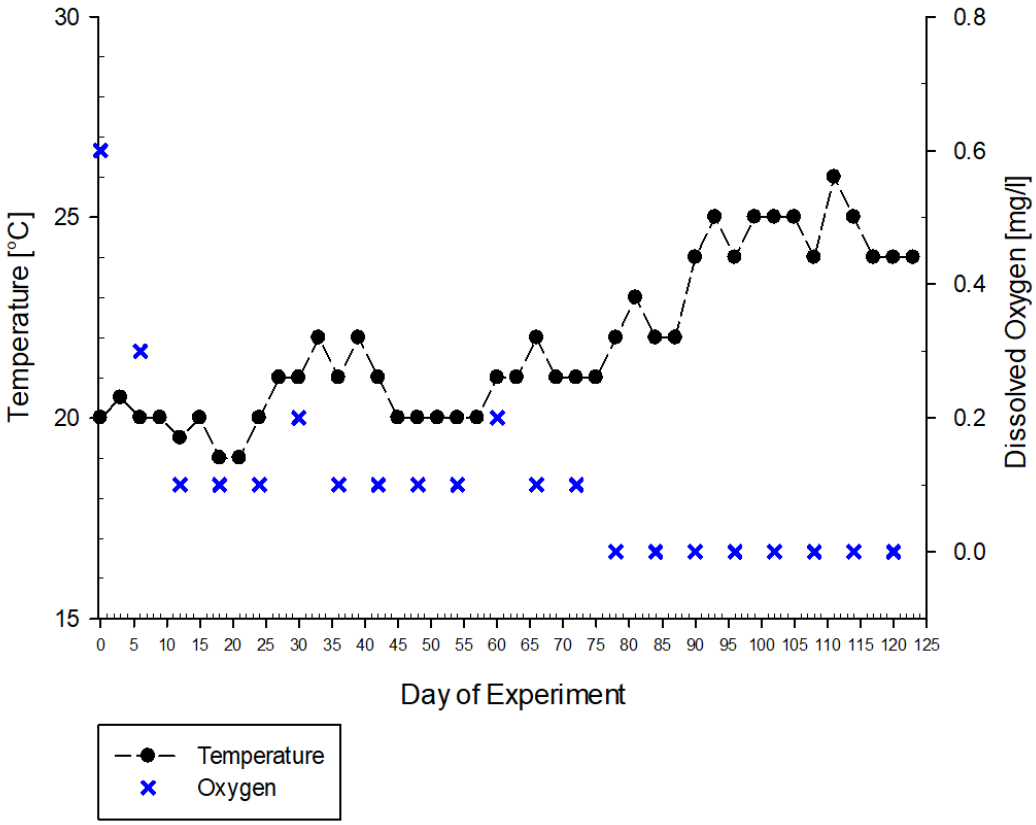


Figure 33: Potassium Reactor: Temperature and dissolved oxygen concentration development.

The pH remained acidic throughout the experiment (Figure 34). The highest pH of 4.6 was measured on DOE 0.

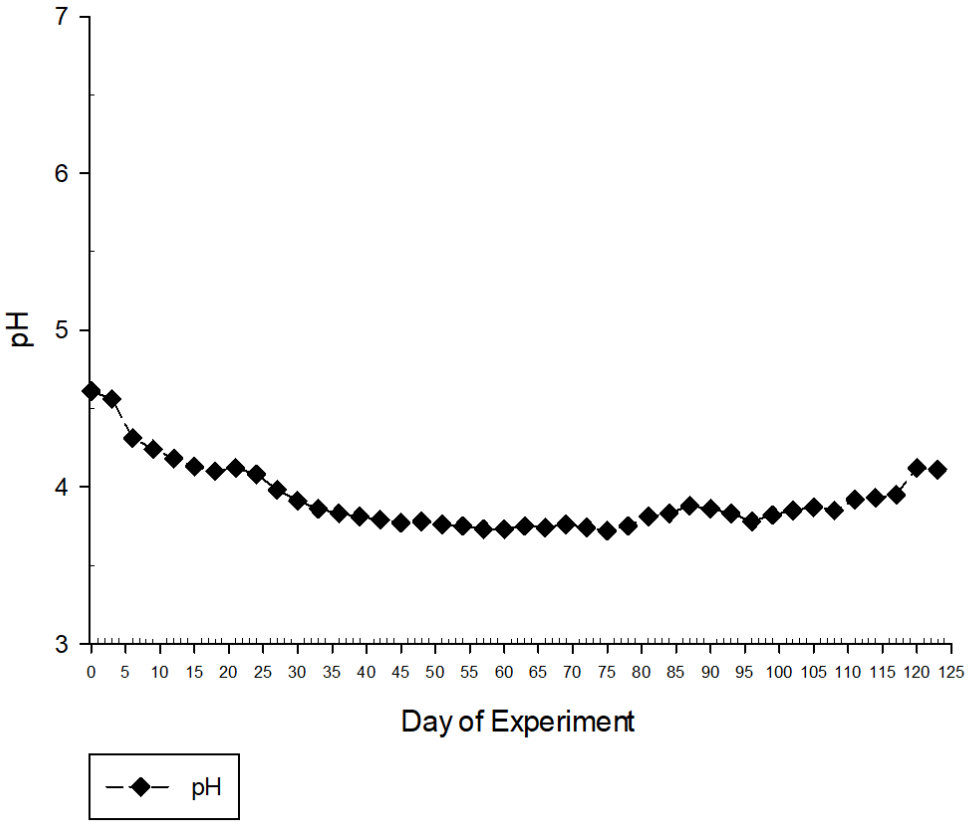


Figure 34: pH development of the Potassium Reactor.

The temporal development of the K^+ concentration clearly represents the addition of potato mix on DOE 0, 24, and 84. The consecutive measurement showed strongly increased K^+ concentrations (Figure 35). The highest K^+ concentration was measured on DOE 123 with 2520 ± 20 mg/l; this corresponds to a mineralization rate of 87 % of the added K by potato mix.

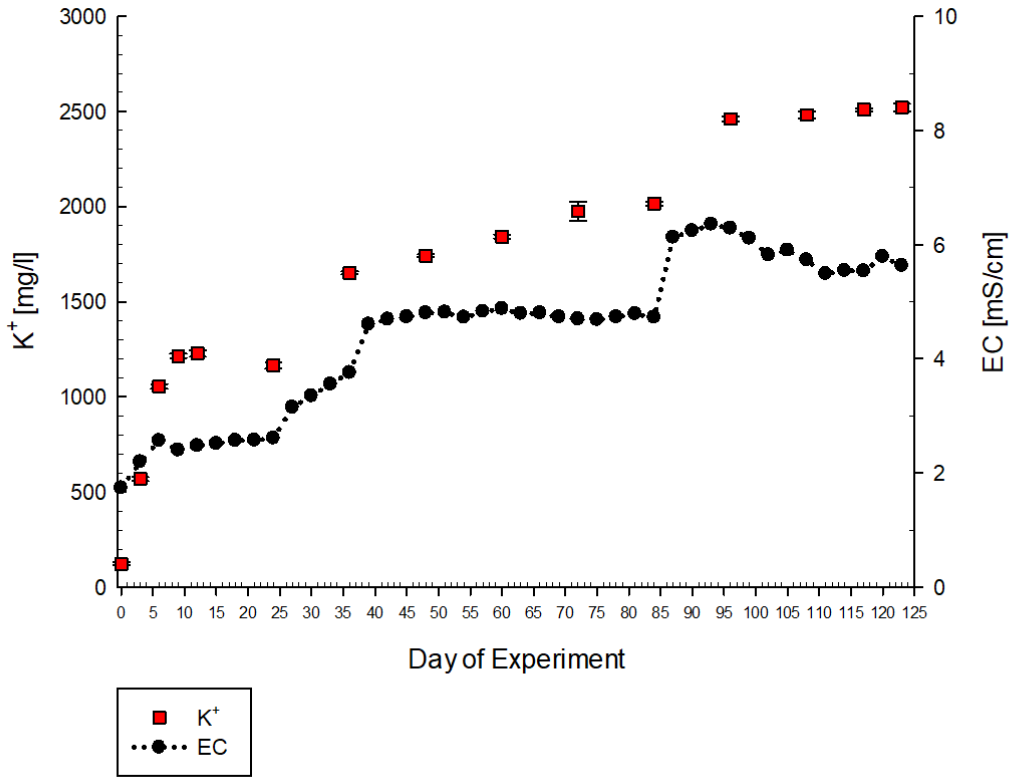


Figure 35: Potassium Reactor: Potassium (K^+) and electrical conductivity (EC) concentration development.

A linear regression was conducted for the correlation between K^+ as dependent and EC as explanatory variable, with a high coefficient of determination ($R^2 = 0.9$). The scatter plot and the linear regression are presented in Figure 36.

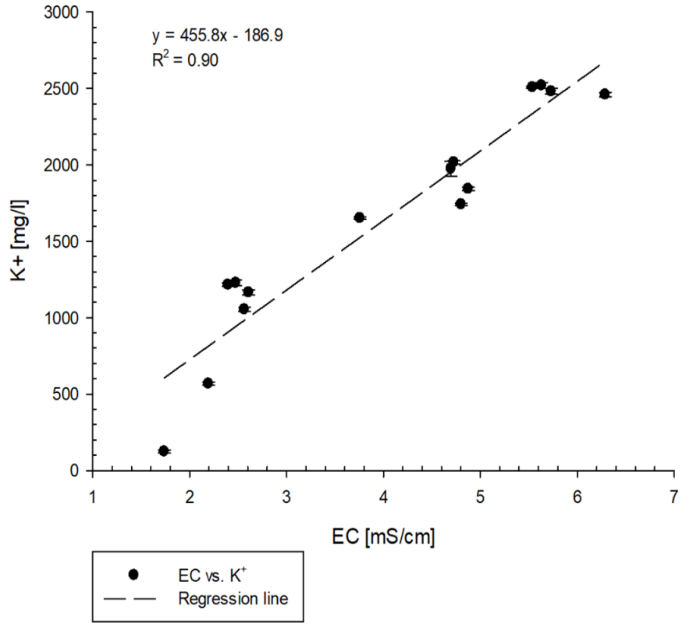


Figure 36: Electrical conductivity vs. Potassium:Scatter plot and fitted linear regression line with formula for potassium (K^+) as dependent and electrical conductivity (EC) as explanatory variable.

As in the *P-Reactor* also in the *K-Reactor*, mold formed (Figure 37) - the first time it was observed on DOE 21. Mold was only removed at the beginning as the formation started.

Figure 37: Potassium Reactor on day of experiment 7 (left) and 75 (right) with visible mold formation.

5.1.5 Final Analysis

The final analysis (Table 19), where all essential macro- and micronutrients for plant growth, except for Cl and Ni, were measured in each reactor's solution, revealed that the solutions of *NH_4^+-Reactor II* and the *K-Reactor* had the highest nutrient concentration of the respective nutrient of interest (Table 19). In *NO_3^--Reactor II,* a slightly higher Ca concentration than NO_3^--N concentration was measured. In the *P-Reactor,* a 2.6 times higher Ca concentration than PO_4^{3-}-P concentration was measured. Also, the NH_4^+-N concentration was comparatively high compared to the PO_4^{3-}-P concentration (Table 19).

Substantial Mg concentrations were measured in all produced solutions, while S concentrations were below 10 mg/l in each solution, except for the *K-solution*. All other measured essential nutrients were present in at least one of the solutions (Table 19).

Na^+ concentration was highest in the solution of the *NH_4^+-Reactor II* and lowest in the *K-solution* (Table 19).

Table 19: Concentrations of measured essential macronutrients, micronutrients, and sodium in the produced bioponic nutrient solutions of the different reactors on day of experiment 123.

Nutrient	NH_4^+-Reactor II	NO_3^--Reactor II	P-Reactor	K-Reactor
		[mg/l]		
NH_4^+-N	**1155.9 ± 3.3**	30.3 ± 0.4	105.9 ± 1.4	97.4 ± 2.2
NO_3^--N	0.9 ± 0.1	**68.9 ± 1.3**	0.3 ± 0.1	0.3 ± 0.1
PO_4^{3-}-P	2.5 ± 0.0	0.0 ± 0.0	**166.0 ± 2.9**	8.6 ± 0.2
K	86.4 ± 0.0	47.9 ± 0.0	36.0 ± 3.1	**2520.0 ± 20.4**
Ca	180.5 ± 0.5	77.2 ± 0.2	429.5 ± 0.5	27.3 ± 1.9
Mg	81.1 ± 0.1	11.9 ± 0.2	15.4 ± 0.0	130.5 ± 5.5
S	7.4 ± 0.3	3.9 ± 0.2	3.7 ± 0.0	82.8 ± 4.7
B	0.50 ± 0.01	0.41 ± 0.00	0.48 ± 0.01	1.29 ± 0.15
Fe	4.94 ± 0.18	0.52 ± 0.01	4.05 ± 0.02	62.80 ± 2.70
Zn	0.90 ± 0.00	0.79 ± 0.01	0.87 ± 0.01	2.10 ± 0.14
Cu	0.00 ± 0.00	0.12 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Mn	0.30 ± 0.01	0.78 ± 0.00	0.24 ± 0.00	1.56 ± 0.04
Mo	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.10 ± 0.03
Na	25.3 ± 1.2	21.7 ± 0.5	19.7 ± 0.9	11.0 ± 2.8

5.1.6 Composition of the Bioponic Nutrient Solution

Considering NO_3^--N, NH_4^+-N, PO_4^{3-}-P, and K^+ concentrations of the final analysis (Table 19), the ***B**NS* and ***SB**NS* mixing ratios were made (Table 20). The mean values of the mineral solutions listed in Table 4 served as reference for the mixing ratios. From the beginning, it was planned not to use the *NH_4^+-solution,* and since the other solutions provided more than enough NH_4^+-N, this plan was adhered to.

Table 20: Mixing ratios of the bioponic stock solutions to produce the bioponic (BNS) (left) and spiked bioponic nutrient solution (SBNS) (right). The SBNS was spiked with $CaNO_3$ added by mineral B stock solution.

Mixing Ratio BNS		
NO_3^--solution	621.30	ml
P-solution	297.00	ml
K-solution	81.70	ml

Mixing Ratio SBNS		
B stock solution	1.75	ml
P-solution	297.00	ml
K-solution	93.50	ml
Desalinated water	607.75	ml

The concentrations of the ***B**NS* and ***SB**NS*, if mixed as shown in the tables above, are presented in Table 21. Also, the nutrient concentrations of the ***M**NS* are presented in the table. Compared to the ***M**NS*, a considerable lack of NO_3^-, S, and Mo is apparent in the ***B**NS,* while Zn and Mn were 6.9 and 6.4 times higher concentrated, respectively (Table 21). No Mo was present in the ***B**NS* and the ***SB**NS*, while Cu was absent only in the ***SB**NS*. The mixing ratio of the ***B**NS* resulted in a Na^+ concentration of 20.2 mg/l.

Table 21: Nutrient concentrations of the used solutions. Mineral (MNS), bioponic (BNS), and spiked bioponic nutrient solution (SBNS). Comparison of nutrient concentrations of bioponic (BNS/MNS) and spiked bioponic solution (SBNS/MNS) divided by mineral nutrient solution concentrations.

Nutrient	Mineral nutrient solution	Bioponic nutrient solution	BNS/MNS	Spiked bioponic nutrient solution	SBNS/MNS
	[mg/l]	[mg/l]		[mg/l]	
NH_4^+-N	28.0	58.2	2.1	40.6	1.5
NO_3^--N	196.0	42.9	0.2	196.1	1.0
PO_4^{3-}-P	61.9	50.0	0.8	50.1	0.8
K	236.6	246.5	1.0	246.3	1.0
Ca	160.3	177.7	1.1	410.7	2.6
Mg	24.3	22.6	0.9	16.8	0.7
S	32.2	10.3	0.3	8.8	0.3
B	0.27	0.50	1.9	0.30	1.11
Fe	2.79	6.70	2.4	7.10	2.54
Zn	0.13	0.90	6.9	0.50	3.85
Cu	0.03	0.10	3.3	0.00	0.00
Mn	0.11	0.70	6.4	0.20	1.82
Mo	0.05	0.00	0.0	0.00	0.00

5.2 Hydroponic Plant Cultivation Experiment

The following section presents the results of the hydroponic cultivation experiment in a deep-water culture system. The ***B**NS* and ***SB**NS* were tested against an ***M**NS* on lettuce. Since one plant was wilted and less vigorous for every treatment at the end of the experiment (Figure 46 p.72, Figure 47 p.73, Figure 48 p.74), the respective plant was excluded from the evaluation. Consequently, all following figures, tables, and statistical evaluations are based on each treatment's four remaining lettuce plants.

5.2.1 pH

After three to four days in the deep-water culture containers, the measured pH of the exchanged nutrient solution differed slightly between the ***B****NS* and the ***SB****NS* (Figure 38). The pH had increased compared to the pH of the added solution of 6.0. On the contrary, the pH of the ***M****NS* decreased compared to the added solution's pH (Figure 38).

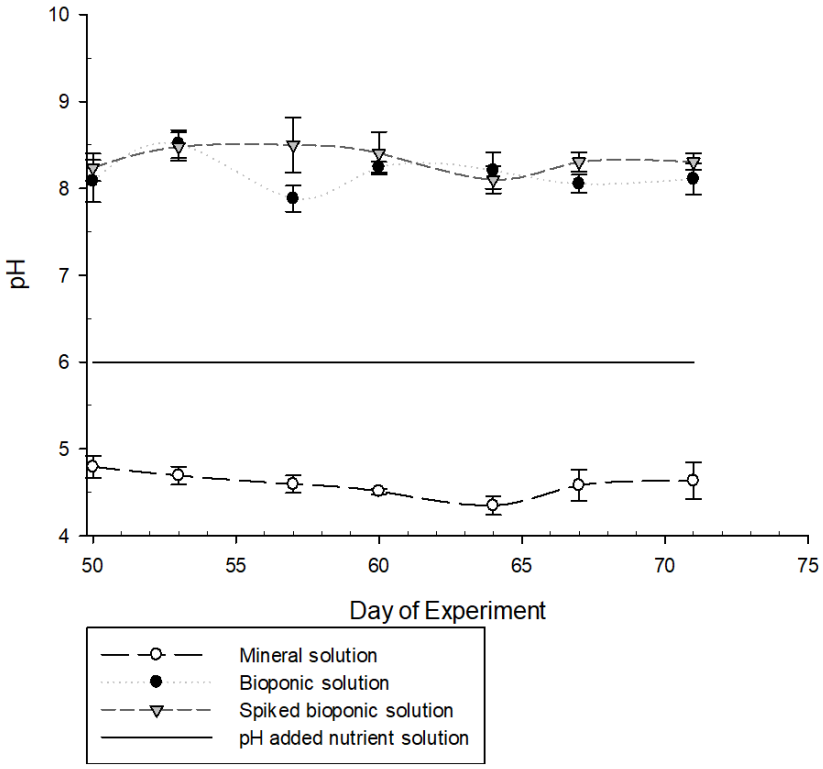


Figure 38: pH of the nutrient solutions on exchange days after three or four days in the deep-water culture containers.

5.2.2 Electrical Conductivity

Overall, the EC of the added ***B****NS* and ***SB****NS* had higher EC than the added ***M****NS* (Figure 39). The highest EC was measured for the ***B****NS*. Also, higher changes in the EC of the added solution were measured in the ***B****NS* and ***SB****NS*.

For the ***M****NS*, the EC of the remaining solution after three or four days in the deep water culture containers was higher than the EC of the added solution (Figure 39). This was the case for each interval. The strongest increase in EC between added and remaining ***M****NS* was measured in the interval DOE 64 to DOE 67. The EC development of the ***B****NS* showed an opposite trend; the EC decreased during four of the seven intervals. In the ***SB****NS*, the EC decreased during the first four intervals and increased in the last three, compared to the EC of the added solution (Figure 39).

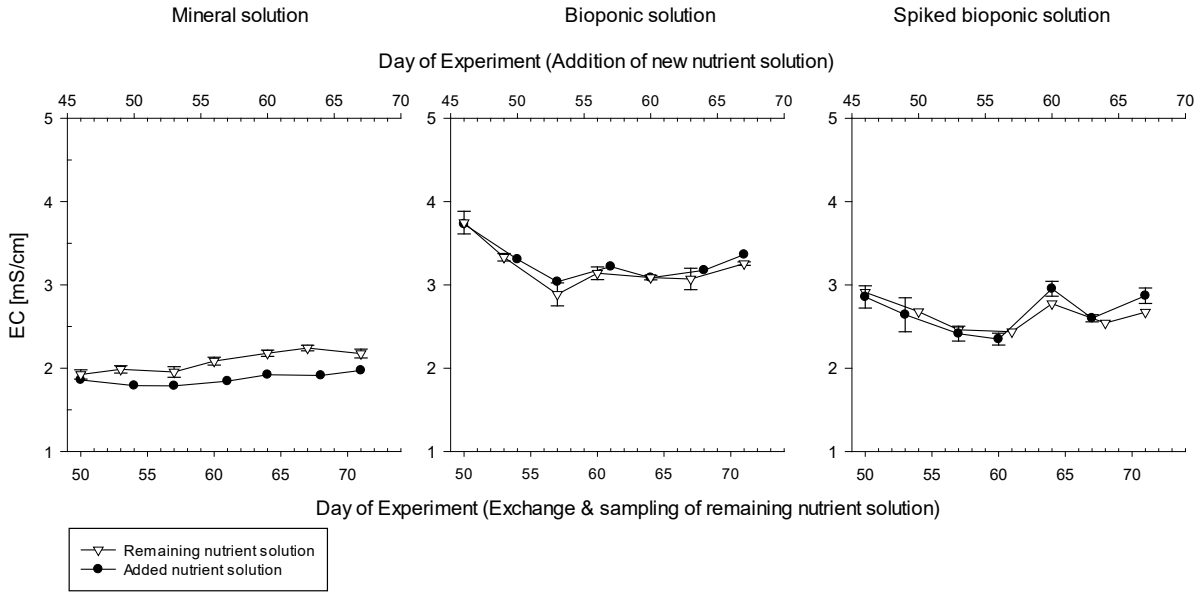


Figure 39: Electrical Conductivity (EC) of added and remaining nutrient solution in the deep-water culture containers after intervals of three or four days for each tested solution. Days nutrient solution was added are shown on the upper x-axis. "Added nutrient solution" refers to this axis. Days the nutrient solution was exchanged and sampled are shown on the lower x-axis, "Remaining nutrient solution" refers to this axis.

5.2.3 Evapotranspiration

Throughout the experiment, higher evapotranspiration was measured for the plants grown in mineral nutrient solution (*PG**M**)* than for the plants grown in bioponic nutrient solution (*PG**B**)* or the plants grown in spiked bioponic nutrient solution (*PG**SB**)* (Figure 40). Recurring fluctuations were measured for the evapotranspiration of the *PG**M***. From DOE 64, the *PG**SB*** had higher evapotranspiration rates than the *PG**B*** (Figure 40).

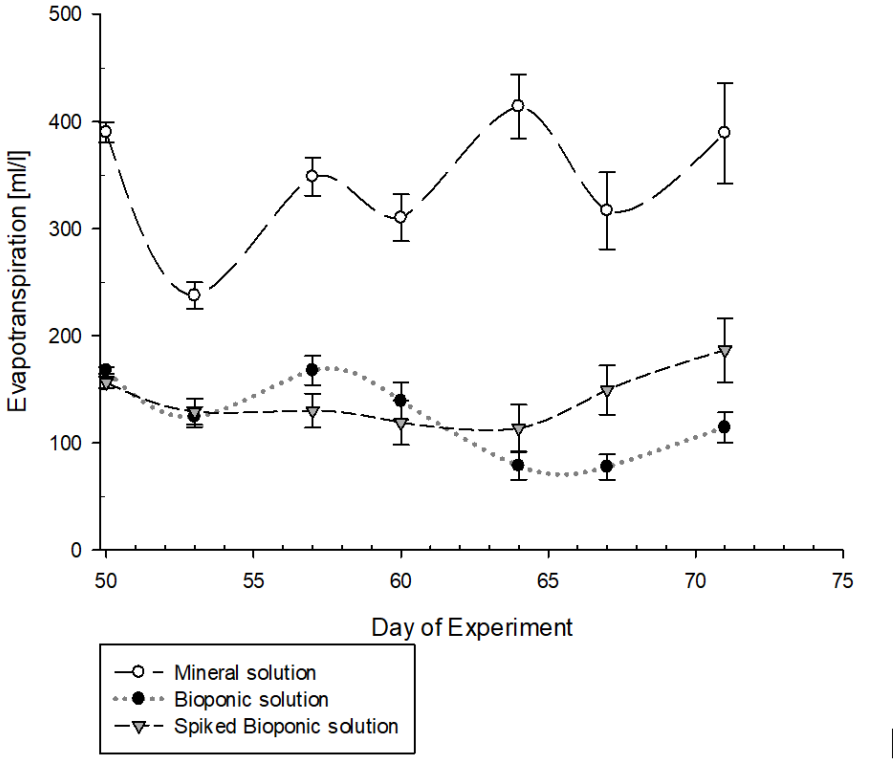


Figure 40: Evapotranspiration of the lettuce plants grown in mineral, bioponic, or spiked bioponic nutrient solution within three or four days. Measured on the day the nutrient solution was exchanged. The first measurement on day of experiment 50 corresponds to evapotranspiration within four days.

5.2.4 Plant Mass Development

In the initial phase, as all lettuce plants were supplied with ***M****NS*, the plants showed an exponential increase in total FM (Figure 41). DOE 46 FM is not considered here because only three days had passed since the last measurement and not a week as in the other measurements in the initial phase. At none of the measurement days of the initial phase, the means of the FM of the plants of the pre-mineral, pre-bioponic, and pre-spiked-bioponic groups differed significantly.

The exponential FM increase continued for the plants fertilized beyond the initial phase with ***M****NS* (Figure 41). This growth lasted until about DOE 60, then slowed down. The *PG**B*** or *PG**SB*** from DOE 46 onwards showed barely any further exponential FM increase, and if it lasted only a few days (*PG**B*** DOE 46 - 53). Initially, the *PG**B*** showed an increase in total FM until DOE 57, followed by stagnation and finally a decrease in FM (Figure 41). A significant ($p=0.005$) FM difference was detected on DOE 60 compared to the *PG**M***.

The *PG**SB*** showed almost no FM increase until DOE 57; from DOE 46 to 57, the FM increased by 7.9 ± 3.1 g per plant. Thereupon, the total FM increased stronger (Figure 41). A significant difference to the *PG**M*** was measured from DOE 57 ($p=0.009$) onwards. The masses of the *PG**B*** and *PG**SB*** showed no significant differences.

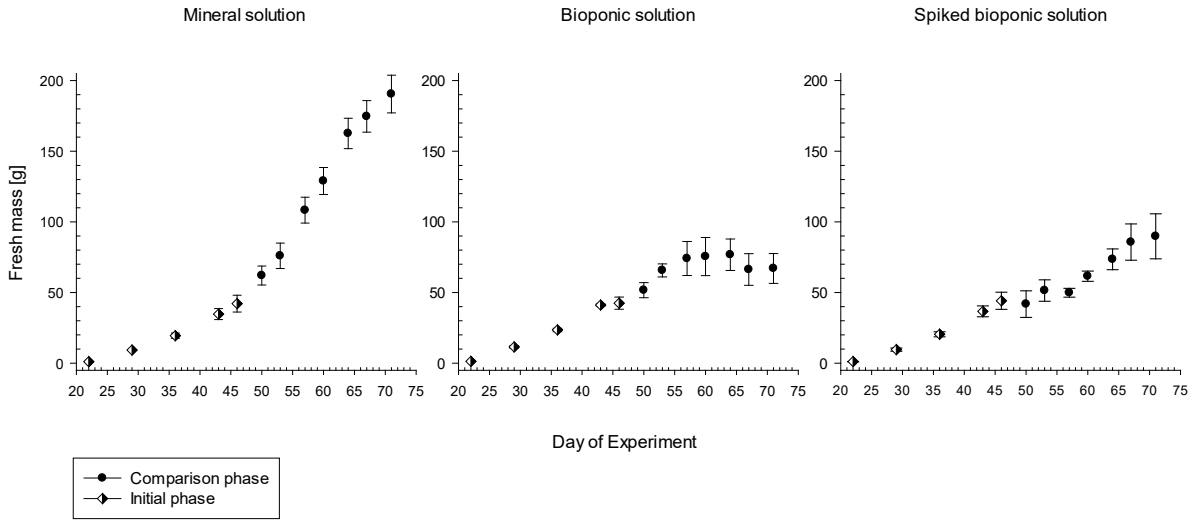


Figure 41: Cumulated fresh mass development of the lettuce plants, grown in mineral, bioponic, or spiked bioponic nutrient solution. Including roots and shoots. Inclusively until day of experiment 46, all plants were fertilized with mineral nutrient solution.

The weekly FM increases (Figure 42) also depicted the trends mentioned above. The decrease in the exponential growth of the *PG**M*** in the sixth (DOE 57 - 64) and seventh week of the experiment is visible. Noticeable for the *PG**B*** and *PG**SB*** was the substantial decrease in FM increase in the week after the ***M**NS* of the initial phase was replaced (Figure 42). The recovery from this decrease was more consistent for the *PG**SB***.

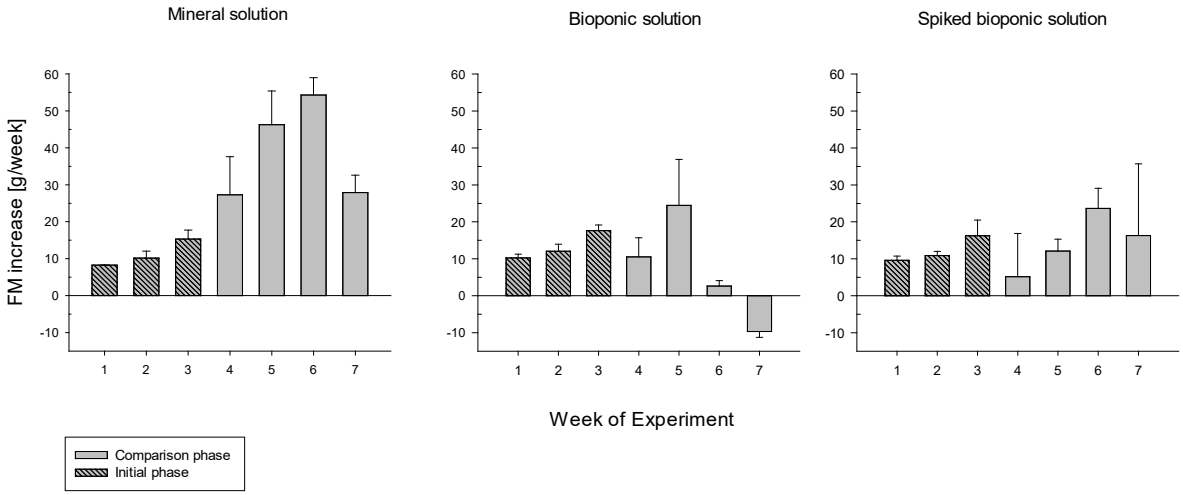


Figure 42: Weekly total fresh mass (FM) increases of the lettuce plants grown in mineral, bioponic, or spiked bioponic nutrient solution. Inclusively until week three, all plants were fertilized with mineral nutrient solution.

On final harvest, the lettuce shoot FM of the *PG**B*** was 21 % of the *PG**SB*** 36 % of the *PG**M*** (Figure 43).

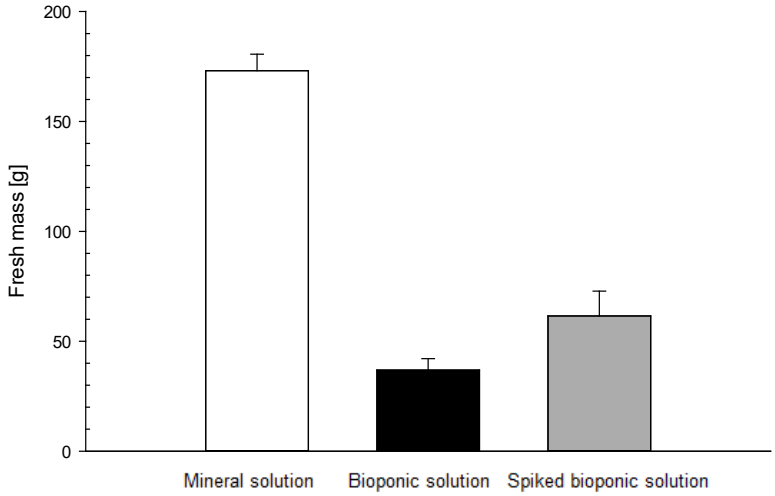


Figure 43: Harvested average lettuce shoot fresh mass after the plants grown in mineral, bioponic, or spiked bioponic nutrient solution for 25 days.

The low FM shoot-to-root ratio (S/R ratio) of 1.2 ± 0.2 of the *PG**B*** (Table 22) showed that nearly half of the total FM consisted of roots on DOE 71. Also, the FM S/R ratio of the *PG**SB*** was with 2.2 ± 0.3 low. The S/R ratio calculated for the DM showed an increase of 2.8 times for the *PG**B*** compared to the FM S/R ratio (Table 22). In contrast, the S/R ratio of the *PG**M*** increased only by 1.1 times. The highest percentage of DM, with 13.2 % in the shoot, was measured for the *PG**B***. Contrarily, the roots of the *PG**B*** had the lowest DM portion (Table 22).

Table 22: Shoot and root dry (DM) and fresh masses (FM) of the lettuce plants grown in mineral, bioponic, or spiked bioponic nutrient solution measured on DOE 71. S/R ratio = Shoot/Root ratio, Shoot mass divided by root mass.

	Mineral solution		
	Shoot [g]	**Root** [g]	**S/R ratio**
FM	173.1 ± 7.5	17.4 ± 1.4	9.9 ± 0.4
DM	15.2 ± 0.2	1.4 ± 0.1	10.9 ± 0.2
DM [%]	8.8	8.0	

	Bioponic solution		
	Shoot [g]	**Root** [g]	**S/R ratio**
FM	36.9 ± 5.2	30.1 ± 5.3	1.2 ± 0.2
DM	4.9 ± 0.4	1.5 ± 0.3	3.3 ± 0.1
DM [%]	13.2	5.0	

	Spiked bioponic solution		
	Shoot [g]	**Root** [g]	**S/R ratio**
FM	61.5 ± 11.3	28.3 ± 3.4	2.2 ± 0.3
DM	5.2 ± 0.5	1.9 ± 0.2	2.7 ± 0.2
DM [%]	8.5	6.7	

5.2.5 Anatomical Development of the Lettuce Plants

In the initial phase, the plants showed no differences in their development. All plants had brown tip burn symptoms on the younger leaves at the end of the initial phase.

In the comparison phase, the development of the plants strongly diverged, depending on the nutrient solution they were fertilized with. Additionally, to the change in nutrient solution, a failure in O_2 supply occurred directly at the start of the comparison phase. A hose attached to the air pump and connected it to the deep-water culture containers disconnected from its mount, most probably due to the pump's vibration. All 15 deep-water containers filled with nutrient solution were without O_2 addition for a maximum of 1.5 days. On DOE 47, the *PG**B*** and *PG**SB*** showed wilted limp leaves, particularly the older ones, were affected (Figure 44).

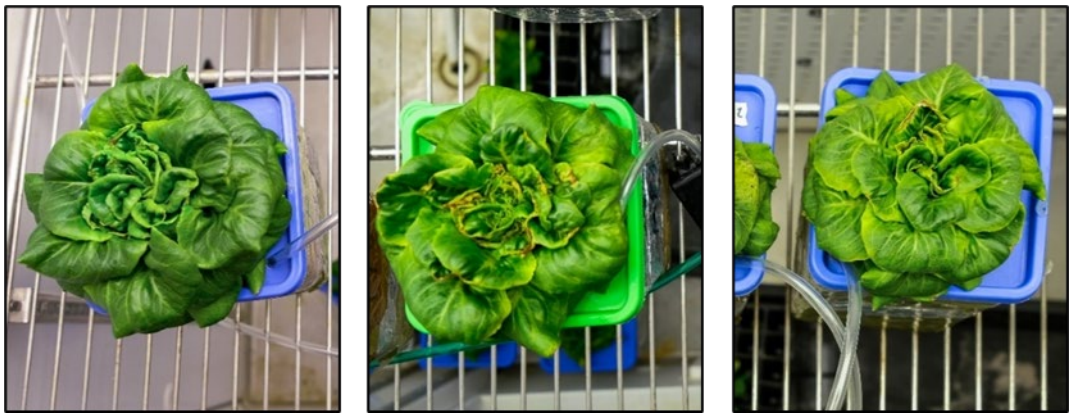


Figure 44: Lettuce plants on day of experiment 47. Grown in mineral (left), bioponic (middle), and spiked bioponic nutrient solution (right).

After one week of the comparison phase, most of the tips of these leaves were necrotic or covered by brown, necrotic spots (Figure 45). Whereas the *PG**B*** developed some new leaves, the *PG**SB*** remained in a stagnating stage (Figure 45). This changed after DOE 57; from then on, the *PG**SB*** developed more new leaves than the *PG**B*** (Figure 47, Figure 48). The leaves of the *PG**B*** and the *PG**SB*** had smaller areas than those of the *PG**M***, visible in Figure 46, Figure 47, and Figure 48.

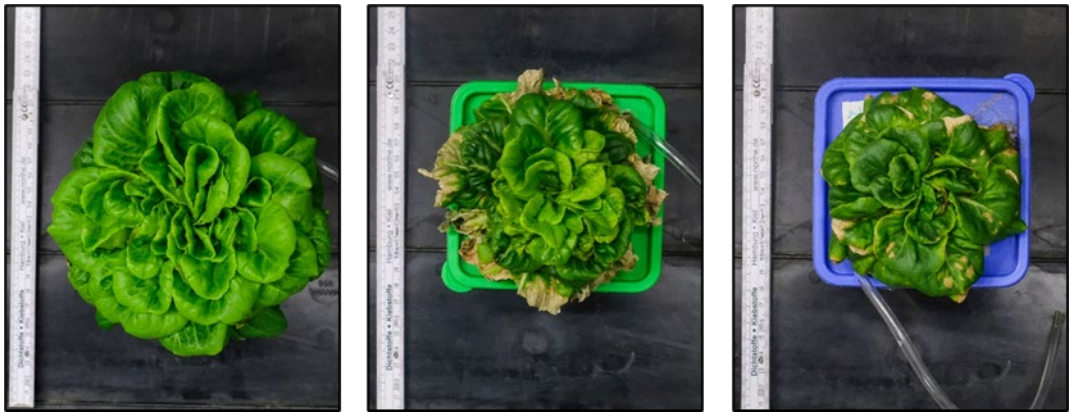


Figure 45: Lettuce plants on day of experiment 53. Grown in mineral (left), bioponic (middle) and spiked bioponic nutrient solution (right).

The roots of the *PG**B***, *PG**SB***, and *PG**M*** showed substantial differences. Whereas the roots of the *PG**M*** were clean and white (Figure 46), the roots of the *PG**B*** and *PG**SB*** were long and thin, brown-discolored, and covered by a slimy biofilm (Figure 47, Figure 48). The biofilm was more pronounced on the roots of the *PG**B***. During the exchange of nutrient solution twice a week, parts of the roots of the *PG**B*** and *PG**SB*** detached. A few more young, healthy roots were found on the *PG**B*** compared to the *PG**SB*** until DOE 57. This changed in the following course of the experiment; more healthy roots were found on the *PG**SB*** (Figure 47, Figure 48).

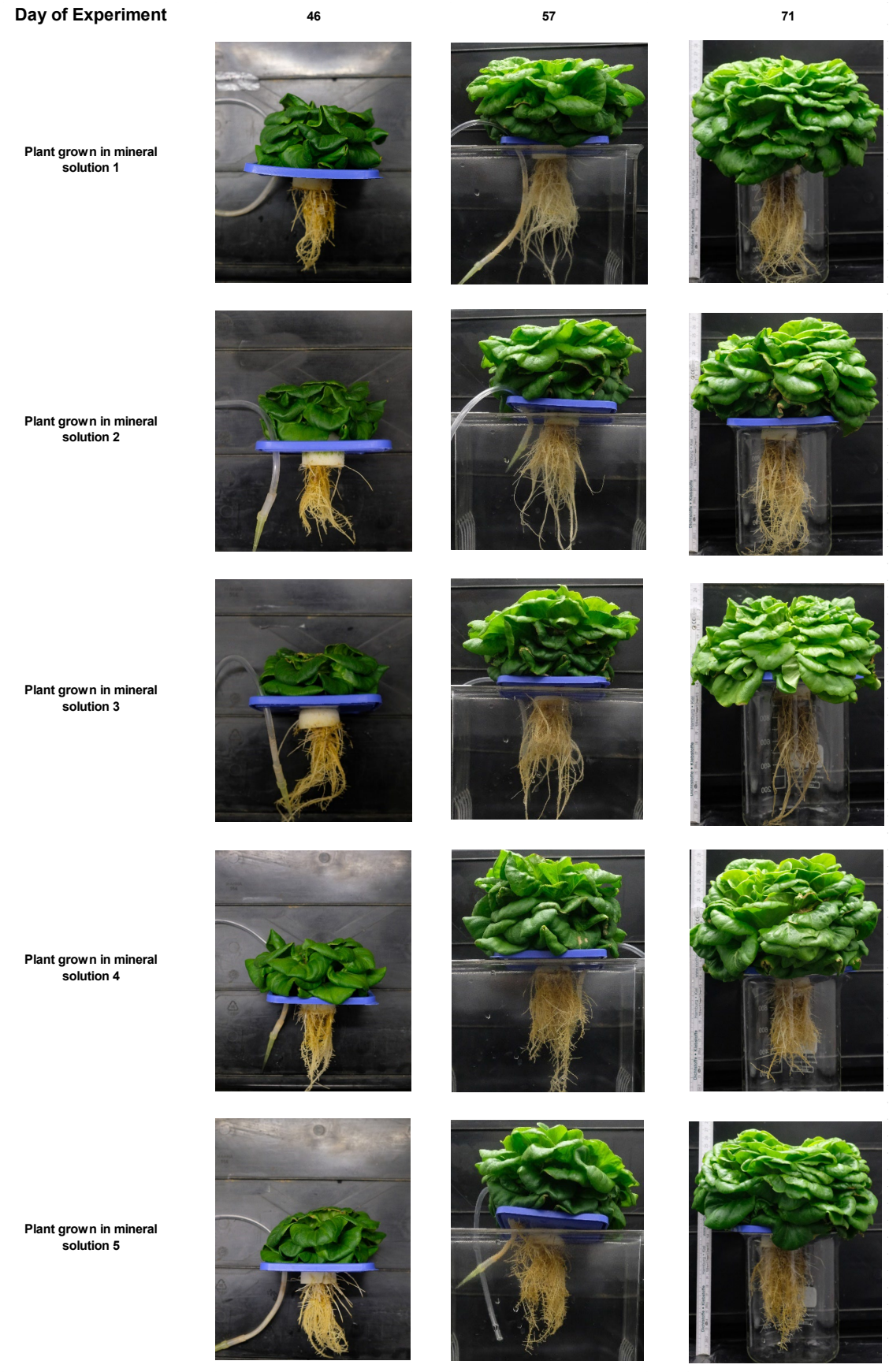


Figure 46: The five lettuce plants grown in mineral nutrient solution on day of experiment 46, as the comparison between the three different nutrient solutions started, 57 and harvest day 71. Plant 3 was excluded from the evaluation.

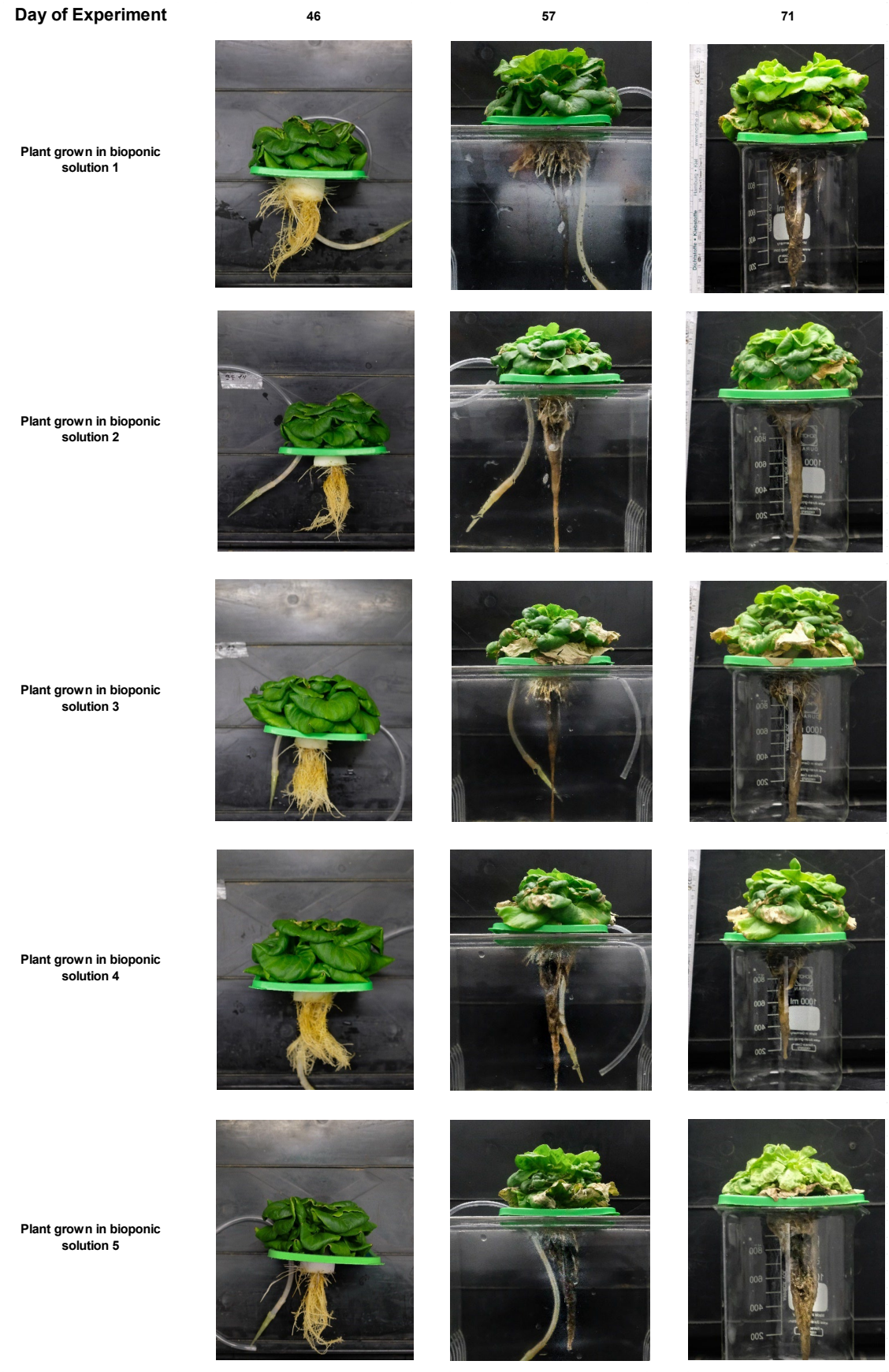


Figure 47: The five lettuce plants grown in bioponic nutrient solution on day of experiment 46, as the comparison between the three different nutrient solutions started, 57 and harvest day 71. Plant 5 was excluded from the evaluation.

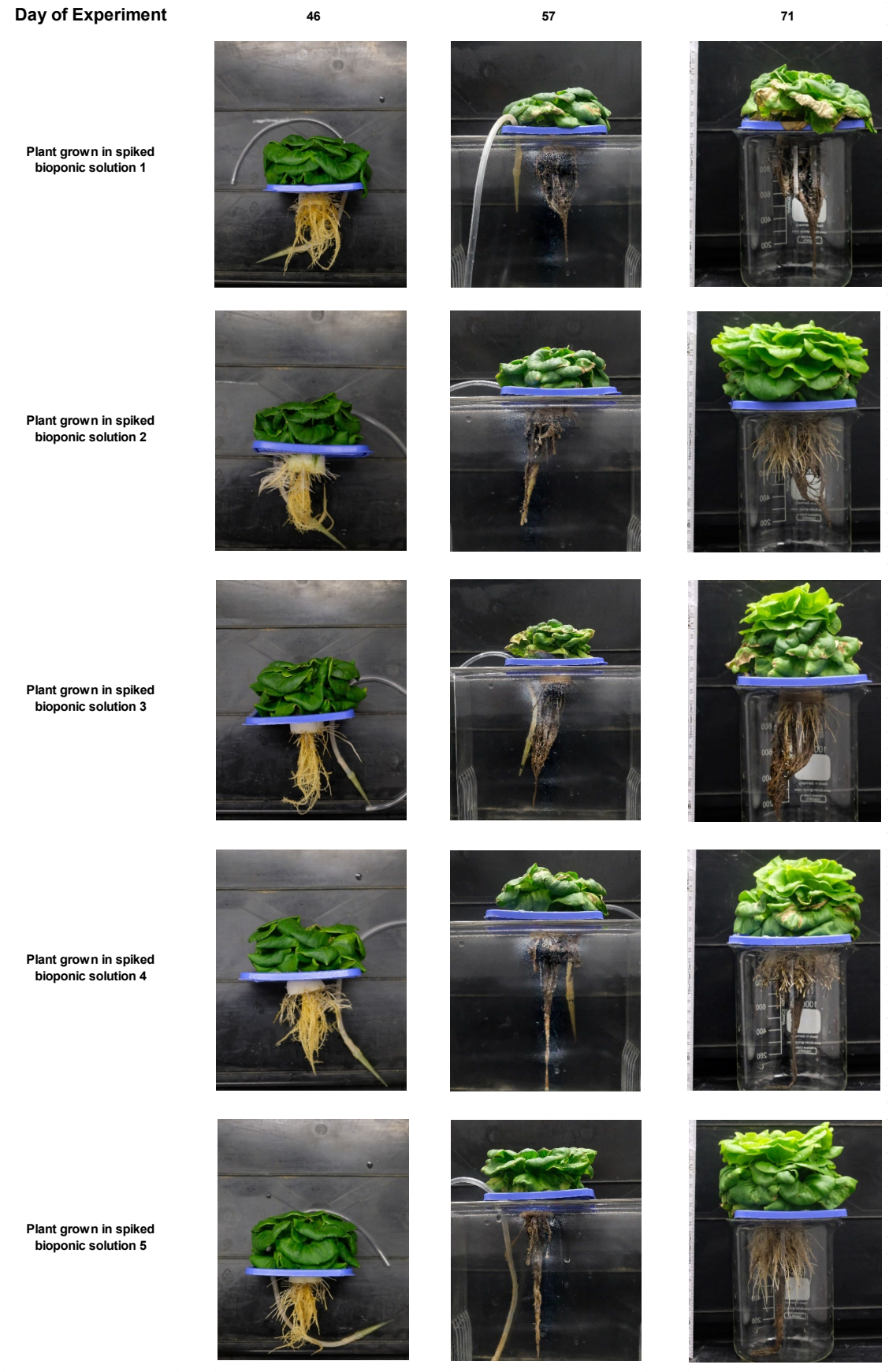


Figure 48: The five lettuce plants grown in spiked bioponic nutrient solution on day of experiment 46, as the comparison between the three different nutrient solutions started, 57 and harvest day 71. Plant 1 was excluded from the evaluation.

5.2.6 Nutrient Mass Development and Reduction

This section presents the added total mass of NH_4^+, NO_3^-, PO_4^{3-}, and K^+ (added mass), added by the respective nutrient solution, and the mass measured after four or three days (remaining mass), as the solution was replaced. Because samples of the added ***B****NS* and ***SB****NS* were taken only on DOE 53, 60, and 67, the added masses on DOE 50, 57, and 64 were calculated using mean values and the added volume. The added mass on DOE 46 was the mass that was obtained by the mixing ratios (Table 21) and the added volume. The added mass of nutrients in the ***M****NS* is based on the recipe. Every time the nutrient solution was replaced, one liter of new nutrient solution was added; thus, apparent changes in the added mass correspond to changes in nutrient concentration. Nutrient reduction between added mass and remaining mass within the intervals is equated with uptake by the plant for simplicity and to avoid misunderstanding. In the discussion, this point is taken up again (Chapter 6.2.4).

In the initial phase, there were no considerable differences in nutrient uptake between the pre-mineral, pre-bioponic, and pre-spiked-bioponic groups in the weekly intervals (Figure 49, Figure 50, Figure 51). All groups had the low percentual uptake in the last short interval of the initial phase in common (DOE 43 – 46). The following presents the nutrient mass development of the added nutrients and the uptake during the intervals in the comparison phase according to treatment (***M****NS*, ***B****NS*, ***SB****NS*).

5.2.6.1 Mineral Nutrient Solution

No substantial changes were observed in the added mass of each nutrient in the ***M****NS* (Figure 49). The most substantial percentual uptake of added NH_4^+, NO_3^-, and K^+ by the lettuce plants was measured in the interval DOE 46 to 50, as the added NH_4^+ mass decreased by 54.5 %, the NO_3^- mass by 40.3 % and K^+ mass by 49.2 %. During the comparison phase, the nutrient uptake by the plants showed recurring fluctuations, most pronounced for NH_4^+ (Figure 49). Toward the experiment's end, the nutrient uptake was slightly reduced. This trend was visible for all nutrients except PO_4^{3-}. Within the comparison phase, the lowest percentual uptake for all nutrients was measured in the penultimate interval from DOE 64 – 67.

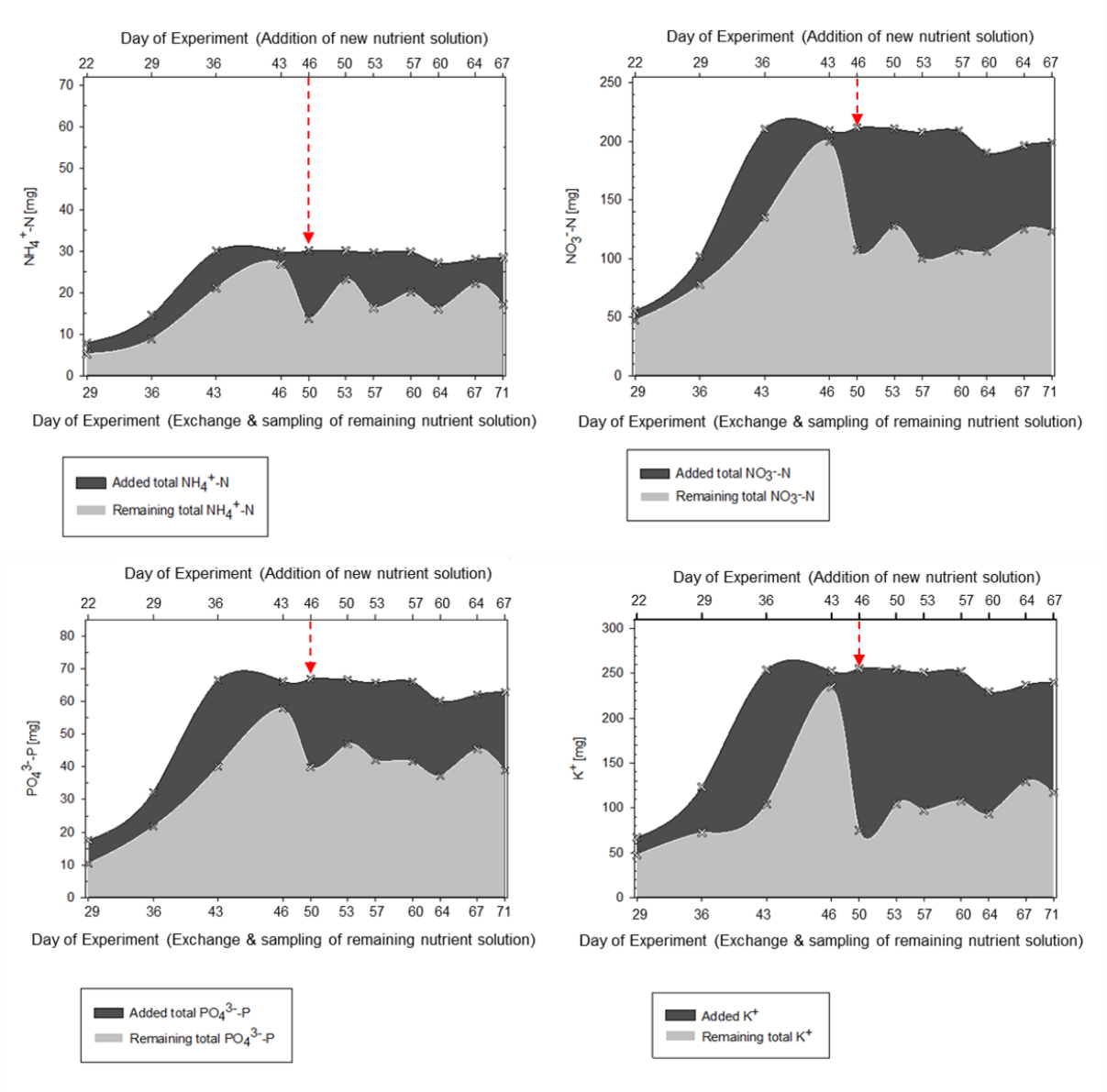


Figure 49: Added and remaining nutrient masses of the mineral solution. Days nutrient solution was added are shown on the upper x-axis; added nutrient mass refers to this axis. Days the nutrient solution was exchanged and sampled are shown on the lower x-axis; remaining nutrient mass refers to this axis. Stacked symbols (x) represent the added and remaining mass of nutrients within an interval of three or four days. The difference between two symbols corresponds to the nutrient uptake by the plant. The red dotted arrow marks the start of the comparison phase and the added nutrient mass on that day. For presentation reasons, no linear axis labels were selected. All values are mean values (n=4); no standard deviation was included for presentation reasons.

5.2.6.2 Bioponic Nutrient Solution

In the ***B****NS*, the only added nutrient mass that remained constant throughout the comparison phase was K^+; the added mass of all other nutrients decreased compared to the added mass on DOE 46 (Figure 50). The percentual decrease was most severe for NO_3^-; curiously, even higher NO_3^- masses were measured in the remaining nutrient solution on DOE 64, 67, and 71 as added on DOE 60, 64, and 67, respectively. The most substantial percentual decrease of added PO_4^{3-} and NH_4^+ mass was from DOE 50 to 53 (Figure 50).

The strongest uptake of nutrient mass was either measured in interval DOE 53 – 57 for NH_4^+, NO_3^-, and K^+ with a nutrient mass uptake of 73.9 %, 90.0 %, and 25.2 %, respectively, or DOE 57 – 60 for PO_4^{3-} with 83.6 % (Figure 50). The percentual and total NH_4^+ uptake was stronger by the *PG**B*** compared to the *PG**M*** (Figure 49) or *PG**SB*** (Figure 51).

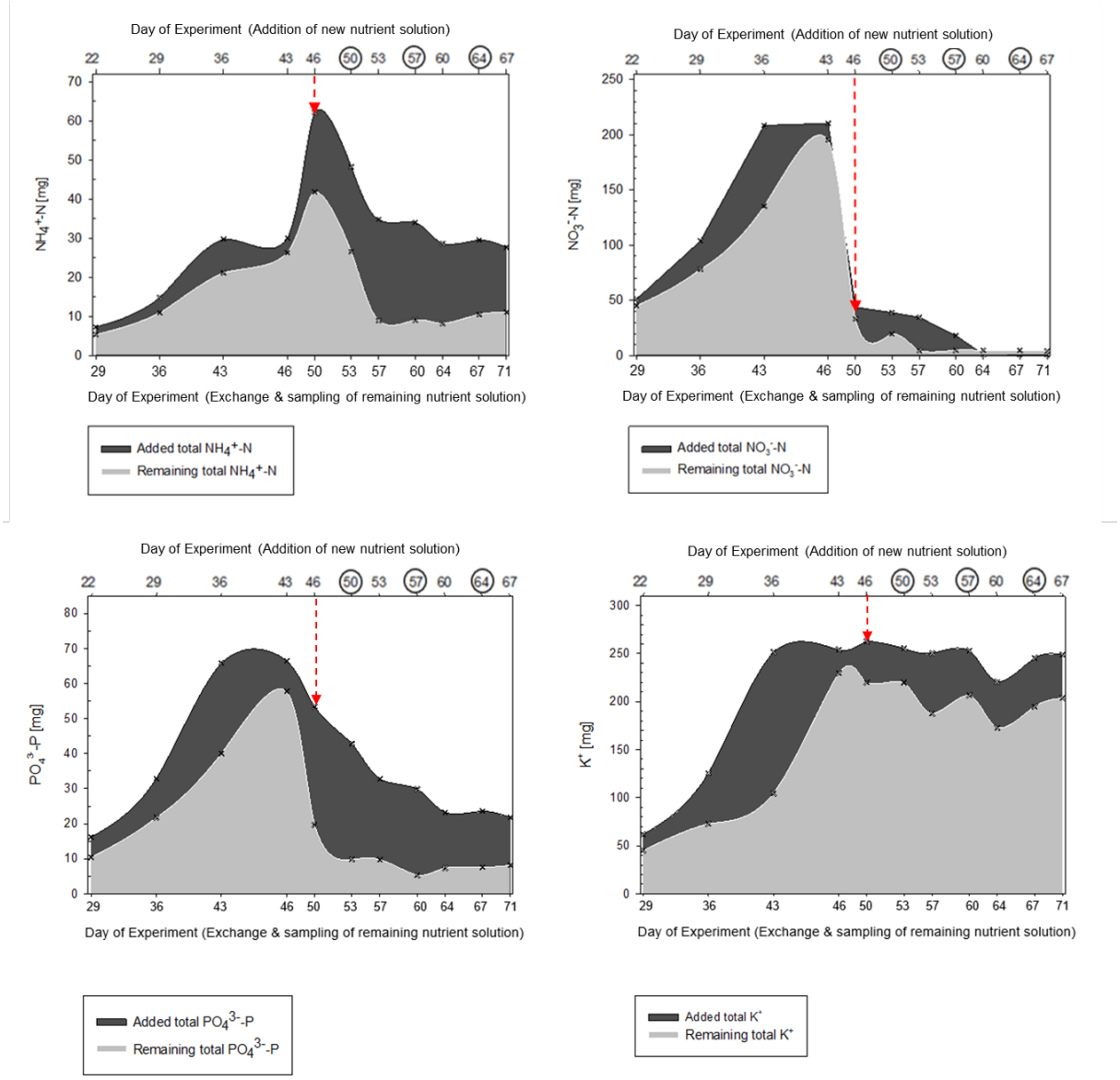


Figure 50: Added and remaining nutrient masses of the bioponic solution. Days nutrient solution was added are shown on the upper x-axis; added nutrient mass refers to this axis. Days the nutrient solution was exchanged and sampled are shown on the lower x-axis; remaining nutrient mass refers to this axis. Stacked symbols (x) represent the added and remaining mass of nutrients within an interval of three or four days. The difference between two symbols corresponds to the nutrient uptake by the plant. The red dotted arrow marks the start of the comparison phase and the added nutrient mass on that day. For presentation reasons, no linear axis labels were selected. All values are mean values (n=4); no standard deviation was included for presentation reasons. Circled days represent days added nutrient mass was calculated by means.

5.2.6.3 Spiked Bioponic Nutrient Solution

As for the ***B**NS*, also for the ***SB**NS*, the added mass of K^+ remained most stable (Figure 51). Added NO_3^- mass, added by $CaNO_3$, and added NH_4^+ mass showed fluctuations. On DOE 60,

only 62 % of the NH_4^+ mass added one week before (DOE 53) was added. A strong decrease in added mass was measured for PO_4^{3-}, which was very pronounced between DOE 46 and 53 (Figure 51). The decrease was stronger than in the ***B**NS* (Figure 50).

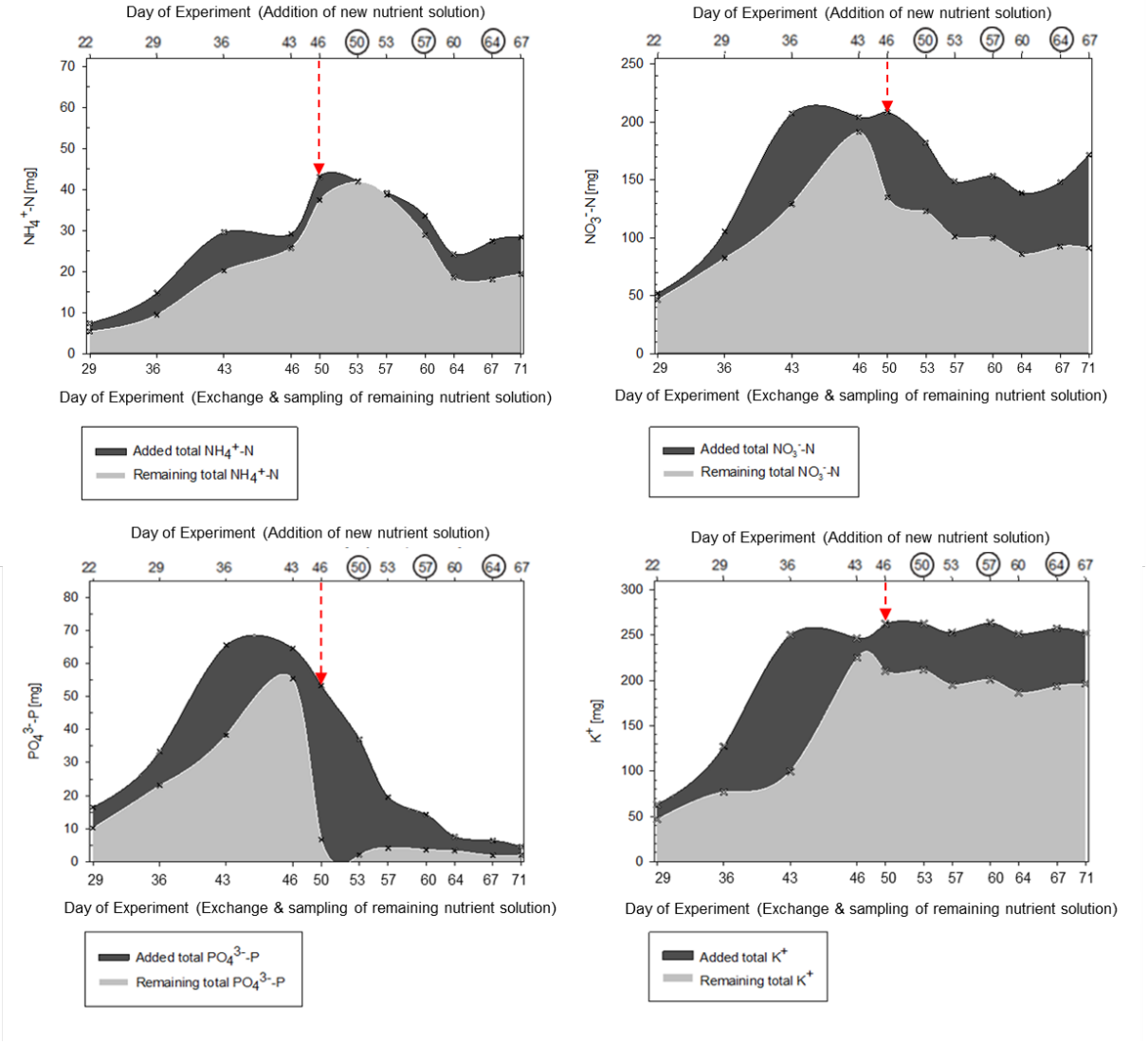


Figure 51: Added and remaining nutrient masses of the spiked bioponic solution. Days nutrient solution was added are shown on the upper x-axis; added nutrient mass refers to this axis. Days the nutrient solution was exchanged and sampled are shown on the lower x-axis; remaining nutrient mass refers to this axis. Stacked symbols (x) represent the added and remaining mass of nutrients within an interval of three or four days. The difference between two symbols corresponds to the nutrient uptake by the plant. The red dotted arrow marks the start of the comparison phase and the added nutrient mass on that day. For presentation reasons, no linear axis labels were selected. All values are mean values (n=4); no standard deviation was included for presentation reasons. Circled days represent days added nutrient mass was calculated by means.

The NH_4^+ uptake of the *PG**SB*** was very low at the beginning of the comparison phase (Figure 51). However, the NH_4^+ uptake increased in later intervals. The lowest uptake was measured in the DOE 50 – 53 interval; only 0.2 % of added NH_4^+ mass was taken up. The same applied to K^+; only 19.5 % of the added K^+ was taken up in this interval. The lowest NO_3^- uptake with 32.1 % of the added mass was measured in the interval DOE 53 - 57.

The highest NH_4^+ and NO_3^- uptake was measured in the penultimate or last interval, respectively. K^+ uptake was highest in the interval DOE 60 – 64 (25.8 % of added K^+). For PO_4^{3-} the highest uptake was measured already in interval DOE 50 – 53 (Figure 51).

5.2.7 Leaf Elemental Analysis

The analysis of the lettuce leaves harvested at the end of the cultivation revealed mainly lower nutrient concentrations in the leaves of the *PG**B*** and *PG**SB*** compared to the *PG**M*** (Table 23). The lowest nutrient concentrations in the leaves of the *PG**B***, compared to the leaves of the *PG**M***, were measured for P, Ca, and Mg. For each of these nutrients, 0.6 times the concentration of the leaves of the *PG**M*** was measured. For the *PG**SB***, the lowest leaf nutrient concentration with 0.5 times the leaf nutrient concentration of the *PG**M*** was measured for Zn. P concentration was 0.6 times the concentration of the leaves of the *PG**M*** (Table 23).

Table 23: Results of the elemental analysis of the lettuce shoots. Plants grown either in mineral, bioponic, or spiked bioponic nutrient solution. Comparison of the nutrient concentrations of plants grown in bioponic (B/M) and plants grown in spiked bioponic nutrient (SB/M) divided by concentrations of the plants grown in mineral solution (M).

Element	Mineral	Bioponic	B/M	Spiked bioponic	SB/M
		Macronutrients			
	[g/kg]	[g/kg]		[g/kg]	
C	401.5 ± 4.3	421.0 ± 2.6	1.0	409.2 ± 3.6	1.0
N	49.6 ± 1.0	33.1 ± 4.2	0.7	42.4 ± 7.1	0.9
P	8.1 ± 0.8	4.7 ± 0.7	0.6	4.4 ± 0.4	0.6
K	48.6 ± 4.0	43.3 ± 2.8	0.9	57.6 ± 9.7	1.2
Ca	12.9 ± 1.3	8.2 ± 1.2	0.6	12.6 ± 1.2	1.0
Mg	2.8 ± 0.2	1.6 ± 0.2	0.6	1.8 ± 0.2	0.7
S	2.6 ± 0.1	1.7 ± 0.3	0.7	1.8 ± 0.2	0.7
		Micronutrients			
	[mg/kg]	[mg/kg]		[mg/kg]	
B	26.8 ± 3.2	21.4 ± 2.6	0.8	29.5 ± 4.7	1.1
Fe	78.2 ± 8.6	80.7 ± 27.3	1.0	60.3 ± 7.5	0.8
Zn	33.5 ± 4.5	22.3 ± 3.8	0.7	18.2 ± 0.7	0.5
Cu	4.3 ± 0.6	5.4 ± 1.5	1.2	3.9 ± 0.8	0.9
Mn	20.6 ± 1.4	21.6 ± 5.5	1.0	18.5 ± 5.0	0.9
Mo	10.4 ± 1.6	7.2 ± 1.0	0.7	7.1 ± 1.1	0.7

5.2.8 Allocation of Nutrients

The total nutrient mass available for a single lettuce plant during the initial and comparison phase differed only marginally for K^+ for the three treatments (Table 24). In contrast, a *PG**SB*** had only 51 %, a *PG**B*** 65 % of the available P of a *PG**M***. Only 46 % of the N mass of a *PG**M***

was available for a *PG**B***. The total mass of each main nutrient measured in the shoot of a *PG**M*** was many times higher than in a *PG**B*** or a *PG**SB*** (Table 24).

Table 24: Allocation of nutrients. Added and remaining nutrient masses in the mineral, bioponic and spiked bioponic solutions, measured nutrient masses and undetected nutrient masses. All values are mean values (n=4) referring to one single lettuce plant.

	Mineral solution		
Allocation	**N**	**P**	**K**
	[mg/plant]		
Added	2288.8 ± 9.4	632.5 ± 7.8	2417.0 ± 9.1
Remaining in solution	1447.8 ± 46.0	422.0 ± 13.1	1569.0 ± 66.5
Shoot	757.8 ± 9.1	125.1 ± 9.6	754.1 ± 61.2
Undetected	83.2 ± 47.9	85.4 ± 25.9	93.9 ± 107.5

	Bioponic solution		
Allocation	**N**	**P**	**K**
	[mg/plant]		
Added	1059.8 ± 17.3	408.5 ± 18.1	2447.3 ± 52.4
Remaining in solution	710.3 ± 47.5	197.8 ± 11.7	1867.3 ± 114.6
Shoot	167.8 ± 16.3	23.8 ± 10.9	211.5 ± 29.0
Undetected	181.8 ± 31.5	187.0 ± 6.4	368.5 ± 90.7

	Spiked bioponic solution		
Allocation	**N**	**P**	**K**
	[mg/plant]		
Added	2039.3 ± 20.1	322.8 ± 29.1	2490.8 ± 59.7
Remaining in solution	1441.5 ± 47.1	149.8 ± 22.2	1842.5 ± 47.4
Shoot	228.3 ± 19.9	22.5 ± 6.0	289.8 ± 11.9
Undetected	369.5 ± 43.0	150.5 ± 12.6	358.5 ± 33.0

The undetected nutrient masses, which were neither measured in the remaining nutrient solution nor in the shoots of the plants, were much higher in the bioponic and spiked bioponic treatments. About the same P and K masses were undetectable for the bioponic and spiked bioponic treatments. In contrast, the undetected N mass was two times higher in the spiked bioponic than in the bioponic treatment (Table 24).

5.3 Post-Experimental Analysis of the Reactor Solutions

The *NO_3^--Reactor II* samples taken on the day the cultivation experiment ended revealed a substantial reduction in NO_3^- and NH_4^+ concentration (Table 25) compared to the concentrations measured in the final analysis of the mineralization experiment (Table 19). NO_3^- concentration had decreased by 89 % compared to the concentration measured in the final analysis of the mineralization experiment (Table 19). Also, the NH_4^+ concentration of the *P-Reactor* had decreased (Table 25), whereas the PO_4^{3-} concentration had increased. The other nutrients showed no substantial concentration changes.

Table 25: Post-experimental analysis of the reactor solutions after the cultivation experiment.

Nutrient	**NO_3^--Reactor II**	**P-Reactor**	**K-Reactor**
		[mg/l]	
NH_4^+-N	2.7 ± 0.0	75.8 ± 1.1	101.5 ± 2.7
NO_3^--N	7.4 ± 0.1	0.2 ± 0.0	0.3 ± 0.0
PO_4^{3-}-P	0.1 ± 0.0	193.6 ± 2.5	6.4 ± 0.1
K^+	37.6 ± 0.1	39.8 ± 1.7	2507.8 ± 27.8

If the *NO_3^--, P-, and K-solutions* with the nutrient concentrations measured after the cultivation experiment (Table 25) were mixed according to the ratio used to mix the ***B**NS* (Table 20), almost no NO_3^--N would be present in the solution (Figure 52). PO_4^{3-}-P and K^+ concentrations changed not drastically. As well as the NH_4^+-N concentration, they were within the range of the reference mineral nutrient solutions (Table 4).

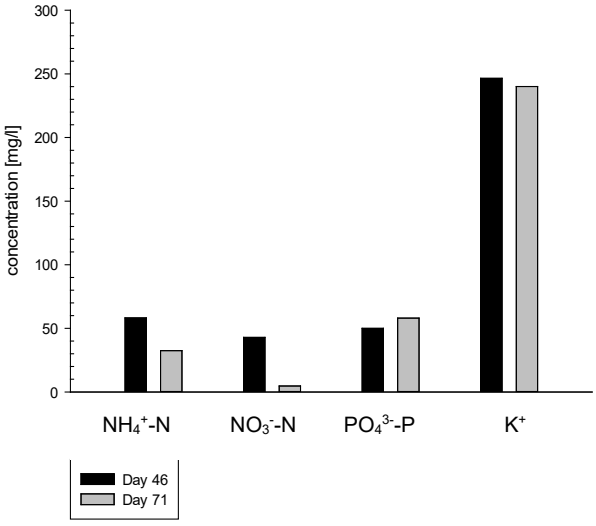


Figure 52: Calculated changes in nutrient concentrations in the bioponic solution between day of experiment 46 and 71. Mixed by the bioponic stock solutions according to the ratio set on day of experiment 46.

6. Discussion

In the following, the results of the previous chapter are discussed. First, the mineralization of the nutrients in the individual reactors for producing a bioponic nutrient solution is discussed, then the hydroponic experiment results.

6.1 Production of a Bioponic Nutrient Solution

The results of the *NH_4^+-*, *NO_3^--*, *P-*, and *K-Reactors* are discussed in separate sections. In the end, the fertilizing quality of the produced ***B****NS* is assessed regarding nutrient availability and balance.

6.1.1 NH_4^+-Reactors

Both *NH_4^+-Reactors* reached very high NH_4^+ concentrations. With a maximum mineralization rate of 90 and 98 % for *Reactor I* and *II*, respectively, almost all added N by organic residues was mineralized into NH_4^+-N.

However, it took twice as long to reach a concentration of over 1000 mg/l NH_4^+-N in *Reactor I* compared to *Reactor II* (Figure 21). One reason for this time difference could be the different temperatures measured in the two reactors. The start temperature of *Reactor I* was 3 °C lower than the start temperature of *Reactor II*, and it took twice as long to reach the intended 30 °C in *Reactor I* than in *Reactor II* (Figure 19). Temperature strongly influences the activity and growth of MOs (Stefanakis et al., 2014). At higher temperatures, the activity is generally increased. The strong temperature increase in *Reactor I* between DOE 24 and 33 (Figure 19) depicts the correlation between temperature, anaerobic digestion, and the associated NH_4^+ production. The NH_4^+ measurements next to this period (DOE 24 and 36) showed the most substantial concentration increase for *Reactor I* (Figure 21). Even though the temperature optimum for anaerobic digestion is between 35 – 70 °C (Meegoda et al., 2018), which could not be fully achieved with available means, every degree closer to the optimum temperature range increases the activity of the involved MOs, and thus NH_4^+ release. Therefore, higher NH_4^+ concentrations may have been reached more rapidly in *Reactor I*.

The difference in temperature between the two reactors, although the same heating rod was used, was very likely caused by the different starting dates. While *Reactor I* was started in April 2022, as the mean outside temperature in Stuttgart was 8.6 °C (DWD, 2023). *Reactor II* was started in June, as the mean temperature was 19.7 °C (DWD, 2023). Since the hall where the experiments were conducted was unheated, the outside temperature significantly affected the reactors' temperature. The heating device was unsuitable for quickly heating the 45 l of water to 30 °C.

A second reason for the faster N mineralization into NH_4^+ from the organic residues in *Reactor II* could be the reuse of the mash bags already used in *Reactor I*. No new compost as source of MOs was added to *Reactor II*. However, compost contains mainly aerobic (Neklyudov et al., 2008) and only a few anaerobic MOs (Fuchs, 2010). In the first run, these anaerobic MOs had to grow and settled, among other things, on the mash bags. These were reused in the second run so that a well-established community of anaerobic MOs, such as ammonifying bacteria, could be present from the beginning. Thus, the anaerobic digestion process occurred faster, and N was mineralized faster into NH_4^+.

The substantial NH_4^+ increase in *Reactor I* between DOE 24 and 36 is also reflected by the increasing pH between DOE 21 and 33 (Figure 20). The release of NH_3 in the acidogenesis increases pH (Körner, 2009). This increase is visible despite the performed pH control. If pH adjustment was conducted for *Reactor I*, the pH was lowered only to 6.5, and during the three days between the adjustments, the pH had increased again. For *Reactor II*, the pH was lowered more drastically; therefore, the pH rarely exceeded 7, and pH regulation was less often necessary.

The high mineralization rate of N into NH_4^+ in both reactors can likely be attributed to the low C:N ratio of blood meal of approximately 3.5 (Lazicki et al., 2020). The preliminary experiment's highest achieved N mineralization rate was 12.5 % (Appendix I). Instead of blood meal, the primary N source was goat manure with a C:N ratio of 9.5-19.9 (Ansah et al., 2019). Lazicki et al. (2020) examined different organic fertilizers, among them blood meal, on their N mineralization rate in a soil incubation experiment. After 84 days, over 70 % of the initially added N by blood meal was measured in mineral form. They attributed the high mineralization rate to the low C:N ratio of blood meal. Other tested fertilizers with higher C:N ratios had lower mineralization rates due to immobilization by MOs, which fed on the C contained (Lazicki et al., 2020). Calderón et al. (2005) and Gale et al. (2006) reported an immobilization of N at C:N ratios higher than 15 - many times above the C:N ratio of blood meal. The addition of glucose in the present study, which was done to feed the heterotrophic MOs in the *NH_4^+-Reactors,* and the addition of acetic acid, additionally adding C, had no adverse effect on the mineralization rate in terms of immobilization. The even higher mineralization rates in this study, as reported by Lazicki et al. (2020) can be attributed, for instance, to differences in medium and time.

The anaerobic digestion of blood meal, as used in the present study, is very well suitable to mineralize the contained N into NH_4^+ and produce a digestate especially rich in NH_4^+. The nutrient with the second highest concentration in the digestate, Ca, had only 0.1 times the concentration of NH_4^+-N (Table 19).

6.1.2 NO_3^--Reactors

No major differences were observed in temperature, DO, and pH for the two *NO_3^--Reactors* (Figure 24, Figure 25). The reactors differed only in the method of establishing a nitrifying community, in the mass of added glucose per liter and day (Chapter 4.1.4.2), and in the NH_4^+ concentration at the beginning of the transfer. One of these differences, or a combination, must be responsible for the higher nitrification in *Reactor II* (Figure 26, Figure 27).

Despite the higher NH_4^+-N concentrations at the beginning of the transfer, which exceeded the recommendations by Shinohara et al. (2011) for optimum nitrification of 31.5 mg/l N, *NO_3^--Reactor II* yielded higher NO_3^- concentrations than *Reactor I*. However, the NH_4^+-N concentration of *NO_3^--Reactor II* exceeded the recommendation only during the first two measurements (Figure 27). Apparently, in this period, no nitrification occurred (Figure 27). Also, the possible NH_4^+ concentration that can be transformed into NO_3^- highly depends on the number of nitrifying bacteria and the species (van Niel et al., 1993). Thus, a maximum threshold of NH_4^+ concentration for nitrification is hard to define without knowing the abundance and species of nitrifying bacteria. The determination of MO species is possible with methods like polymer chain reaction or next-generation sequencing (Pavlovic et al., 2011), which are costly and time intensive and would have gone beyond the scope of this thesis. In the present study, possibly resource-conserving methods were applied to cultivate nitrifying bacteria. Whether these methods succeeded or not was only visible in the measured NO_3^- concentrations in both *NO_3^--Reactors* and the NH_4^+ and NO_2^- concentrations in *NO_3^--Reactor II*. It appears that the cultivation of nitrifying bacteria in the “start phase” of each reactor, which aimed to establish a nitrifying community, was not ideal. This is indicated by *Reactor II*'s NH_4^+-NO_2^--, and NO_3^--curves (Figure 27). The curves followed a phenomenon called “New Tank Syndrome” in aquaristics (Roberts & Palmeiro, 2008). It states that in a new environment, bacteria that oxidize NH_4^+ into NO_2^- establish faster than NO_2^--oxidizing bacteria. Therefore, NO_2^- accumulates because too few NO_2^--oxidizing bacteria are present. After a specific time, enough NO_2^--oxidizing bacteria have formed, and NO_2^- is directly transformed into NO_3^-, without accumulation (Roberts & Palmeiro, 2008). Since the “New Tank Syndrome” occurred, it can be assumed that during the start phase of *Reactor II* no sufficient community of nitrifying, or at least NO_2^--oxidizing, bacteria was established. During the start phase of both reactors, only glucose was added to feed the heterotrophic nitrifying bacteria present in sewage sludge (Körner, 2009). Autotrophic nitrifiers, added by compost and sewage sludge, were outnumbered by the heterotrophic nitrifiers due to a high C:N ratio (Samocha & Prangnell, 2019; van Niel et al., 1993) caused by the addition of only glucose. If heterotrophic nitrifiers have optimum growing conditions, they grow fast; however, their nitrification rate is low compared to autotrophic nitrifiers (van Niel et al., 1993).

It appears that the heterotrophic nitrifiers did not find optimum growing conditions during the start phase. For optimum growth, heterotrophic MOs require, besides C also, N, and various micronutrients (Bast, 2001), which were not supplied in the start phase. Therefore, the heterotrophic nitrifying bacteria community did not develop properly. Since for both reactors only glucose was added in the start phase, this applied to both. However, it can be assumed that in *Reactor II* more heterotrophic nitrifying bacteria were present as in *Reactor I* as the NH_4^+-rich solution transfer started. For *Reactor II*, sewage sludge and MO carriers were directly added to the reactor, whereas only the assumed inoculated MO-carriers were transferred in *Reactor I* (Chapter 4.1.4.2). Apparently, only very few nitrifying bacteria had settled on the MO-carriers.

As the NH_4^+-rich solution transfer started, N and some other necessary micronutrients for heterotrophic MO growth were added. The higher concentration of heterotrophic nitrifying bacteria in *Reactor II* probably benefitted from the twice as high glucose addition and grew faster. Thus, a larger nitrifying community was established, leading to the higher NO_3^- concentrations measured in *Reactor II*. The curves in Figure 27 indicate that the experimental period ended, as a sufficiently large population of NO_2^--oxidizing bacteria was established to transform NH_4^+ into NO_3^- without the accumulation of NO_2^-. It can be assumed that if the experimental period had lasted longer and the transfer of *NH_4^+ solution* had continued, NO_3^- concentration would have increased further.

Despite *Reactor II's* higher NO_3^- concentration, only 5 % of the added NH_4^+-N was converted into NO_3^--N. Since also low NH_4^+ and NO_2^- concentrations were measured in the *NO_3^--Reactor II solution* (Figure 27, Table 19), most of the added NH_4^+ must either have been incorporated by MOs or lost to NH_3 outgassing due to ventilation and a pH above or close to 7 (Figure 25). In contrast to the present study, Shinohara et al. (2011) achieved conversion rates of organic N into NO_3^--N of 30.5 to 99.7 %, depending on the C:N ratio of the used organic source. They used chemolithoautotrophic nitrifiers such as *Nitrosomonas* spp. and *Nitrospira* spp. Organic C inhibits these MOs' growth; thus, higher conversion efficiencies were achieved at low C:N ratios by Shinohara et al. (2011). In the present study, nitrification by heterotrophic MOs was targeted. However, autotrophic nitrifiers could have been more appropriate due to the experiences and results of Shinohara et al. (2011) and the independence of organic C, such as glucose, a precious resource, especially in countries of the global South. Furthermore, heterotrophic nitrifiers are considered more difficult to cultivate (Körner, 2009), and some heterotrophic nitrifiers present in sewage sludge of wastewater treatment plants simultaneously perform nitrification and denitrification in aerobic environments (Khanichaidecha et al., 2019). Consequently, it is possible that a part of the produced NO_3^-

was lost to denitrification. This or increased incorporation by MOs can also explain the NO_3^- reduction observed in *Reactor I* from DOE 57 onwards (Figure 26).

The higher start NO_3^- concentration in *Reactor II* (Figure 27) compared to *Reactor I* (Figure 26) can be explained by the sewage sludge added directly into *Reactor II*, whereas only the MO carriers were added into *Reactor I*. Sewage sludge can contain some NO_3^- (Castro et al., 2009), which likely caused the higher start concentration.

To summarize, the nitrification rate in none of the two *NO_3^--Reactors* was adequate to provide a NO_3^- concentration comparable with commercial hydroponic nutrient solutions. Even if *Reactor II*'s *NO_3^--solution* was used independently, without adding the *P*- and *K*- *solutions*, not even 50 % of the NO_3^--N concentration of commercial nutrient solutions (Table 4) would be achieved.

6.1.3 P-Reactor

The increasing temperature measured in the *P-Reactor* is most certainly correlated to the simultaneously increasing ambient temperature (DWD 2023). As for the *NH_4^+-Reactors*, the used heating device seems insufficient to heat the water to 30 °C quickly.

The strongest PO_4^{3-} concentration increase occurred between DOE 24 and 36 (Figure 30). Within this period, the largest temperature increase was observed (Figure 28). Furthermore, from DOE 27 onwards, the pH was continuously below 5.5 (Figure 29). 5.5 is the pH threshold described by Epple and Enax (2018), below which hydroxyapatite ($Ca_5(PO_4)_3(OH)$), the main component of bones, is dissolved. A correlation between increased temperature and low pH seems obvious. However, by lowering the pH below 5.5, it was accepted that anaerobic digestion would not take place completely, since for the last anaerobic digestion step, during which gaseous CH_4 is produced, a neutral pH is necessary (Meegoda et al., 2018). This could have resulted in high TOC concentrations in the *P-solution*.

Despite the low pH, only 19.7 % of the added P was mineralized into PO_4^{3-}-P. This was lower than the P mineralization rate in the preliminary experiment (Appendix I) and this in a trial period almost four times as long. However, the PO_4^{3-} concentration increased until the end of the experiment. It can be concluded that P inherent in bone meal is mineralized only slowly with the used approach. In the preliminary experiment, other organic residues than bone meal were used as P source, which most certainly released P more easily. Perhaps the pure chemical approach, only lowering the pH below 5.5, is not the most effective. MOs known to solubilize P in soil, genera like *Azotobacter, Burkholderia, Enterobacter*, *Erwinia*, or *Flavobacterium* (Bhattacharyya & Jha, 2012) could accelerate P mineralization. However, acidification is also the main mechanism of these MOs to solubilize P (Richardson et al., 2009).

Although no MOs were added via inoculation, the observed mold formation in the *P-Reactor* from DOE 24 onwards indicated the presence of MOs. As a heterotrophic organism, mold consumes N, P, and other nutrients (Nelson 1993; Bajaj et al. 1954) and thus may also have contributed to the low PO_4^{3-} concentrations. Furthermore, mold formation proves that a certain amount of O_2 was present in the anaerobic reactor, which is necessary for mold growth (Nelson 1993). The regular opening and stirring caused the O_2 input into the reactor and prevented, in combination with the low pH, complete anaerobic digestion. It is likely that besides mold, other aerobic, anaerobic, or facultative anaerobic MOs were present in the *P-solution*.

6.1.4 K - Reactor

As expected from the preliminary experiment (Appendix I) and the pre-test (Chapter 5.1.4), K inherent in the dried potato mix was mineralized fast in solution (Figure 35). Precisely, the term mineralization is not accurate in this case since K occurs only as unbound readily soluble K^+ ion in organic material. Thus, simple leaching easily transfers it through cell membranes (Ako et al., 2003; Sardans & Peñuelas, 2015). The ease of leaching combined with the high mobilization rate of 87 % achieved in the present study makes K readily available to prepare nutrient solutions from organic residues. A long digestion process, as applied in this study, is not necessary; this could even have rather negative effects, such as the observed mold growth. As for the *P-Reactor*, the mold indicated a non-perfect anaerobic environment and the possible presence of other MOs.

The low pH observed in the *K-Reactor (*Figure 34*),* although no pH control took place, can be explained by a not completed anaerobic digestion process or by different organic acids, like chlorogenic, proto-catechuic, and caffeic acids, present in potato peels (Onyeneho & Hettiarachchy, 1993). The latter reason certainly had an impact, as the low pH was noticeable directly from the beginning (Figure 34). As for the *P-Reactor*, the continuously low pH and the presence of O_2 most certainly prevented the methanogenesis step of the anaerobic digestion.

6.1.5 Regression of EC vs. Nutrient Concentrations

For EC vs. NH_4^+-N (Figure 22), PO_4^{3-}-P (Figure 31), and K^+ (Figure 36), a linear regression line was fitted, respectively. By this, the possibility of determining the specific nutrient concentration derived from the chosen organic residue by the easy measurement of EC should be evaluated.

The coefficient of determination of the regression line was higher for EC vs. NH_4^+-N and EC vs. K^+ as for EC vs. PO_4^{3-}-P. The final analysis results (Table 19) explain the lower R^2 of PO_4^{3-}-P. For the *NH_4^+-Reactor II* and the *K-Reactor,* the highest concentrations were measured for the nutrient of interest, NH_4^+-N, and K^+, respectively. Whereas for the *P-Reactor*, the highest concentration was not measured for PO_4^{3-}-P, but for Ca. NH_4^+-N concentrations were also

comparatively high compared to the PO_4^{3-}-P concentration. This means that the concentrations of the desired nutrient most strongly influenced the EC of the *NH_4^+-Reactor* and the *K-Reactor*. In contrast, besides PO_4^{3-}-P, Ca, and NH_4^+-N concentrations also strongly influenced the *P-Reactor's* EC.

The regression lines of EC vs. NH_4^+-N and EC vs. K^+ can serve as a reference for the concentration of the respective nutrient. By this, an easy and cheap measurement method for similar experiments could be established. However, the amount of added acid, if used, must be considered, and the results are based on one (*K-Reactor*) or two (*NH_4^+-Reactor*) repetitions only and need further verification. Furthermore, it must be considered that organic residues can vary greatly in their elemental composition. In the case of both plant and animal residues, the species and nutrition have a major influence on this. Nazifa et al. (2021) compared the element content of fresh animal blood of different livestock species. Whereas the N content mainly ranged between 14 and 25 %, other elements like K and Na differed strongly depending on the species. Nazifa et al. (2021) reported K^+ concentrations of 118 mg/l in pig blood and 667.9 mg/l in cow blood. While K is an essential nutrient for all higher plants, Na, which has some beneficial functions in the plant, can cause severe growth reductions at higher concentrations. Especially in hydroponics, Na can severely impair plant growth (Khan et al., 2021). Nazifa et al. (2021) reported Na^+ concentrations of 94 mg/l in pig, 1650 mg/l in swine, and 2380 mg/l in cow blood. When used in a hydroponic system, the measured Na^+ concentrations, especially of the latter two, would make it impossible to grow almost any crop. (Morgan, 2021). Furthermore, the high Na^+ concentration would influence the EC and thus reduce the correlation between EC and the plant essential nutrients. The study of Nazifa et al. (2021) illustrates how extremely variable the nutrient composition of organic residues can be and that using EC as a measure of nutrient concentration is hardly applicable in bioponic systems. A prerequisite for using EC in bioponic systems would be a constant and known nutrient composition of the organic residues used, which is nearly impossible.

6.1.6 Fertilizing Quality of the Produced Bioponic Nutrient Solution

To evaluate the fertilizing quality, the nutrient concentrations of the produced ***B****NS* and ***SB****NS* (Table 21) were compared to the nutrient range of reference mineral solutions (Table 4).

PO_4^{3-}-P and K^+ concentrations met exactly the reference table's mean. Ca, B, Zn, Mn, and Mo concentrations were in the range of the reference table, as well as Mg. However, the Mg concentration was at the lower limit of the wide range of the reference table (Table 4). Cu and Fe concentrations were above the range, whereas S was below. In addition to the essential nutrients analyzed, Na was also analyzed. The concentration of 22.2 mg/l Na^+ should not

severely impair plant growth in hydroponic systems. Morgan (2021) mentions that Na^+ in hydroponic nutrient solutions generally should not exceed 50 mg/l.

The two N forms, NH_4^+ and NO_3^-, differed strongly in their ratio from the reference range. NH_4^+-N concentration was more than two times higher than recommended, whereas NO_3^--N concentration was only 0.3 times the recommendation. Especially the lack of NO_3^-, and the surplus of NH_4^+ could cause poor plant growth. Due to the unbalanced NH_4^+:NO_3^- ratio, the produced ***B****NS* cannot be considered balanced regarding the main nutrients N, P, and K.

However, compared to other ***B****NS* used in hydroponic systems, the main nutrient composition was comparatively balanced, especially regarding P and K. Many other bioponic solutions had substantially lower P and K concentrations, which were additionally unbalanced (Bergstrand et al., 2020; Kechasov et al., 2021; Krishnasamy et al., 2012; Liedl et al., 2004). Of the bioponic nutrient solutions listed in Table 7 and Table 8, only the solution of Pelayo Lind et al. (2021), who used anaerobic digestate of plant material nitrified with an MBBR, also had a balanced P and K ratio. In addition, their nutrient solution had a more balanced NH_4^+:NO_3^- ratio. Compared to the aerobically produced nutrient solutions listed in Table 8, the NH_4^+ concentration of the ***B****NS* produced in the present study is comparatively high. The NH_4^+ concentration can rather be compared with the anaerobically produced nutrient solutions (Table 7). The reason for this is the use of the anaerobic P and K solutions, which had relatively high NH_4^+ concentrations (Table 19). By aerating these solutions, the NH_4^+ concentration could have been lowered. However, this might have negatively affected the P concentration since MOs' P uptake is strongly increased under aerobic conditions (Shapiro, 1967).

The concentrations of the main nutrients (N, P, K) in the ***SB****NS* met the range of the reference table (Table 4); only NH_4^+-N was 1 mg/l above the range. However, none of the other macro- and micronutrients met the range. Whereas Ca had about two times the recommended mean concentration, also Fe and Zn were too high, Mg, S, B, Cu, Mn, and Mo were below the range. Whereas for micronutrients, different mineral solutions can also show variances in concentrations - the ***M****NS* by Taiz and Zeiger used in the present study matched the micronutrient range of the reference table only for Cu – all macronutrients of the solution by Taiz and Zeiger were within the range of the reference table.

The ***SB****NS* can be considered balanced regarding the main nutrients. However, replacing the bioponic *NO_3^--solution*, containing essential micronutrients for plant growth, with the $CaNO_3$ solution resulted in a reduction in micronutrients and an oversupply of Ca. Ca has antagonistic effects on the uptake of many other nutrients, for instance, K, Fe, P, and Mg (Goddek et al., 2019). These imbalances could result in poor plant growth.

Based on the results presented, Hypothesis 1 must be rejected. Although the key messages of the preliminary experiment were implemented and resulted in N mineralization rates into NH_4^+-N of over 95 % and a K mineralization rate of 87 %, which were above the aimed rates of 50 % and 80 %, respectively, the nitrification rate of NH_4^+-N into NO_3^--N of only 5 % and the P mineralization rate of 19.7 % were below the intended 50 % and 30 %. The ***B****NS* was not balanced regarding the main nutrients N, P, and K due to a substantial lack of NO_3^-.

6.2 Test of the Bioponic Nutrient Solution on Lettuce

The results of cultivating lettuce in the deep water culture system are discussed in this second part of the discussion.

6.2.1 pH

In hydroponics, the pH of the nutrient solution is usually influenced by the absorption of nutrients by plant roots. If the plant absorbs nutrient ions, the plant releases protons (H^+) or hydroxyls (OH^-) by the root to maintain its ionic equilibrium (Goddek et al., 2019). The decrease in pH of the ***M****NS* (Figure 38) during the cultivation trial was probably caused by higher uptake of cations than anions. This is supported by the NH_4^+, K^+, NO_3^-, and PO_4^{3-} uptake by the *PG****M*** (Figure 49). In each interval, the reduction, and thus the uptake by the plant, of the sum of measured cations was higher than that of anions. Especially the high uptake of K^+ had a strong impact.

For the ***B****NS* and ***SB****NS*, it is doubtful that the strong pH increase (Figure 38) was caused by a higher uptake of anions than cations. More likely is that microbial activity caused the increase in pH. This observation is in line with Williams and Nelson (2016), who related the high pH in bioponic systems to MO activities. MOs can alter their environment, including pH, to create optimum conditions for their growth (Ratzke & Gore, 2018). Most MOs prefer a neutral pH (Werner, 1991); thus, it is possible that MOs increased the initial pH of six of the ***B****NS* and ***SB****NS* to improve their growth conditions. High pH is commonly reported for bioponic nutrient solutions and reduces the availability of specific nutrients for the plant in hydroponics (Krishnasamy et al., 2012; Liedl et al., 2004; Mupambwa et al., 2019; Phibunwatthanawong & Riddech, 2019). The high pH of the ***B****NS* and ***SB****NS* impeded the absorption of Cu^{2+}, Zn, B, Fe^{2+}, Mn^{2+}, PO_4^{3-}, Ca^{2+}, and Mg^{2+} due to the formation of insoluble compounds and precipitation (Goddek et al., 2019).

The high pH in addition to the deficiency of several nutrients in the ***B****NS* and especially in the ***SB****NS,* can partly explain the deficiencies measured in the lettuce shoots of the *PG****B*** and *PG****SB*** (Table 23). Compared to recommendations for lettuce before harvest compiled by Hartz et al. (2007), the *PG****B*** lacked Mg and S, whereas the *PG****SB*** lacked Mg, S, Zn, and Cu. Also,

the comparatively low P concentrations measured in *PG**B*** and *PG**SB*** can be attributed, among other things, to the high pH of the ***B**NS* and ***SB**NS*.

6.2.2 Electrical Conductivity

Contrary to the expectations that by uptake of nutrients by the plants, the EC decreases, the EC of the ***M**NS* increased in the deep water culture system (Figure 39). This can be explained by the highly concentrated ***M**NS*, which was replaced already after a maximum of four days. The evapotranspiration was high (Figure 40), and the nutrient uptake was comparatively low; thus, the solution became more concentrated.

In the ***B**NS*, the EC showed no such distinct pattern (Figure 39). Due to the low nutrient concentration and low evapotranspiration (Figure 40) the EC decreased during most intervals. The reduction of EC in the added ***B**NS* from DOE 46 to 53 was caused by a reduction of nutrient concentration in the added ***B**NS* (Figure 50). Whereas in the first half of the comparison phase, the EC of the ***SB**NS* was higher in the added solution as in the remaining solution, the opposite was the case in the second half (Figure 39). Toward the end of the experiment, the evapotranspiration of the *PG**SB*** increased (Figure 40). As for the ***M**NS*, the nutrient solution became more concentrated. The changes in EC between added and exchanged solutions of the ***B**NS* and ***SB**NS* were probably caused by the fluctuating plant growth and the associated changes in nutrient uptake during the experiment.

The higher initial EC of the ***SB**NS* and especially of the ***B**NS* were caused by higher concentrations of plant non-essential salts, for instance, Na^+ (Table 19) (Williams & Nelson, 2016). The highest EC of all three treatments was measured in the ***B**NS*, indicating the highest concentration of plant non-essential salts. Further analysis would have been necessary to determine other non-essential salts in the ***B**NS* besides Na. However, it can be assumed that other non-essential salts were present in the solution since it is doubtful that the relatively low concentration of Na^+ of 20.2 mg/l in the ***B**NS* alone was responsible for the high EC.

As elaborated in Section 6.1.5 EC is less reliable for nutrient concentration determination in bioponic solutions since every organic nutrient source contains a different amount of nutrients and plant non-essential salts. For a better understanding of EC development in the ***B**NS* used, longer intervals in which the nutrient solution was not exchanged would probably have been beneficial.

6.2.3 Reduction of Nutrient Mass in the Added Bioponic and Spiked Bioponic Solutions

Substantial reductions in NH_4^+ and PO_4^{3-} added mass occurred for the ***B**NS* (Figure 50) and the ***SB**NS* (Figure 51) during the experimental period. Beyond this, the ***B**NS*'s added NO_3^-

concentration reduced drastically during the experiment, whereas in the ***SB****NS*, only some NO_3^- fluctuations were measured, which were nonetheless stronger than in the ***M****NS* (Figure 49).

The reductions in added NO_3^- and NH_4^+ are clearly related to the reductions in the *NO_3^--* and *P-solution* measured in the remaining nutrient solutions in the reactors in the post-experiment analysis (Table 25). In contrast, PO_4^{3-} concentration in the *P-solution* had even increased (Table 25). There are several possible causes for the reduction in added nutrients. The most probable for NO_3^- and NH_4^+ reductions are microbial activities or changes in pH in the *P-* and *NO_3^--solution* before use in the hydroponic system, indicated by the changes observed by the post-experiment analysis. On the contrary, for the low PO_4^{3-} concentrations, it is more likely that PO_4^{3-} precipitated or was incorporated by MOs during the long storage period of the samples before analysis.

First, it must be emphasized that the added nutrient mass on DOE 46 was based on the mixing ratios. Especially for PO_4^{3-}, the most substantial decreases in added mass in the ***B****NS* and ***SB****NS* occurred between DOE 46 and the following measurement day, DOE 53 (Figure 50, Figure 51). It is possible that already on DOE 46, a lower nutrient mass was added than expected by the calculations; for instance, by mixing the solutions, PO_4^{3-} could have precipitated. However, further substantial PO_4^{3-} reductions probably occurred during the long storage period of the samples. Indications for this theory are the high PO_4^{3-} concentrations measured in the remaining *P-solution* during the post-experiment analysis (Table 25) and the lower added PO_4^{3-} mass in the ***SB****NS* than in the ***B****NS*, visible from DOE 53 onwards (Figure 50, Figure 51). Theoretically, the same mass of PO_4^{3-} was added to the ***B****NS* and the ***SB****NS* (Table 19). However, the ***SB****NS* contained more than twice the concentration of Ca^{2+} as the ***B****NS*. In sufficient high quantity, Ca^{2+} and metal cations, like Fe^{3+}, or Al^{3+}, form, in combination with soluble PO_4^{3-}, insoluble forms, which precipitate (Stefanakis et al., 2014). An increase in pH increases precipitation (Goddek et al., 2019). It cannot be ruled out that even in the cool-stored samples, an increase in pH occurred and led, in combination with Ca^{2+} cations, to P precipitation. Since the precipitated P was not resolubilized before analysis, low PO_4^{3-} concentrations were measured in the added ***B****NS* and especially in the ***SB****NS*. In addition, P uptake by molds or other MOs could also have reduced P concentrations in the stored samples.

The reduction of added NO_3^- mass in the ***B****NS* was very pronounced. On DOE 60 and 67, almost no NO_3^- was measured in the added solution (Figure 50). This reduction is related to the low NO_3^- concentration measured in the post-experiment analysis of the *NO_3^--solution* (Table 25, Figure 52). The *NO_3^--solution* was further aerated until it was used in the hydroponic system to prevent denitrification. However, many heterotrophic nitrifiers perform nitrification

and denitrification in aerobic conditions (Körner, 2009). Since no further NH_4^+-rich solution was added to the *NO_3^--Reactor II* after the end of the mineralization experiment, the curves in Figure 27 continued until NH_4^+ was used up. Consequently, there was no further nitrification by the heterotrophic nitrifiers but only denitrification. The capability of the heterotrophic nitrifiers to denitrify can explain the decrease in NO_3^- and NH_4^+ in the added ***B**NS*, also visible in the post-experiment analysis of the *NO_3^--solution*. The ability of some heterotrophic nitrifiers to nitrify and denitrify in aerobic conditions questions the utility of these MOs for the desired purpose of accumulating NO_3^-, as already discussed in Section 6.1.2. Whereas in wastewater treatment, this ability is highly desirable, it is not helpful to produce a nutrient solution containing high NO_3^- concentrations.

Compared to the ***B**NS* (Figure 50), only minor fluctuations were measured in the NO_3^- mass of the added ***SB**NS* (Figure 51). However, they were higher than in the ***M**NS*. In the stored samples of the ***SB**NS*, most likely processes occurred, probably initiated by MO present in the *P-* and *K-solutions*, which reduced NO_3^- during the long storage period. This also occurred in the stored samples of the ***B**NS*.

The reduction of NH_4^+ concentration in the added ***B**NS* and ***SB**NS* is related to the decrease in NH_4^+ concentration in the *NO_3^--solution* and in the *P-solution*, measured in the post-experiment analysis (Table 25). The cause for the reduction in NH_4^+ in the *NO_3^--solution* is explained above. In the *P-solution*, microbial activities probably reduced the NH_4^+ concentration.

6.2.4 Uptake of Nutrients by the Plants

A reduction in nutrient mass in a hydroponic system is usually equivalent to nutrient uptake by the cultivated plant. This certainly applied to large parts for the reduction between added and remaining nutrient mass in the ***M**NS* (Figure 49). The strong fluctuations measured in the nutrient uptake visible in Figure 49 especially pronounced for NH_4^+, were caused by the different times of the intervals. In the four-day intervals, more nutrients were taken up by the plants. The difference in interval time was also visible in the fluctuation in evapotranspiration of the ***M**NS* (Figure 40).

Dalastra et al. (2020) measured comparable N and P concentrations but lower K concentrations in lettuce shoots as in the present study (Table 23). They used an NFT system with a flow rate of 1.0 l/min. In the roots, they measured 37.6, 15.1, and 15.2 mg/g DM for N, P, and K, respectively. Using these concentrations and the mean root DM of the *PG**M*** of the present study, root nutrient masses of 52 mg N, 23 mg P, and 29 mg K per plant were calculated. Therefore, a share of the undetected nutrient masses (Table 24) is located in the roots. Nonetheless, 31 mg N, 62 mg P, and 65 mg K per plant are still missing.

On the one hand, this can be explained by different nutrient root concentrations as measured by Dalastra et al. (2020). For instance, the K shoot concentration measured in the present study was higher than that measured by Dalastra et al. (2020); it is possible that the K root concentration was also higher. Additionally, the different hydroponic systems could impact nutrient shoot and root allocation. On the other hand, MOs may also be present and consume nutrients in hydroponic systems operated with mineral solution. Additionally, minor deviations from the recipe may also have occurred when mixing the stock solutions.

For the ***B****NS* and the ***SB****NS*, the nutrient reduction in the respective intervals (Figure 50, Figure 51) cannot be equated with nutrient uptake by the plants for the same reasons elaborated in Section 6.2.3. The MOs present and the increase in pH (Figure 38) reduced the plant available nutrient mass, for instance, by incorporation and precipitation of P. Additionally, the aeration of the ***B****NS* and ***SB****NS* reduced plant-available P since MOs have a strongly increased P uptake under aeration (Shapiro, 1967).

That other processes, like incorporation by MOs or precipitation, reduced the nutrients in the ***B****NS* and ***SB****NS*, is shown by the large nutrient masses that are still missing if the root nutrient masses calculated according to the concentrations stated by Dalastra et al. (2020) are subtracted from the undetected fraction (Table 24). In the ***B****NS* 125 mg N, 164 mg P and 346 mg K and for the ***SB****NS* 298 mg N, 122 mg P, and 329 mg K are still missing per plant. A multiple of what is missing in the ***M****NS*.

6.2.5 Plant Growth

This section discusses the visible and measured differences in the lettuce plants cultivated in ***M****NS*, ***B****NS*, or ***SB****NS* and the respective causes. However, first, the inner leaf tip burn observed on the younger leaves of all plants in the initial phase is addressed. Inner leaf tip burn is a symptom of Ca deficiency in growing plant tissue, which is usually not attributed to low Ca concentration in the nutrient solution, but to the immobility of Ca in the plant (Morgan, 2021). Because Ca is transported in the xylem, the outer, older leaves, which transpire more than younger leaves, receive most Ca. Inner leaf tip burn can be caused by high temperatures, low humidity, or fast vegetative growth, for instance, induced by an oversupply of N (Ashkar & Ries, 1971). Since the first two causes can be excluded (Appendix IV, A-Figure 11), an oversupply of N is assumed. Indeed, it is very well possible that the 100 % ***M****NS* supplied when the inner leaf tip burn appeared was too highly concentrated for the young plants.

After exchanging the ***M****NS* and starting the comparison phase, the *PG**B*** and *PG**SB*** showed poor growth, visible in the significantly lower total FM from DOE 60 respective DOE 57 onwards, compared to the total FM of the *PG**M*** (Figure 41), the weekly FM increase (Figure 42), and appearance (Figure 47, Figure 48). Poor plant growth can have many causes related

to bioponic solutions. These are a less balanced nutrient supply and nutrient deficiencies (Liedl et al., 2004), O_2 deficiency (Krishnasamy et al., 2012), high MO load, development of biofilms (Kano et al., 2021), phytotoxic effects (Garland & Mackowiak, 1990; Garland et al., 1997), and unfavorable pH (Williams & Nelson, 2016). Whether these or other factors were responsible for the poor growth in the present study is elaborated in the following.

The failure in O_2 supply directly at the beginning of the comparison phase affected the *PG**B*** and the *PG**SB*** much stronger (Figure 44) since the load of MOs in the ***B**NS*, and ***SB**NS* was many times higher than in the ***M**NS*. Aerobic MOs, present in the *NO_3^--solution* and in the *P-* and *K-solutions*, indicated by the mold formation (Figure 32, Figure 37), consumed O_2. Therefore, the O_2 availability in the root zone of the plants was reduced. The wilted, limp leaves observed on the *PG**B*** and *PG**SB*** on DOE 47 (Figure 44) may be a symptom of O_2 deficiency in the root zone as the roots enter anaerobic respiration and the plant produces toxic compounds (Schutzki & Cregg, 2007). However, many other causes, besides the O_2 supply failure, could be responsible for the wilted leaves. The change of the initial 100 % ***M**NS* towards ***B**NS* or ***SB**NS* increased stress for the lettuce plants since they had to cope with the drastic change from the nutritionally optimum balanced ***M**NS* to the less balanced ***B**NS* and ***SB**NS*, depicted in Figure 50 and Figure 51. In particular, the oversupply in NH_4^+ and the simultaneous reduction in NO_3^- in the ***B**NS* could have caused detrimental effects, causing symptoms like wilted leaves (Pierpont & Minotti, 1977), dark, brown roots (Pan et al., 2016), and an overall reduced growth (Britto & Kronzucker, 2002) – all observed on the *PG**B***.

6.2.5.1 NH_4^+ Toxicity

Generally, NH_4^+-N should not exceed 25 % of total N in hydroponic nutrient solutions (Savvas et al., 2006). Wenceslau et al. (2021) verified this for iceberg lettuce, where the best growth was obtained at 23 % NH_4^+-N and 77 % NO_3^--N. However, in the used ***B**NS*, NH_4^+-N made 57.6 % of the added N, as NH_4^+-N and NO_3^--N, on the start day of the comparison. In the further course of the experiment, the percentual share of NH_4^+-N even increased (Figure 50). Wenceslau et al. (2021) demonstrated that increasing the NH_4^+-N fraction in a nutrient solution's NH_4^+-N:NO_3^--N ratio, reduces leaf NO_3^- but increases NH_4^+ content. Figure 50 indicates a high NH_4^+ assimilation by the *PG**B***. An excess of NH_4^+ ions in plant tissue causes several adaptations and disturbances within the plant. A deficiency of mineral cations, for instance, K^+, Mg^{2+}, and Ca^{2+}, is frequently reported (Britto & Kronzucker, 2002). Ethylene production is increased and disturbs hormonal homeostasis (Barker, 1999a, 1999b). Furthermore, reduced net photosynthesis rates (Claussen & Lenz, 1999; Takács & Técsi, 1992), oxidative stress (Skopelitis et al., 2006), and uncoupling of photophosphorylation (Britto & Kronzucker, 2002; Gerendas et al., 1997) with accompanied reduced ATP synthesis are associated with high NH_4^+ concentrations in the plant. Torralbo et al. (2019) reported a drastic

reduction in stomatal conductance if NH_4^+ is used as the main N source for durum wheat (*Triticum durum*, var. Amilcar) in hydroponics. A strategy to reduce further NH_4^+ assimilation by the plant was assumed (Torralbo et al., 2019). Ethylene is also involved in the closure of stomata (Desikan et al., 2006), which implies reduced photosynthesis. Consequently, high NH_4^+ concentrations in a hydroponic system have detrimental effects on plant growth. It can be concluded that NH_4^+ toxicity was partly responsible for the poor growth of the *PG**B***.

6.2.5.2 MOs, Biofilm Development and Root Rot

The necrotic leaf edges (Figure 45) evolved from the wilted leaves can either be caused by NH_4^+ toxicity in the case of the *PG**B*** or represent a symptom of severe outer leaf edge burn (Baur & Neuweiler, 2021) in the case of the *PG**SB***. Outer leaf edge burn occurs if the plant's transpiration is higher than the absorbed water by the root, which a limited functioning root system can cause (Baur & Neuweiler, 2021; Mattson, 2015). That this was the case is clearly visible in Figure 47 and Figure 48. Either a thick layer of biofilm covered the roots of the *PG**B*** and *PG**SB*** or, if the biofilm was less pronounced, the roots were brownish, nearly black. This change in color is either a symptom of NH_4^+ toxicity (Pan et al., 2016) or of root rot, caused by pathogens like the oomycote *Pythium* spp., and favored by a lack of O_2 (Abdelsamad et al., 2017; Thaines Bodah, 2017). The detachment of root parts of the *PG**B*** and *PG**SB*** is a further indication of root rot. It can be assumed that the roots of the *PG**B*** and *PG**SB*** were infected by this disease. An infection with *Pythium* spp. drastically reduces the development of root hairs (Desilets & Belanger, 1991), which are crucial for the uptake of water and nutrients. Thus, the uptake of these was reduced for the *PG**B*** and *PG**SB***, which had detrimental effects on plant development.

The biofilm was most pronounced on the roots of the *PG**B***. Besides the images in Figure 47, the low root DM of 4.98 % (Table 22) indicates this since biofilms in aqueous environments can comprise 98 % of water (Flemming & Wingender, 2001); thus, their DM is very low. The distinct biofilm on the roots of the *PG**B*** was caused by a higher C and MO load in the ***B**NS*. How divers and abundant the MO community in bioponic systems can be was shown by Wongkiew et al. (2021), who added chicken manure directly into an NFT system. After lettuce was grown in the system for 35 days, samples of lettuce roots and digested chicken manure were taken and analyzed for microbial communities using next-generation sequencing. The results showed the presence of microbial genera associated with nitrification (*Nitrospira* spp.), phosphorus solubilization, plant growth promotion (*Bacillus* spp.), and organic material degradation (*Nocardiopsis* spp., *Cellvibrio* spp.).

In the present study, MOs were most probably added by the *NO_3^--*, *K-*, and *P-solutions*. Even though no TOC measurements were conducted, it can be assumed that despite the microbial activity in the *NO_3^--Reactor II*, C was present in the *NO_3^--solution* and served as food for

heterotrophic MOs. Also, the *K*- and *P-solutions*, which were part of the ***B****NS* and ***SB****NS*, contained C from the digested residues. However, the C load was lower in the ***SB****NS* since it was diluted with desalinated water. The bioponic solution share in the ***SB****NS* was 60 % of the total volume; the remaining 40 % was water.

Biofilms contain an abundance of MOs, which can have beneficial but also detrimental effects on plant growth. A root biofilm can protect plants from harmful pathogens (Fujiwara et al., 2016; Li et al., 2013), enhance root development by beneficial MOs (Chinta et al., 2014), or host nitrifying bacteria that transform NH_4^+ into NO_3^- (Kano et al., 2021). Contrary, Kano et al. (2021) reported reduced DO concentrations if biofilms were strongly pronounced. Furthermore, biofilms can host *Pythium* spp. and other pathogens (Sutton et al., 2006). In the carried-out deep water culture experiment, these adverse effects dominated in the ***B****NS* and the ***SB****NS*. However, it could be possible that some nitrifying bacteria established on the roots of the *PG**B*** and transformed NH_4^+ into NO_3^-. This would explain the higher measured remaining NO_3^- masses on DOE 64 and 71 as added on DOE 60 and DOE 67 (Figure 50).

6.2.5.3 Water Deficit Stress

Besides the already mentioned adverse effects of MOs in hydroponics, O_2 consumption and biofilm development, MOs consume nutrients, thus reducing the availability for the plant. For example, P is taken up by MOs, this uptake is increased under aerobic conditions (Shapiro, 1967). In addition, P probably precipitated due to the high pH and Ca concentrations in the ***B****NS* and ***SB****NS*. Thus, the P uptake by the plants was reduced, visible in the lower P concentrations of the leaves of the *PG**B*** and the *PG**SB***, compared to the *PG**M*** (Table 23). Consequently, P was limited and not fully available for functions like ATP production (Morgan, 2021). Combined with the previously mentioned reduced transpiration and ATP production caused by NH_4^+ toxicity this can explain the water deficit stress experienced by the *PG**B***, visible in the higher DM share (Table 22). Water deficit stress in a hydroponic system seems unlikely, but in this particular case, the concurrence of several factors induced it, as explained below. The low water potential within a plant, causing the influx of water, is usually maintained by transpiration (Kadereit et al., 2014). If transpiration is reduced, as it can be assumed for the *PG**B***, due to NH_4^+ toxicity, the low water potential within a plant is maintained by root pressure (Lopez & Barclay, 2017). Root pressure is built up by the active transport of inorganic ions into the xylem, which increases the osmotic potential, lowers the water potential, and causes water influx. The active ion transport is energy dependent; it requires ATP (Lopez & Barclay, 2017). Since ATP was limited in the *PG**B***, root pressure was reduced. Combined with the comparatively high EC (Figure 39) of the ***B****NS*, which lowered the water potential outside the plant, the water uptake by the plant was reduced. Additionally, the poorly developed root system, which was probably infected by *Pythium* spp., reduced the water uptake and led to a

water deficit. The water deficit stress, in combination with reduced photosynthesis and N and P availability, explains the smaller leaves of the *PG**B*** since plants try to reduce water losses by reducing leaf area (Garrido et al., 2014).

6.2.5.4 Phytotoxic Compounds

Garland and Mackowiak (1990), Mupambwa et al. (2019), and Phibunwatthanawong and Riddech (2019), highlighted the risk of adding phytotoxins to hydroponic system by the use of bioponic nutrient solutions. These include, besides the already mentioned high NH_4^+ concentrations, different organic compounds like phenolic acids, for instance, chlorogenic acid (Waechter-Kristensen et al., 1999), MOs with phytotoxic attributes (Waechter-Kristensen et al., 1999), heavy metals like Cd or Pb (Mupambwa et al., 2019) or other substances like NO_2^- (Pelayo Lind et al., 2021) which have phytotoxic properties if they exceed a specific concentration (Bergstrand et al., 2020). The risk is especially enhanced if anaerobic digestate without further treatment is used as nutrient solution (Garland et al., 1997).

Some phytotoxic properties, besides NH_4^+ toxicity, could have been present in the ***B****NS* and ***SB****NS*, possibly caused by anaerobic MOs, harmful concentrations of heavy metals, NO_2^- or Cl^-, which were not monitored, or by phenolic acids, like chlorogenic acid, added to the ***B****NS* and ***SB****NS* by the *K-solution* derived from potato peel. Since the phytotoxicity of chlorogenic acid and other compounds is concentration dependent, further in-depth analysis would be necessary to evaluate the potential phytotoxicity of the used ***B****NS* and ***SB****NS*, especially of the anaerobic *P-* and *K-solution*. Vaughan and Ord (1990) showed that different concentrations of phenolic acids reduce or even inhibit root growth in *Pisum sativum.*

6.2.5.5 Fresh Mass Development and Plant Growth Depending on Used Nutrient Solution

How fundamentally important healthy roots are for good plant growth is unquestionable since it is the organ the plant absorbs nutrients and water (Ryan et al., 2016). This was illustrated by the development of the *PG**B*** and *PG**SB***, depending on the presence of healthy, white roots (Figure 47, Figure 48). More white roots were found on the *PG**B*** until DOE 57 than on the *PG**SB***. Likewise, more new leaves were visible on the *PG**B***. That changed in the further course of the experiment; more healthy roots developed on the *PG**SB***, accompanied by new leaves and an increase in total FM (Figure 41). The weekly FM increase also showed this development (Figure 42). Why the *PG**B*** showed a faster recovery at the start of the comparison phase is hard to explain. A theory is that the roots and leaves were so severely damaged that the plants were depending on developing new ones; otherwise, they would have not survived. In contrast, the *PG**SB*** were vigorous enough to maintain a stagnating stage by reducing all functions to a minimum, for instance, transpiration by reduction of stomatal conductance, until they adapted to the new environment. The better growth of the *PG**SB*** and the higher shoot

FM compared to the *PG**B*** towards the end of the experiment can be explained on the one hand by the more balanced nutrient supply and especially by the higher NO_3^- concentrations (Figure 51). On the other hand, the factors that led to reduced plant growth in the ***B**NS*, besides nutrient deficiencies, like O_2 deficiency, high MO load, development of biofilms, and phytotoxic effects, were also present in the ***SB**NS* but less pronounced due to the 40 % dilution with water. Nonetheless, these factors were also present in the ***SB**NS* and led to reduced growth compared to the *PG**M***, visible in the significantly lower FM (Figure 41). The ***SB**NS* dilution was inadequate to reduce these negative effects efficiently. It is possible that a higher dilution, which would have come at the expense of nutrient concentration, would have resulted in better yields for *PG**B*** and *PG**SB***. Many studies highlight the importance of diluting bioponic solutions, especially if anaerobic digestate is used (Krishnasamy et al., 2012; Liedl et al., 2004; Phibunwatthanawong & Riddech, 2019). Phibunwatthanawong and Riddech (2019), for instance, used only a 1 % concentrated anaerobic digestate of molasses, distillery slop, and sugarcane leaves and achieved similar yields when compared to a mineral solution for romaine lettuce (*L. sativa* var. *longifolia*). In the present study, the harvested lettuce shoot FM of the *PG**B*** reached 21 % of the *PG**M*** (Figure 43). Thus, the percentage achieved is lower than achieved by most studies presented in Table 7 and Table 8, where dilutions were frequently used.

The low S/R ratio of the *PG**SB*** and especially of the *PG**B*** (Table 22) is, on the one hand, related to the limited P supply in the ***B**NS* and ***SB**NS*. P deficiency promotes root and simultaneously suppresses shoot growth (Resh, 2013). On the other hand, the repeating cycle of regrowing and dying of roots increased root mass if the dead roots were not completely detached. The formed biofilms also contributed strongly to the low FM S/R ratio, recognizable by the low DM of the roots of the *PG**B*** and *PG**SB*** (Table 22).

The FM increase of the *PG**M*** followed an expected exponential curve until the growth decreased, visible in the total FM on DOE 60 and 64 (Figure 41) and in the FM increase in week six (Figure 42). The evaluated four lettuce plants showed no larger differences in mass, visible at the considerably low standard deviations. A nutrient deficiency was also not present (Figure 49). Since no defect in the hydroponic system occurred, it can be assumed that the plants reached the end of their vegetative growth. Typically, lettuce in hydroponic systems can be harvested after 35 - 40 days (Sharma et al., 2018). In the present study the lettuce plants were cultivated for 49 days in the deep water culture system, more than one week longer than the cultivation period mentioned by Sharma et al. (2018). This could also explain the renewed growth reduction of the *PG**SB*** (Figure 42).

6.2.5.6 Nutrient Deficiencies in Plant Tissue and Concluding Remarks on Plant Growth

The elemental analysis of the dried lettuce leaves conducted at the end of the experiment revealed higher nutrient concentrations in the leaves of the *PG**M*** for most nutrients (Table 23). However, if the measured nutrient concentrations are compared to the optimum range for lettuce leaves before harvest (Hartz et al., 2007), most of the nutrient concentrations in the leaves of the *PG**B*** and *PG**SB*** matched the range. Only Mg and S were below the recommended range in the leaves of the *PG**B***. The leaves of the *PG**SB*** had additional deficits in Zn and Cu, caused by deficiencies in the nutrient solution and by the high pH, as elaborated before. N for the *PG**B*** and *PG**SB*** **was** within the range, however, only total N and no distinction in NH_4^+-N and NO_3^--N was made. This distinction would have been especially interesting for the *PG**B***. The leaves of the *PG**M*** had slightly higher P concentrations than recommended and too low Cu concentrations. The nutrient deficiencies of the *PG**B*** and *PG**SB*** are not very severe; it can be concluded that nutrient deficiency alone was not responsible for the poor growth of the *PG**B*** and *PG**SB***. However, it must be considered that the shoot FM of the *PG**B*** and *PG**SB*** was many times lower than that of the *PG**M*** and what is commercially acceptable. Supposed that the other factors responsible for the reduced growth of the *PG**B*** and *PG**SB***, like high MO load and phytotoxic effects, and perhaps more shoot biomass would have been produced, it can be assumed that, at least the leaves of the *PG**B***, would have shown stronger nutrient deficiencies. This certainly would have applied for N since the provided N by the ***B****NS* was very low at the end (Figure 50). Also, it must be considered that all plants were fertilized with ***M****NS* in the initial phase. A share of the measured leaf nutrient concentrations could arise from this initial nutrient supply.

It appears that other factors, besides nutrient supply, strongly influenced the reduced growth of the *PG**B*** and the *PG**SB***. In the ***B****NS*, the unbalanced NH_4^+:NO_3^- ratio negatively affected the development of the plants. High MO load, with associated O_2 deficiency, nutrient uptake, and biofilm development, potential phytotoxic effects by heavy metals or organic acids, high pH values which reduced nutrient availability, and root infections possibly led to suppressed plant growth of the *PG**B*** and *PG**SB***, with varying degrees of severity.

Hypothesis 2, which states that a bioponic nutrient solution with N, P, and K concentrations similar to commercial nutrient solutions would achieve comparable yields as a mineral solution, must be rejected. The lettuce yield of the plants grown in spiked bioponic solution, which was balanced concerning the main nutrients N, P, and K, was significantly lower than the yield of the plants grown in mineral solution. As described in the previous section, other factors negatively affected plant growth.

6.3 Limitations, Feasibility of the Low-Tec Approach, and Evaluation of the Selected Approach for Producing a Bioponic Nutrient Solution

All results of the mineralization experiment are based on only one reactor. A slightly different approach was chosen for the second run of the *NH_4^+-Reactor*, while several adaptations were made to optimize the nitrification process for the second run of the *NO_3^--Reactor*. It must be emphasized that further replicates are needed to verify the results of the mineralization experiment. However, the results presented provide important information on the mineralization of essential plant nutrients from the selected organic residues. For the hydroponic cultivation trial, a second run would have been of interest, for instance, to test other dilutions.

The hydroponic plant cultivation experiment showed that many more parameters than just nutrient concentrations need to be considered to fully understand plant growth in bioponic nutrient solutions. Analysis of the bioponic nutrient solution for concentrations of heavy metals, phytotoxic substances, and the two essential plant nutrients Cl and Ni, which have not been analyzed due to their absence in Table 4, which served as a reference table for balanced nutrient solutions, would be of great interest. Ni, a heavy metal, and Cl can severely impair plant growth if concentrations are too high. Furthermore, measuring TOC and O_2 concentration during the mineralization and hydroponic experiment would have provided important additional information.

Animal-based organic residues, such as blood and bone meal, are available in arid regions, for example, from goats. However, both are comparatively elaborative in their processing, especially the grinding of bones to bone meal is time and labor-intensive. In addition, the mineralization rate of the P contained in bone meal was low with the selected method. Potato, on the other hand, is relatively rarely grown in arid areas because it is considered drought-sensitive due to its shallow root system. However, there are some more drought-tolerant varieties (Nasir & Toth, 2022), and generally, the peel and skin of fruits and vegetables are rich in K. For example, the peel of the drought-resistant considered sweet potato (Hahn, 1977) could replace potato peel. However, fruit and vegetable peels are also good animal feed and are often used for this purpose (Wu, Di, 2016). Other resources used in the mineralization experiment, such as activated sewage sludge for inoculation, can be replaced by compost, as shown by Shinohara et al. (2011), and possibly better nitrification rates can be achieved by this. Also, the glucose addition for the nitrification process is unnecessary if autotrophic nitrifiers are used. Any floating material with a high surface area, such as sponges, can replace the MO-carriers used. All these measures can further reduce the resources required, thereby enabling the production of a nutrient solution in low-income and resource-limited regions. The technical effort for the production of the nutrient solution was comparatively low. However,

aerobic digestion, which is necessary for nitrification, requires an air pump and thus, a power supply. Electricity is generally essential for the operation of a hydroponic system. Even in the very simple deep water culture system, electricity is necessary to operate an air pump. The plant experiment showed how necessary the air supply is, especially for bioponic solutions.

The chosen approach of digesting organic residues with a high content of a certain main nutrient separately allows a better balance of the bioponic nutrient solution if mineralization and especially nitrification occur sufficiently. However, the selected residues in this study may contain some phytotoxic compounds. This could be ameliorated using other organic residues. Other N-rich residues, for instance, are coffee grounds (Atabani et al., 2018), grape pomace (Kanthak et al., 2022), or human urine (Viskari et al., 2018). However, their use may be restricted by climatic conditions or for ethical and religious reasons. Additionally, the aeration of anaerobic digestate or dilution of the bioponic solution before being used in a hydroponic system could reduce phototoxic properties. Another major problem was the measured nitrate decrease during solution storage before use in the hydroponic system. The reduction could probably be prevented by using different nitrifying bacteria, regular filtration, or by cooling, which is energy intensive and expensive.

7. Conclusion and Outlook

The present study addressed whether a balanced hydroponic nutrient solution can be produced by separate mineralization of N, P, and K-rich organic residues potentially available in arid regions with low technical effort. The produced N, P, and K balanced bioponic solution should achieve comparable lettuce yields as a mineral nutrient solution. A successful outcome could enable hydroponic plant production in resource and financially constrained dry lands, where traditional agriculture is increasingly threatened by desertification and climate change. This could improve food security and dietary diversification in these regions.

A Mineralization and a hydroponic plant cultivation experiment were conducted to assess the feasibility. In the mineralization experiment, organic residues rich in either N, P, or K were mineralized in separate reactors to produce bioponic solutions high in the respective nutrient. These solutions were subsequently mixed in the best ratio according to the measured concentrations to create a nutrient-balanced hydroponic solution. As N, P, and K-rich organic residues, blood meal, bone meal, and potato peel were selected. The anaerobic digestion of all three organic residues showed that this method can achieve high mineralization rates of 98 % of the N contained in blood meal into NH_4^+-N and 87 % of the K contained in potato peels. In contrast, only around 20 % of the P inherent in bone meal was mineralized to plant-available PO_4^{3-}-P in 123 days. Further, the conversion of the produced NH_4^+ from blood meal into NO_3^- in an aerobic process was relatively unsuccessful, with a nitrification rate of 5 %. However, it can be assumed that higher NO_3^- concentrations would have been achieved if the nitrification process had been continued. The low nitrification rate hindered the production of an N, P, and K-balanced bioponic nutrient solution. The bioponic nutrient solution's P and K concentrations matched concentrations used in commercial hydroponic nutrient solutions. However, the produced bioponic solution lacked a substantial amount of NO_3^-, whereas NH_4^+ was too highly concentrated.

The technical effort necessary for producing the nutrient solution was relatively low. However, air pumps were necessary for the aeration of the nutrient solution, essential for nitrification, depending on power supply. The availability of electricity could be the limiting factor in the target regions. However, for hydroponics in general, power supply is often essential. Furthermore, the used organic residues blood meal and bone meal are comparatively complicated in the production, and potato peel, if available, is also a suitable feed for animals.

Besides the bioponic nutrient solution, a spiked bioponic nutrient solution was tested against a mineral nutrient solution on lettuce in a deep water culture hydroponic system. The spiked bioponic nutrient solution was composed of the produced bioponic *P*- and *K-solution* and amended with $CaNO_3$ to a NO_3^- concentration as present in the mineral control solution. Using

the spiked bioponic nutrient solution allowed answering the second part of the research question of whether a bioponic nutrient solution with balanced N, in this case through mineral support, P, and K concentrations, can produce comparable lettuce yields to a mineral nutrient solution.

On final harvest, after the lettuce plants were grown for 25 days in the three different nutrient solutions, the plants grown in spiked bioponic solution had produced 36 %, the plants grown in pure bioponic solution 23 % of the shoot fresh mass of the plants grown in mineral solution. Consequently, a bioponic nutrient solution prepared with the organic residues and methods used in this study cannot provide lettuce yields comparable to a mineral solution, even when spiked with mineral NO_3^-. Many different reasons could have been responsible for this beyond nutrient availability and balance. These were very likely a high microorganism load, which caused oxygen deficiency in the root zone, nutrient reduction and biofilm development, potential phytotoxic properties by heavy metals or organic acids, high pH values that reduced nutrient availability, and infections by pathogens.

The mineralization and the plant cultivation experiments have provided new insights into the production and use of bioponic nutrient solutions and have revealed further research opportunities in the still new research field of bioponics.

The key findings of the mineralization experiment are a high rate of mineralization of N into NH_4^+ from blood meal and K from potato peel. Especially K from potato peel was readily available in solution after a short time without elaborative methods. In this way, a possibility of replacing K of mineral origin in hydroponic nutrient solutions was demonstrated. In contrast, P mineralization rate from bone meal into PO_4^{3-} and the nitrification of NH_4^+ into NO_3^- were below expectations. This could be a starting point for further research addressing faster P-mineralization from bone meal. Possible approaches could be the use of microorganisms or experiments to investigate the influence of pH in more detail. Nitrification rates also need to be improved to take advantage of the great potential of blood meal as an N source for bioponic nutrient solutions. For example, the influence of autotrophic instead of heterotrophic nitrifiers or different NH_4^+ concentrations on the nitrification rate could be examined here. Further findings of the mineralization experiment were the presence of essential macro- and micronutrients, besides N, P, and K, in the produced bioponic solutions and a high correlation between EC and NH_4^+ and K^+ with the used organic residues.

The most important finding of the hydroponic plant cultivation experiment is that besides an imbalanced nutrient supply in the bioponic solution - high NH_4^+ and low NO_3^- concentrations were particularly detrimental - other factors impeded good plant growth. On the one hand, these were phytotoxic compounds potentially present in the used bioponic solutions. More comprehensive analysis and phytotoxicity tests of the bioponic nutrient solution would be

necessary to clarify this. If these analyses confirm phytotoxicity, measures like dilution of the bioponic solution or the use of other organic residues could be considered. In order to further increase sustainability, residues of plant origin like N-rich grape pomace or coffee grounds or excreta, such as human urine, would be of high interest.

On the other hand, microorganisms present in the bioponic solution had an adverse effect on plant growth. They were most likely responsible for reducing the NO_3^- concentration in the stored bioponic nutrient solution before it was used in the hydroponic system. Furthermore, they reduced oxygen and nutrient availability for the plants in the hydroponic system. In particular, the behavior of PO_4^{3-} in the combined presence of microorganisms and oxygen requires further attention. The high microbial load of the bioponic solution manifested itself in forming biofilms on the roots of the lettuce plants, potentially hosting plant pathogens. These results demonstrate the significant effect microorganisms can have in hydroponic systems and that reducing them in bioponic solutions may be of great interest. A first step towards this aim would be the reduction of C, the feed for heterotrophic microorganisms. Therefore, appropriate measures with monitoring TOC must be applied. Furthermore, removing microorganisms from the bioponic solution with adequate measures like ultraviolet disinfection, heating, or hydrogen peroxide disinfection, common practices in commercial hydroponic cultivation, could significantly improve plant growth. Any of these disinfection methods would compromise the low-tech approach of this study. It appears that low-tech and producing a bioponic nutrient solution, and hydroponics, in general, are difficult to reconcile. However, the necessity for a well-balanced bioponic nutrient solution that produces good yields is high, not only in the low-tec context of this study but due to the reduction in fertile land by climate change and unsustainable mineral fertilizers, in hydroponic plant production in general.

References

Abdelsamad, N. A., Noor., Baumbach, J., Bhattacharyya, M. K., & Leandro, L. F. (2017). Soybean Sudden Death Syndrome Caused by Fusarium virguliforme is Impaired by Prolonged Flooding and Anaerobic Conditions. *Plant Disease*, *101*(5), 712–719. doi:10.1094/PDIS-04-16-0534-RE

Ako, P. A. E., Adebanjo, A. S., Fadipe, A. L., & Ndamitso, M. M. (2003). Extractability of Potassium from Some Organic Manures in Aqueous Medium and the Effect of pH, Time and Concentration. *Journal of Applied Sciences and Environmental Management*, *7*(1), 51–56. doi:10.4314/jasem.v7i1.17165

AlShrouf, A. (2017). Hydroponics, Aeroponic and Aquaponic as Compared with Conventional Farming. *American Scientific Research Journal for Engineering, Technology, and Sciences*, *27*(1), 247–255.

Ansah, K. O., Antwi, C., Osafo, E. L., Enning, S., & Adu-Dapaah, H. (2019). Manure characteristics of small ruminants fed agro by-products in the guinea savannah agro-ecological zone of Ghana. *Ghana Journal of Agricultural Science*, *54*(1), 67–76. doi:10.4314/gjas.v54i1.7

Arnon, I. (1992). *Agriculture in Dry Lands: Principles and Practice*. *Developments in agricultural and managed-forest ecology: Vol. 26*. Elsevier Science.

Arvanitoyannis, I. (2008). *Waste management for the food industries*. *Food science and technology international series*. Academic Press.

Ashkar, S. A., & Ries, S. K. (1971). Lettuce Tipburn as Related to Nutrient Imbalance and Nitrogen Composition. *American Society for Horticultural Science*, *96*(4), 448–452. doi:10.21273/JASHS.96.4.448

Atabani, A. E., Mercimek, S. M., Arvindnarayan, S., Shobana, S., Kumar, G., Cadir, M., & Al-Muhatseb, A. H. (2018). Valorization of spent coffee grounds recycling as a potential alternative fuel resource in Turkey: An experimental study. *Journal of the Air & Waste Management Association*, *68*(3), 196–214. doi:10.1080/10962247.2017.1367738

Barbosa, G. L., Gadelha, F. D. A., Kublik, N., Proctor, A., Reichelm, L., Weissinger, E., Wohlleb, G. M., & Halden, R. U. (2015). Comparison of Land, Water, and Energy Requirements of Lettuce Grown Using Hydroponic vs. Conventional Agricultural Methods. *International Journal of Environmental Research and Public Health*, *12*(6), 6879–6891. doi:10.3390/ijerph120606879

Barker, A. V. (1999a). Ammonium accumulation and ethylene evolution by tomato infected with root-knot nematode and grown under different regimes of plant nutrition. *Communications in Soil Science and Plant Analysis*, *30*(1-2), 175–182. doi:10.1080/00103629909370194

Barker, A. V. (1999b). Foliar ammonium accumulation as an index of stress in plants. *Communications in Soil Science and Plant Analysis*, *30*(1-2), 167–174. doi:10.1080/00103629909370193

Basosi, R., Spinelli, D., Fierro, A., & Jez, S. (2014). *Fertilizers: Components, Uses in Agriculture and Environmental Impacts: Mineral Nitrogen Fertilizers: Environmental Impact of Production and Use*. Nova.

Bast, E. (2001). *Mikrobiologische Methoden: Eine Einführung in grundlegende Arbeitstechniken* (2nd ed.). Spektrum Akademischer Verlag.

Baur, B., & Neuweiler, R. (2021). *Physiologische Störungen bei Salaten* (Agroscope Merkblatt No. 130). Wädenswil. Eidgenössische Departement für Wirtschaft, Bildung und Forschung.

Bergstrand, K.-J., Asp, H., & Hultberg, M. (2020). Utilizing Anaerobic Digestates as Nutrient Solutions in Hydroponic Production Systems. *Sustainability*, *12*(23), 10076. doi:10.3390/su122310076

Bhattacharyya, P. N., & Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World Journal of Microbiology & Biotechnology*, *28*(4), 1327–1350. doi:10.1007/s11274-011-0979-9

Botheju, D., Svalheim, Ø., & Bakke, R. (2010). Digestate Nitrification for Nutrient Recovery. *The Open Waste Management Journal*, *3*(1), 1–12. doi:10.2174/1876400201003010001

Britannica. (2023a). *"hydroponics".* Encyclopedia Britannica. https://www.britannica.com/topic/hydroponics

Britannica. (2023b). *"salt".* Encyclopedia Britannica. https://www.britannica.com/science/salt-acid-base-reactions

Britto, D. T., & Kronzucker, H. J. (2002). NH4+ toxicity in higher plants: a critical review. *Journal of Plant Physiology*, *159*(6), 567–584. doi:10.1078/0176-1617-0774

Brown, P. H., M. Welch, R., & Cary E., E. (1987). Nickel: A Micronutrient Essential for Higher Plants. *Plant Physiology*, *85*(3), 801–803. doi:10.1104/pp.85.3.801

Calderón, F. J., McCarty, G. W., & Reeves, J. B. (2005). Analysis of manure and soil nitrogen mineralization during incubation. *Biology and Fertility of Soils*, *41*(5), 328–336. doi:10.1007/s00374-005-0843-x

Castro, E., Mañas, M. P., & Las Heras, J. de (2009). Nitrate content of lettuce (Lactuca sativa L.) after fertilization with sewage sludge and irrigation with treated wastewater. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *26*(2), 172–179. doi:10.1080/02652030802425334

Chinta, Y. D [Yufita D.], Kano, K., Widiastuti, A., Fukahori, M., Kawasaki, S., Eguchi, Y., Misu, H., Odani, H., Zhou, S., Narisawa, K., Fujiwara, K., Shinohara, M., & Sato, T. (2014). Effect of corn steep liquor on lettuce root rot (Fusarium oxysporum f.Sp. Lactucae) in hydroponic cultures. *Journal of the Science of Food and Agriculture*, *94*(11), 2317–2323. doi:10.1002/jsfa.6561

Claussen, W., & Lenz, F. (1999). Effect of ammonium or nitrate nutrition on net photosynthesis, growth, and activity of the enzymes nitrate reductase and glutamine synthetase in blueberry, raspberry and strawberry. *Plant and Soil*, *208*, 95–102. doi:10.1023/A:1004543128899

Constantino, N. N., Mastouri, F., Damarwinasis, R., Borrego, E. J., Moran-Diez, M. E., Kenerley, C. M., Gao, X., & Kolomiets, M. V. (2013). Root-expressed maize lipoxygenase 3 negatively regulates induced systemic resistance to Colletotrichum graminicola in shoots. *Frontiers in Plant Science*, *4*, 1–12. doi:10.3389/fpls.2013.00510

Cordell, D., Drangert, J.-O., & White, S. (2009). The story of phosphorus: Global food security and food for thought. *Global Environmental Change*, *19*(2), 292–305. doi:10.1016/j.gloenvcha.2008.10.009

Dalastra, C., Teixeira Filho, M. C. M., Da Silva, M. R., Nogueira, T. A. R., & Fernandes, G. C. (2020). Head lettuce production and nutrition in relation to nutrient solution flow. *Horticultura Brasileira*, *38*(1), 21–26. doi:10.1590/s0102-053620200103

Dannehl, D., Schuch, I., Gao, Y., Cordiner, S., & Schmidt, U. (2016). Effects of hypochlorite as a disinfectant for hydroponic systems on accumulations of chlorate and phytochemical compounds in tomatoes. *European Food Research and Technology*, *242*(3), 345–353. doi:10.1007/s00217-015-2544-5

Desikan, R., Last, K., Harrett-Williams, R., Tagliavia, C., Harter, K., Hooley, R., Hancock, J. T., & Neill, S. J. (2006). Ethylene-induced stomatal closure in Arabidopsis occurs via AtrbohF-mediated hydrogen peroxide synthesis. *The Plant Journal : For Cell and Molecular Biology*, *47*(6), 907–916. doi:10.1111/j.1365-313X.2006.02842.x

Desilets, H., & Belanger, R. R. (1991). An In Vitro System for Studying the Effects of Pythium ultimum Metabolites on Pelargonium X hortorum. *Phytopathology*, *81*(2), 202–206. doi:10.1094/Phyto-81-202

DIN 38406-E23-2 (1993). Kationen Bestimmung von Ammonium Stickstoff mit Fließanalyseverfahren.

Dunn, B., & Singh, H. (2016). *Electrical Conductivity and pH Guide for Hydroponics* (Technical Report). Oklahoma State University. doi:10.13140/RG.2.2.20271.94885

DWD. (2023). *Klimadaten Deutschland: Station: Stuttgart/Echterdingen.* Deutscher Wetterdienst. https://www.dwd.de/DE/leistungen/klimadatendeutschland/klimadatendeutschland.html#buehneTop

El-shinawy, M., Abd-Elmoniem, E. M., & Abou Hadid, A. F. (1999). The use of organic manure for lettuce plants grown under NFT conditions. *Acta Horticulturae*(491), 315–318. doi:10.17660/ActaHortic.1999.491.47

Epple, M., & Enax, J. (2018). Moderne Zahnpflege aus chemischer Sicht. *Chemie in Unserer Zeit*, *52*(4), 218–228. doi:10.1002/ciuz.201800796

Flemming, H.-C., & Wingender, J. (2001). Biofilme - die bevorzugte Lebensform der Bakterien: Flocken, Filme und Schlämme. *Biologie in Unserer Zeit*, *31*(3), 169–180. doi:10.1002/1521-415X(200105)31:3%3C169::AID-BIUZ169%3E3.0.CO;2-U

Fuchs, J. G. (2010). Interactions Between Beneficial and Harmful Microorganisms: From the Composting Process to Compost Application. In H. Insam, I. Franke-Whittle, & M. Goberna (Eds.), *Microbes at Work* (pp. 213–229). Springer Berlin Heidelberg. doi:10.1007/978-3-642-04043-6_11

Fujiwara, K., Iida, Y., Someya, N., Takano, M., Ohnishi, J., Terami, F., & Shinohara, M. (2016). Emergence of Antagonism Against the Pathogenic Fungus Fusarium oxysporum by Interplay Among Non-Antagonistic Bacteria in a Hydroponics Using Multiple Parallel Mineralization. *Journal of Phytopathology*, *164*(11-12), 853–862. doi:10.1111/jph.12504

Future Market Insights. (2023). *Hydroponics Market Size to be valued at US$ 2.7 Billion in 2023 and is further expected to cross a revenue of US$ 5.7 Billion by 2033 end.* Future Market Insights Global and Consulting Pvt. Ltd. https://www.futuremarketinsights.com/reports/hydroponics-market

Gale, E. S., Sullivan, D. M., Cogger, C. G., Bary, A. I., Hemphill, D. D., & Myhre, E. A. (2006). Estimating plant-available nitrogen release from manures, composts, and specialty products. *Journal of Environmental Quality*, *35*(6), 2321–2332. doi:10.2134/jeq2006.0062

Garland, J. L., & Mackowiak, C. L. (1990). Utilization Of The Water Soluble Fraction Of Wheat Straw As A Plant Nutrient Source. *NASA Technical Memorandum*, *103497*, 1–19.

Garland, J. L., Mackowiak, C. L., Strayer, R. F., & Finger, B. W. (1997). Integration of waste processing and biomass production systems as part of the KSC breadboard project. *Advances in Space Research*, *20*(10), 1821–1826. doi:10.1016/S0273-1177(97)00847-8

Garrido, Y., Tudela, J. A., Marín, A., Mestre, T., Martínez, V., & Gil, M. I. (2014). Physiological, phytochemical and structural changes of multi-leaf lettuce caused by salt stress. *Journal of the Science of Food and Agriculture*, *94*(8), 1592–1599. doi:10.1002/jsfa.6462

Gerendas, J., Zhu, Z., Bendixen, R., Ratcliffe, G. R., & Sattelmacher, B. (1997). Physiological and Biochemical Processes Related to Ammonium Toxicity in Higher Plants. *Z. Pflanzenernähr. Bodenk.*, *160*, 239–251.

Goddek, S., Joyce, A., Kotzen, B., & Burnell, G. M. (2019). *Aquaponics Food Production Systems*. Springer International Publishing. doi:1007/978-3-030-15943-6

Hahn, S. K. (1977). Sweet Potato. In *Ecophysiology of Tropical Crops* (pp. 237–248). Elsevier. doi:10.1016/B978-0-12-055650-2.50013-7

Hartz, T. K., Johnstone, P. R., Williams, E., & Smith, R. F. (2007). Establishing Lettuce Leaf Nutrient Optimum Ranges Through DRIS Analysis. *HortScience*, *42*(1), 143–146. doi:10.21273/HORTSCI.42.1.143

Hayden, A. L. (2006). Aeroponic and Hydrogen Systems for Medicinal Herb, Rhizome, and Root Crops. *HortScience*, *41*(3), 536–538. doi:10.21273/HORTSCI.41.3.536

ISO 11732 (1997). Water quality – Determination of ammonium nitrogen by flow analysis (CFA and FIA) and spectrometric detection.

ISO 13395 (1996). Water quality – Determination of nitrite nitrogen and nitrate nitrogen and the sum of both by Flow Analysis (CFA and FIA) and spectrometric detec□on.

ISO DIN EN ISO 15681-2 (2001). Water quality – Determination of orthophosphate and total phosphorus by flow analysis CFA and FIA - Part 2: Method by Continuous Flow Analysis (CFA).

Jan, S., Rashid, Z., Ahngar, T. A., Iqbal, S., Naikoo, M. A., Majeed, S., Bhat, T. A., Gul, R., & Nazir, I. (2020). Hydroponics – A Review. *International Journal of Current Microbiology and Applied Sciences*, *9*(8), 1779–1787. doi:10.20546/ijcmas.2020.908.206

Janesch, E., Pereira, J., Neubauer, P., & Junne, S. (2021). Phase Separation in Anaerobic Digestion: A Potential for Easier Process Combination? *Frontiers in Chemical Engineering*, *3*, Article 711971, 1–16. doi:10.3389/fceng.2021.711971

Jesus, R. H. G. de, Souza, J. T. de, Puglieri, F. N., Piekarski, C. M., & Francisco, A. C. de (2021). Biodigester location problems, its economic–environmental–social aspects and techniques: Areas yet to be explored. *Energy Reports*, *7*, 3998–4008. doi:10.1016/j.egyr.2021.06.090

Kadereit, J. W., Körner, C., Kost, B., & Sonnewald, U. (2014). *Strasburger − Lehrbuch der Pflanzenwissenschaften*. Springer Berlin Heidelberg. doi:10.1007/978-3-642-54435-4

Kano, K., Kitazawa, H., Suzuki, K., Widiastuti, A., Odani, H., Zhou, S., Chinta, Y. D [Yufita Dwi], Eguchi, Y., Shinohara, M., & Sato, T. (2021). Effects of Organic Fertilizer on Bok Choy Growth and Quality in Hydroponic Cultures. *Agronomy*, *11*(3), 491. doi:10.3390/agronomy11030491

Kanthak, S., Pauly, J., & Zimmer, S. (2022). *Leuchtturmprojekt: Optimierung organischer Stoffkreisläufe im Luxemburger Weinbau*. L-6225 Altrier. Institut fir Biologische Landwirtschaft an Agrarkultur Luxemburg a.s.b.l. (IBLA).

Kechasov, D., Verheul, M. J., Paponov, M., Panosyan, A., & Paponov, I. A. (2021). Organic Waste-Based Fertilizer in Hydroponics Increases Tomato Fruit Size but Reduces Fruit Quality. *Frontiers in Plant Science*, *12*, Article 680030, 1–20. doi:10.3389/fpls.2021.680030

Khan, S., Purohit, A., & Vadsaria, N. (2021). Hydroponics: current and future state of the art in farming. *Journal of Plant Nutrition*, *44*(10), 1515–1538. doi:10.1080/01904167.2020.1860217

Khanichaidecha, W., Nakaruk, A., Ratananikom, K., Eamrat, R., & Kazama, F. (2019). Heterotrophic nitrification and aerobic denitrification using pure-culture bacteria for wastewater treatment. *Journal of Water Reuse and Desalination*, *9*(1), 10–17. doi:10.2166/wrd.2018.064

Kjeldahl, J. (1883). Neue Methode zur Bestimmung des Stickstoffs in organischen Koerpern. *Zeitschrift Für Analytische Chemie*(22), 366 - 282. doi:10.1007/BF01338151

Körner, I. (2009). *Stickstoffhaushalt bei der Kompostierung: Bilanzen, Gehalte, Umsetzungs- und Austragsprozesse*. Zugl.: Hamburg-Harburg, Techn. Univ., Inst. für Abfall-Ressourcen-Wirtschaft und Inst. für Umwelttechnik und Energiewirtschaft (IUE), Habil.-Schr., 2008. *Hamburger Berichte Abfallwirtschaft: Vol. 33*. Verl. Abfall aktuell.

Krishnasamy, K., Nair, J., & Bäuml, B. (2012). Hydroponic system for the treatment of anaerobic liquid. *Water Science and Technology : A Journal of the International Association on Water Pollution Research*, *65*(7), 1164–1171. doi:10.2166/wst.2012.031

Law, J. P. (1969). Nutrient Removal from Enriched Waste Effluent by the Hydroponic Culture of Cool Season Grasses. *Federal Water Quality Administration*, 1–33.

Lazicki, P., Geisseler, D., & Lloyd, M. (2020). Nitrogen mineralization from organic amendments is variable but predictable. *Journal of Environmental Quality*, *49*(2), 483–495. doi:10.1002/jeq2.20030

Lee, S., & Lee, J. (2015). Beneficial bacteria and fungi in hydroponic systems: Types and characteristics of hydroponic food production methods. *Scientia Horticulturae*, *195*, 206–215. doi:10.1016/j.scienta.2015.09.011

Li, S., Zhang, N., Zhang, Z., Luo, J., Shen, B., Zhang, R., & Shen, Q. (2013). Antagonist Bacillus subtilis HJ5 controls Verticillium wilt of cotton by root colonization and biofilm formation. *Biology and Fertility of Soils*, *49*(3), 295–303. doi:10.1007/s00374-012-0718-x

Liedl, B. E., Cummins, M., Young, A., & Williams, M. L. (2004). Hydroponic Lettuce Production Using Liquid Effluent from Poultry Waste Bioremediation as a Nutrient Source. *Acta Horticulturae*, *659*, 721–728. doi:10.17660/ActaHortic.2004.659.93

Liu, Y., & Whitman, W. B. (2008). Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Annals of the New York Academy of Sciences*, *1125*, 171–189. doi:10.1196/annals.1419.019

Lopez, F. B., & Barclay, G. F. (2017). Plant Anatomy and Physiology. In *Pharmacognosy* (pp. 45–60). Elsevier. doi:10.1016/B978-0-12-802104-0.00004-4

MacDonald, D. I., & Boyack, J. R. (1969). Density, Electrical Conductivity, and Vapor Pressure of Concentrated Phosphoric Acid. *Journal of Chemical and Engineering Data*, *14*(3), 380–384. doi:10.1021/je60042a013

Mackowiak, C. L., Garland, J. L., Strayer, R. F., Finger, B. W., & Wheeler, R. M. (1996). Comparison of aerobically treated and untreated crop residue as a sorce of recycled nutrients in a recirculating hydroponic system. *Advances in Space Research*, *18*(1/2), 281–287. doi:10.1016/0273-1177(95)00817-x

Matsushita, N., & Matoh, I. (1991). Characterization of Na+ exclusion mechanisms of salt-tolerant reed plants in comparison with salt-sensitive rice plants. *Physiologia Plantarum*, *83*, 170–176. doi:10.1111/j.1399-3054.1991.tb01298.x

Mattson, N. S. (2015). Tipburn of hydroponic lettuce. *E-GRO Alert*, *4*(31), 1–7.

Meegoda, J. N., Li, B., Patel, K., & Wang, L. B. (2018). A Review of the Processes, Parameters, and Optimization of Anaerobic Digestion. *International Journal of Environmental Research and Public Health*, *15*(10), 1–16. doi:10.3390/ijerph15102224

Mesmel, R. E., & Holmes, H. F. (1992). pH, Definition and Measurement at High Temperatures. *Journal of Solution Chemistry*, *21*(8), 725–744. doi:10.1007/BF00651506

Middleton, N., & Thomas, D. (1997). *World Atlas of Desertification* (2nd ed.). United Nations Environment Programme.

Mirzabaev, A., Wu, J., Evans, J., García-Oliva, F., Hussein, I., Iqbal, M. H., Kimutai, J., Knowles, T., Meza, F., Nedjraoui, D., Tena, F., Türkes, M., Vázquez, R. J., & Weltz, M. (2019). *Desertification: Climate Change and Land: an IPCC special report on climate change,*

desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. doi:10.1017/9781009157988.005
Moomen, A.-W., & Dewan, A. (2017). Assessing the spatial relationships between mining and land degradation: evidence from Ghana. *International Journal of Mining, Reclamation and Environment*, *31*(7), 505–518. doi:10.1080/17480930.2016.1188253
Mordor Intelligence. (2023). *Hydrokulturmarkt - Wachstum, Trends und Prognosen (2023 - 2028)*. https://www.mordorintelligence.com/de/industry-reports/hydroponics-market#faqs
Morgan, L. (2021). *Hydroponics and protected cultivation: A practical guide*. CAB International.
Mowa, E. (2015). Organic manure for vegetable production under hydroponic conditions in arid Namibia. *Int. Science Technol. J. Namibia*, *5*, 3–12.
Mupambwa, H. A., Namwoonde, A. S., Liswaniso, G. M., Hausiku, M. K., & Ravindran, B. (2019). Biogas digestates are not an effective nutrient solution for hydroponic tomato (Lycopersicon esculentum L.) production under a deep water culture system. *Heliyon*, *5*(10), e02736. doi:10.1016/j.heliyon.2019.e02736
Nahalkova, J., Fatehi, J., Olivain, C., & Alabouvette, C. (2008). Tomato root colonization by fluorescent-tagged pathogenic and protective strains of Fusarium oxysporum in hydroponic culture differs from root colonization in soil. *FEMS Microbiology Letters*, *286*(2), 152–157. doi:10.1111/j.1574-6968.2008.01241.x
Nasir, M. W., & Toth, Z. (2022). Effect of Drought Stress on Potato Production: A Review. *Agronomy*, *12*(3), 635. doi:10.3390/agronomy12030635
Nazifa, T. H., Saady, N. M. C., Bazan, C., Zendehboudi, S., Aftab, A., & Albayati, T. M. (2021). Anaerobic Digestion of Blood from Slaughtered Livestock: A Review. *Energies*, *14*(18), 1–25. doi:10.3390/en14185666
Neklyudov, A. D., Fedotov, G. N., & Ivankin, A. N. (2008). Intensification of composting processes by aerobic microorganisms: A review. *Applied Biochemistry and Microbiology*, *44*(1), 6–18. doi:10.1134/S000368380801002X
Nkoa, R. (2014). Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. *Agronomy for Sustainable Development*, *34*(2), 473–492. doi:10.1007/s13593-013-0196-z
Ødegaard, H., Rusten, B., & Westrum, T. (1994). A new moving bed biofilm reactor - applications and results. *Water Sci Technol*, *29*(10), 157–165. doi:10.2166/wst.1994.0757
Onyeneho, S. N., & Hettiarachchy, N. S. (1993). Antioxidant activity, fatty acids and phenolic acids compositions of potato peels. *Journal of the Science of Food and Agriculture*, *62*(4), 345–350. doi:10.1002/jsfa.2740620406
Pan, W. L., Madsen, I. J., Bolton, R. P., Graves, L., & Sistrunk, T. (2016). Ammonia/Ammonium Toxicity Root Symptoms Induced by Inorganic and Organic Fertilizers and Placement. *Agronomy Journal*, *108*(6), 2485–2492. doi:10.2134/agronj2016.02.0122
Pavlovic, M., Konrad, R., Huber, I., & Busch, U. (2011). Identifizierung von Mikroorganismen. *DLR Spezial*, 9–16.
Pelayo Lind, O., Hultberg, M., Bergstrand, K.-J., Larsson-Jönsson, H., Caspersen, S., & Asp, H. (2021). Biogas Digestate in Vegetable Hydroponic Production: pH Dynamics and pH Management by Controlled Nitrification. *Waste and Biomass Valorization*, *12*(1), 123–133. https://doi.org/10.1007/s12649-020-00965-y
Phibunwatthanawong, T., & Riddech, N. (2019). Liquid organic fertilizer production for growing vegetables under hydroponic condition. *International Journal of Recycling of Organic Waste in Agriculture*, *8*(4), 369–380. doi:10.1007/s40093-019-0257-7
Pierpont, R. A., & Minotti, P. L. (1977). Effects of Calcium Carbonate on Ammonium Assimilation by Tomato Seedlings. *J. Amer. Soc. Hort. Sci.*, *102*(1), 20–30. doi:10.21273/JASHS.102.1.20
Poudyal, S., & Cregg, B. M. (2019). Irrigating Nursery Crops with Recycled Run-off: A Review of the Potential Impact of Pesticides on Plant Growth and Physiology. *HortTechnology*, *29*(6), 716–729. doi:10.21273/HORTTECH04302-19
Rader, H. B., & Karlsson, M. G. (2006). Northern Field Production of Leaf and Romaine Lettuce using a High Tunnel. *HortTechnology*, *16*(4), 649–654.
RAL-Gütesicherung Kompost. (2022). *Jahreszeugnis 2022 Kirchheimer Qualitätskompost.*
Ratzke, C., & Gore, J. (2018). Modifying and reacting to the environmental pH can drive bacterial interactions. *PLoS Biology*, *16*(3), 1-20. doi:10.1371/journal.pbio.2004248

Resh, H. M. (2013). *Hydroponic food production: A definitive guidebook for the advanced home gardener and the commercial hydroponic grower* (7th ed.). CRC Press.
Richardson, A. E., Barea, J.-M., McNeill, A. M., & Prigent-Combaret, C. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant and Soil*, *321*(1-2), 305–339. doi:10.1007/s11104-009-9895-2
Roberts, H., & Palmeiro, B. S. (2008). Toxicology of aquarium fish. *The Veterinary Clinics of North America. Exotic Animal Practice*, *11*(2), 359-373. doi:10.1016/j.cvex.2007.12.005
Ryan, P. R., Delhaize, E., Watt, M., & Richardson, A. E. (2016). Plant roots: understanding structure and function in an ocean of complexity. *Annals of Botany*, *118*(4), 555–559.
Samocha, T. M., & Prangnell, D. I. (2019). System Treatment and Preparation. In *Sustainable Biofloc Systems for Marine Shrimp* (pp. 119–131). Elsevier. doi:10.1016/B978-0-12-818040-2.00006-X
Sapkota, S [Sundar], Sapkota, S [Sanjib], & Liu, Z. (2019). Effects of Nutrient Composition and Lettuce Cultivar on Crop Production in Hydroponic Culture. *Horticulturae*, *5*(4), 72. doi:10.3390/horticulturae5040072
Sardans, J., & Peñuelas, J. (2015). Potassium: a neglected nutrient in global change. *Global Ecology and Biogeography*, *24*(3), 261–275. doi:10.1111/geb.12259
Savvas, D., Passam, H. C., Olympios, C., Nasi, E., Moustaka, E., Mantzos, N., & Barouchas, P. (2006). Effects of Ammonium Nitrogen on Lettuce Grown on Pumice in a Closed Hydroponic System. *HortScience*, *41*(7), 1667–1673. doi:10.21273/HORTSCI.41.7.1667
Scheurer, M., Sacher, F., Brauch, & Heinz-Jürgen. (2014). *Studie zur Bedeutung von Nitrifikations- und Ureaseinhibitoren für die Roh- und Trinkwasserbeschaffenheit*. DVGW Technisch-wissenschaftlicher Verein.
Schnitkey, G., Paulson, N., Zulauf, C., Swanson, K., Colussi, J., & Baltz, J. (2022). Weekly Farm Economics: Nitrogen Fertilizer Prices and Supply in Light of the Ukraine-Russia Conflict. *Farmdoc Daily*, *45*(12), 1–7.
Schutzki, R. E., & Cregg, B. (2007). Abiotic Plant Disorders: Symptoms, Signs and Solutions A Diagnostic Guide to Problem Solving. *Michigan State Universtity Extension Bulletin*, *E-2996*, 1–16.
Shahbandeh, M. (2021). *Projected market share of indoor farming technology worldwide in 2019 and 2024, by growing method.* Statista. https://www.statista.com/statistics/1088007/global-indoor-farming-technology-market-value-by-growing-method/
Shapiro, J. (1967). Induced rapid release and uptake of phosphate by microorganisms. *Science*, *155*(3767), 1269–1271. doi:10.1126/science.155.3767.1269
Sharma, N., Acharya, S., Kumar, K., Singh, N., & Chaurasia, O. P. (2018). Hydroponics as an advanced technique for vegetable production: An overview. *Journal of Soil and Water Conservation*, *17*(4), 364. doi:10.5958/2455-7145.2018.00056.5
Shinohara, M., Aoyama, C., Fujiwara, K., Watanabe, A., Ohmori, H., Uehara, Y., & Takano, M. (2011). Microbial mineralization of organic nitrogen into nitrate to allow the use of organic fertilizer in hydroponics. *Soil Science and Plant Nutrition*, *57*(2), 190–203. doi:10.1080/00380768.2011.554223
Skopelitis, D. S., Paranychianakis, N. V., Paschalidis, K. A., Pliakonis, E. D., Delis, I. D., Yakoumakis, D. I., Kouvarakis, A., Papadakis, A. K., Stephanou, E. G., & Roubelakis-Angelakis, K. A. (2006). Abiotic stress generates ROS that signal expression of anionic glutamate dehydrogenases to form glutamate for proline synthesis in tobacco and grapevine. *The Plant Cell*, *18*(10), 2767–2781. doi:10.1105/tpc.105.038323
Stefanakis, A., Akratos, C. S., & Tsihrintzis, V. A. (2014). *Vertical flow constructed wetlands: Eco-engineering systems for wastewater and sludge treatment* (First edition). Elsevier.
Sun, Z., Zhang, J., Yang, J., Li, J., Wang, J., & Hu, X. (2019). Acclimation of 2-chlorophenol-biodegrading activated sludge and microbial community analysis. *Water Environment Research*, *91*, Article 4, 273–280. doi:10.1002/wer.1049
Sutton, J. C., Sopher, C. R., Owen-Going, T. N., Liu, W., Grodzinski, B., Hall, J. C., & Benchimol, R. L. (2006). Etiology and epidemiology of Pythium root rot in hydroponic crops: current knowledge and perspectives. *Summa Phytopathologica*, *32*(4), 307–321. doi:10.1590/S0100-54052006000400001

Szekely, I., & Jijakli, M. H. (2022). Bioponics as a Promising Approach to Sustainable Agriculture: A Review of the Main Methods for Producing Organic Nutrient Solution for Hydroponics. *Water*, *14*(23), 3975. doi:10.3390/w14233975

Taiz, L., & Zeiger, E. (2006). *Plant physiology* (4th ed.). Sinauer Associates, Inc.

Takács, E., & Técsi, L. (1992). Effects of NO3-/NH4+ Ratio on Photosynthetic Rate, Nitrate Reductase Activity and Chloroplast Ultrastructure in three Cultivars of Red Pepper (Capsicum annuum L.). *Journal of Plant Physiology*, *140*(3), 298–305. doi:10.1016/S0176-1617(11)81082-5

Thaines Bodah, E. (2017). Root Rot Diseases in Plants: A Review of Common Causal Agents and Management Strategies. *Agricultural Research & Technology: Open Access Journal*, *5*(3). doi:10.19080/ARTOAJ.2017.05.555661

Tikasz, P., MacPherson, S., Adamchuk, V., & Lefsrud, M. (2019). Aerated chicken, cow, and turkey manure extracts differentially affect lettuce and kale yield in hydroponics. *International Journal of Recycling of Organic Waste in Agriculture*, *8*(3), 241–252. doi:10.1007/s40093-019-0261-y

Torralbo, F., González-Moro, M. B., Baroja-Fernández, E., Aranjuelo, I., & González-Murua, C. (2019). Differential Regulation of Stomatal Conductance as a Strategy to Cope With Ammonium Fertilizer Under Ambient Versus Elevated CO2. *Frontiers in Plant Science*, *10*, 597. doi:10.3389/fpls.2019.00597

U.S. Geological Survey. (2021). *Mineral Commodity Summaries*. Reston, VA. U.S. Geological Survey. doi:10.3133/mcs2021

van Niel, E., Arts, P., Wesselink, B. J., Robertson, L. A., & Kuenen, J. G. (1993). Competition between heterotrophic and autotrophic nitrifiers for ammonia in chemostat cultures. *FEMS Microbiology Ecology*, *11*(2), 109–118. doi:10.1111/j.1574-6968.1993.tb05802.x

Vaughan, D., & Ord, B. (1990). Influence of Phenolic Acids on Morphological Changes in Roots of Pisum sativum. *Journal of the Science of Food and Agriculture*, *52*(2), 289–299. doi:10.1002/jsfa.2740520302

Velazquez-Gonzalez, R. S., Garcia-Garcia, A. L., Ventura-Zapata, E., Barceinas-Sanchez, J. D. O., & Sosa-Savedra, J. C. (2022). A Review on Hydroponics and the Technologies Associated for Medium- and Small-Scale Operations. *Agriculture*, *12*(5), 646. doi:10.3390/agriculture12050646

Villalón, L. A. (Ed.). (2021). *Oxford handbooks*. *The Oxford handbook of the African Sahel* (First edition). Oxford University Press.

Viskari, E.-L., Grobler, G., Karimäki, K., Gorbatova, A., Vilpas, R., & Lehtoranta, S. (2018). Nitrogen Recovery With Source Separation of Human Urine—Preliminary Results of Its Fertiliser Potential and Use in Agriculture. *Frontiers in Sustainable Food Systems*, *2*, Article 32, 1–14. doi:10.3389/fsufs.2018.00032

Voroney, P. (2019). Soils for Horse Pasture Management. In *Horse Pasture Management* (pp. 65–79). Elsevier. doi:10.1016/B978-0-12-812919-7.00004-4

Waechter-Kristensen, B., Caspersen, S., Adalsteinsson, S., & Sundin, P. (1999). Organic Compounds and Micro-organisms in closed, Hydroponic Culture: Occurrence and Effects on Plant Growth and Mineral Nutrition. *Acta Horticulturae*, *481*, 197–204. doi:10.17660/ActaHortic.1999.481.20

Wang, L. K., Shammas, N. K., & Hung, Y.-T. (2007). *Biosolids treatment processes*. *Handbook of environmental engineering: Vol. 6*. Humana Press.

Wenceslau, D., Daniele de Souza Lauton Fatima de Oliveira, Hudson, d. O. R., Guilherme, F. F., Luiz, A. A. G., Erica, C. A. L., & Gustavo, C. (2021). Nitrate concentration and nitrate/ammonium ratio on lettuce grown in hydroponics in Southern Amazon. *African Journal of Agricultural Research*, *17*(6), 862–868. doi:10.5897/AJAR2020.15087

Werner, H. (1991). *Medizinische Mikrobiologie mit Repetitorium*. Gruyter, Walter de GmbH. doi:10.1515/9783112327326

Williams, K. A., & Nelson, J. S. (2016). Challenges of using organic fertilizers in hydroponic production systems. *Acta Horticulturae*(1112), 365–370. doi:10.17660/ActaHortic.2016.1112.49

Wongkiew, S., Koottatep, T., Polprasert, C., Prombutara, P., Jinsart, W., & Khanal, S. K. (2021). Bioponic system for nitrogen and phosphorus recovery from chicken manure: Evaluation of manure loading and microbial communities. *Waste Management*, *125*, 67–76. doi:10.1016/j.wasman.2021.02.014

Wu, S., Feng, X., & Wittmeier, A. (1997). Microwave Digestion of Plant and Grain Reference Materials in Nitric Acid or a Mixture of Nitric Acid and Hydrogen Peroxide for the Determination of Multi-

elements by Inductively Coupled Plasma Mass Spectrometry. *Journal of Analytical Atomic Spectrometry*, *12*(8), 797–806. doi:10.1039/A607217H

Wu, Di (2016). Recycle Technology for Potato Peel Waste Processing: A Review. *Procedia Environmental Sciences*, *31*, 103–107. doi:10.1016/j.proenv.2016.02.014

IIX. Appendix

Appendix Table of Contents

Appendix List of Figures

Appendix List of Tables

Appendix I - Preliminary Experiment

This preliminary experiment tested a first approach to produce a nutrient-balanced hydroponic solution regarding N, P, and K concentrations from organic residues.

The approach was to mix organic residues in a particular ratio depending on their N, P, and K concentration with subsequent digestion. Therefore, two recipes were created, consisting of different compositions of organic residues. They were digested either aerobically or anaerobically with a pH adjusted to 6.5 or unadjusted.

The objective was to determine if this approach could produce a balanced bioponic nutrient solution. In addition, information should be collected on the most appropriate method for mineralizing the main nutrients and the possible mineralization rates. This approach should be feasible in arid regions with low technical effort and available materials.

The material and methods, results, and a short conclusion of the preliminary experiment are presented. As in the main part, the reference crop lettuce (*Lactuca sativa*) was selected, and the same recommended N, P, and K concentrations for a hydroponic nutrient solution from Sapkota (N: 250 P: 56 K: 300 mg/l) were used as reference. The CFA system (Chapter 4.3.4) was used for the nutrient analysis of the solutions produced in this experiment.

A.1. Material and Methods

A.1.1 Organic Sources

The organic residues goat manure, bone meal, potato- and banana peel were selected for the preliminary experiment. Goat manure was obtained from the Wilhelma Zoo 70376 Stuttgart, bone meal was bought from Beckmann & Brehm GmbH 27243 Beckeln, potato peel was provided by the restaurant "Speisekammer West" in 70193 Stuttgart, and banana peel was collected from commercially available bananas.

The N, P, and K concentrations of bone meal, goat manure, and potato peel were analyzed using the methods described in Chapter 4.3. For the nutrient concentrations of banana peel, literature values were used.

A-Table 1: Nutrient content of organic residues used in the preliminary experiment in percentage of dry mass [DM]. All are based on conducted analyses except banana peel. FM: Fresh mass.

Organic Residue	N [% DM]	P [% DM]	K [% DM]	DM [% FM]
Bone meal	7.0 ± 0.0	20.1 ± 0.3	0.0 ± 0.0	95.2 ± 0.1
Goat manure	1.3 ± 0.0	0.3 ± 0.0	0.6 ± 0.1	35.1 ± 0.4
Potato peel	2.5 ± 0.1	0.3 ± 0.0	1.7 ± 0.1	16.5 ± 0.7
Banana peel *	0.8 ± 0.5	0.3 ± 0.0	6.8 ± 1.0	13.6 ± 0.3

**Based on the literature values published by Anhwange et al. (2009); Archibald (1949); Jambhale and Gohatre (2019)*

A.1.2 Recipes

The two recipes are presented in A-Table 2 and A-Table 3. The first Recipe (R1) contained exclusively animal residues.

A-Table 2: Recipe 1. Exclusively based on the animal residues bone meal and goat manure. Used fresh mass (FM) of the used organic residues and the expected Nitrogen (N), Phosphorus (P), and Potassium (K) masses calculated according to A-Table 1. Deviations refer to the deviation of the respective total nutrient mass from the optimum for lettuce cultivation in hydroponics, as stated by Sapkota (2019).

Organic residue	FM	Nutrient mass		
		N	P	K
	[mg]	[mg]	[mg]	[mg]
Bone meal	1380	92	264	0
Goat manure	87700	409	80	191
Total		**501**	**344**	**191**
Reference		250	56	300
Deviation [%]		200	615	64

Due to low achievable K concentrations of R1, if compared to the optimum K concentration for lettuce cultivation, a second recipe was developed. Recipe 2 (R2) was based on R1, supplemented with K-rich potato- and banana peel. Both recipes refer to the fresh mass of the organic residues.

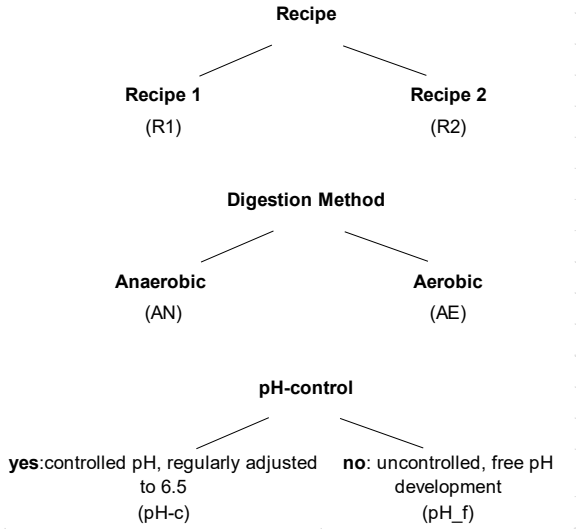
A-Table 3: A- Recipe 2. Used fresh mass (FM) of the used organic residues and the expected Nitrogen (N), Phosphorus (P), and Potassium (K) masses calculated according to A-Table 1 content. Deviations refer to the deviation of the respective total nutrient mass from the optimum for lettuce cultivation in hydroponics, as stated by Sapkota (2019).

Organic residue	FM	Nutrient mass		
		N	P	K
	[mg]	[mg]	[mg]	[mg]
Bone meal	1440	96	276	0
Goat manure	28500	133	26	62
potato peel	60000	246	26	164
banana peel	21200	23	9	196
Total		**497**	**336**	**422**
Reference		250	56	300
Deviation [%]		199	600	141

Since it can be assumed that only a proportion of the added nutrients bound in organic residues could be mineralized into plant-available forms, the potentially achievable concentration was set much higher than the reference by Sapkota et al. (2019). More precisely, the achievable N concentration was 200 % higher for R1 and 199 % higher for R2 than the reference. The higher amount was also chosen since gaseous N losses as NH_3 can occur. P concentration differed by 615 (R1) and 600 % (R2) from the reference. These high concentrations were chosen since P is firmly bound in organic material (Epple & Enax, 2018; Sardans & Peñuelas, 2015); thus, a slow and low mineralization of P was expected.

A.1.3 Treatments

Eight different treatments with three repetitions each were tested. Three different parameters were tested, these were recipe, digestion method, and pH-control:



A-Figure 1: The tested three parameters with two different parameter values each.

The total 24 repetitions were:

A-Table 4: All 24 repetitions used in the preliminary experiment. AN: Anaerobic digestion AE: Aerobic digestion pH_c: pH adjusted to 6.5 pH_f: No pH adjustment.

Recipe	Digestion	pH-control	Repetition
1	AN	pH_c	1
1	AN	pH_c	2
1	AN	pH_c	3
1	AN	pH_f	1
1	AN	pH_f	2
1	AN	pH_f	3
1	AE	pH_c	1
1	AE	pH_c	2
1	AE	pH_c	3
1	AE	pH_f	1
1	AE	pH_f	2
1	AE	pH_f	3
2	AN	pH_c	1
2	AN	pH_c	2
2	AN	pH_c	3
2	AN	pH_f	1
2	AN	pH_f	2
2	AN	pH_f	3
2	AE	pH_c	1
2	AE	pH_c	2
2	AE	pH_c	3
2	AE	pH_f	1
2	AE	pH_f	2
2	AE	pH_f	3

A.1.4 Reactor Set-Up

The experiment was conducted at the Fraunhofer IGB Stuttgart. The reactors, 24 black 5 l buckets, were placed in two separated acrylic glass enclosures – the twelve AN reactors in one and the twelve AE reactors in the other, to avoid potential interferences between the two digestion systems. Both enclosures were equipped with a fume hood. The AN reactors were closed with a lid, while a ventilation plate aerated each unsealed AE reactor (A-Figure 2) with 13 cm Ø from Pondlife. Six ventilation plates were connected via flexible hoses, respectively.

In turn, the air distributor was connected to a compressed air supply. Both compressed air supply taps were turned on to the same degree.

Each reactor was filled with 3 l of desalinated water to rule out any effects of tap water ingredients, such as hardness. The corresponding fresh mass of organic residues was weighted regarding the recipes (A-Table 2, A-Table 3). Potato and banana peel were cut into small pieces, the other ingredients were used as they were. All ingredients were filled in 145-micron mesh bags from manufacturer Baven. 24 mesh bags, twelve filled with R1 ingredients and twelve with R2 ingredients, were prepared and distributed to the reactors. The mesh bags for the AN reactors were weighted with ethanol disinfected, washed stones to prevent floating on the surface and accompanying mold formation. The water level in each reactor was marked with a waterproofed pen to allow refilling to the same level.

A-Figure 2: Left: Anaerobic Reactors. Right: Aerobic Reactors

A.1.5 pH Control

Half of the reactors were manually pH-controlled (pH_c), while in the other half, the pH was uncontrolled/free (pH_f). Every third day the pH was measured using the wtw pH/ION 340i portable pH meter and adjusted to 6.5 by adding HCL or NaOH for the pH_c reactors. For the uncontrolled pH_f reactors, no adjustment was made.

A.1.6 Inoculation

To raise the number of nitrifying bacteria and thus the nitrification rate, the AE reactors were inoculated with activated sludge from the Institute for Sanitary Engineering, Water Quality and Waste Management in 70569 Stuttgart. According to Scheurer et al. (2014), 0.8 g DM of sewage sludge per liter is sufficient for a good nitrification process. The DM content of the used sewage sludge was determined to be 4.08 %. As each reactor was filled with 3 l of water, 60 g of FM sewage sludge was added to each AE reactor.

A.1.7 Experimental Procedure

The experimental period lasted 31 days, from 20/01/2022 (DOE 0) to 20/02/2022 (DOE 31). If necessary, the water level was refilled every third day with desalinated water. The pH using wtw pH meter of the solutions in the reactors was measured, and 15 ml samples were taken and filtered with syringe filters (Minisart NML Plus, GF, CA, 28 mm, 0,2 µm). The samples were stored at 4 °C until analysis. The storing period never lasted longer than two weeks. The pH adjustment of the pH_c reactors and temperature measurements of the solutions were made every third day.

The stored samples were analyzed for their NH_4^+-N, NO_3^--N, PO_4^{3-}-P, and K^+ concentrations. K^+ was analyzed using the Jenway™ PFP7 flame photometer. NO_3^-, NH_4^+, and PO_4^{3-} were analyzed with the CFA system.

A.1.8 Statistics

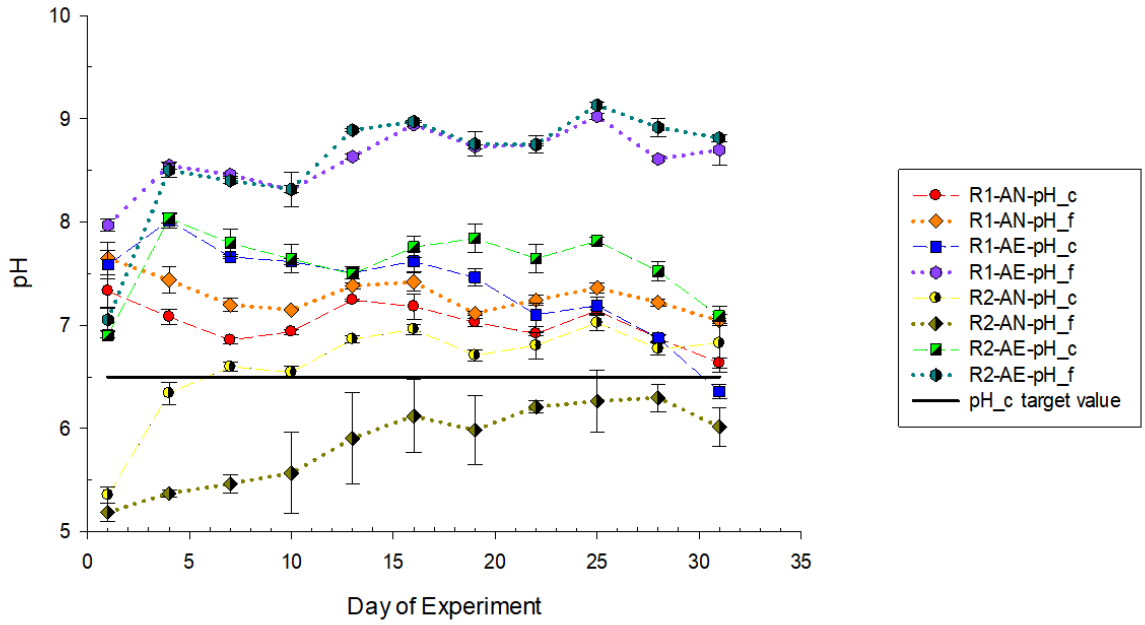
A mixed ANOVA over time was conducted for NH_4^+-N, NO_3^--N, PO_4^{3-}-P, and K^+ concentrations with subsequent post hoc Tuckey analyses with a confidence level of 0.95. The Greenhouse–Geisser correction method was applied for the mixed ANOVA to adjust for lack of sphericity. A significance level of p=0.05 was chosen. To conduct the ANOVA R-Studio 2022.12.0 Build 353, Posit Software, PBC.

A.2 Preliminary Experiment Results

In this chapter, the results of the preliminary experiment are presented. The temporal development of pH, temperature, and concentrations of NH_4^+-N, NO_3^--N, PO_4^{3-}-P, and K^+ are depicted.

A.2.1 pH

Differences in pH between the recipes and digestion methods were apparent. Especially the lower pH values of R2 compared to R1 on DOE 1 were notable (A-Figure 3). The highest pH was measured for the AE-pH_f treatments of both recipes. On DOE 31, their pH value was close to nine, whereas all other treatments, pH controlled or not, had a pH between six (R2-AN-pH_f) and seven (R2-AE-pH_f).

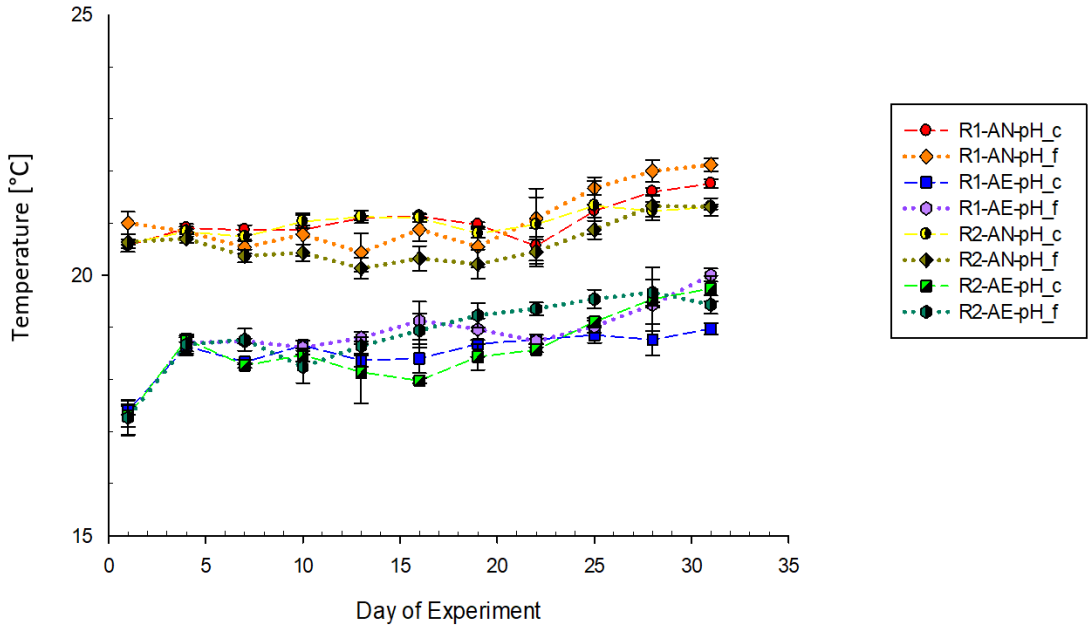


A-Figure 3: pH development of all treatments. pH_c target value refers to the targeted pH of the treatments with adjusted (pH_c) pH.

The pH of the *AE-pH_f* treatments increased from DOE 1 to DOE 31, independently of recipe. Furthermore, the pH of the two *R2-AN* treatments increased throughout the experiment. The pH of all other treatments decreased.

A.2.2 Temperature

The temperature data showed a clear distinction between AN and AE treatments (A-Figure 4). Higher temperatures were measured in the AN treatment. Throughout the experimental period, the temperature increased for all treatments.



A-Figure 4: Temperature development of all treatments.

A.2.3 Nitrogen

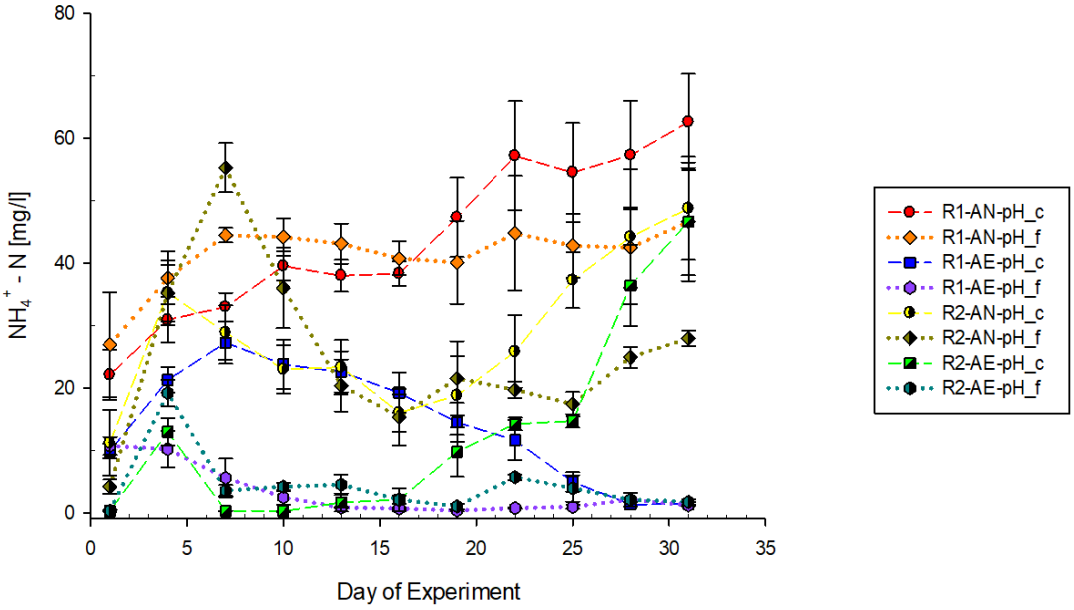
This section presents the results of the concentration development of the measured N forms, NH_4^+ and NO_3^-.

The different treatments had a significant influence on the NH_4^+-N concentration. The mixed ANOVA showed a significant interaction effect (p-value <0.001) of all parameters, recipe, digestion, pH-control, and DOE. For the conduction of the mixed ANOVA, a root transformation of the measured NH_4^+-N concentrations was performed to ensure normality and equality of variances.

The NH_4^+ concentration rose for all treatments except for *R1-AE-pH_f* between DOE 1 and DOE 4 (A-Figure 5). In the following, the concentration of many treatments decreased again. A clear trend was observed from DOE 10 onwards, as the NH_4^+-N concentration of all treatments decreased, except for *R1-AN-pH_f* and *R1-AN-pH_c*. The concentration of these two treatments remained at the already comparatively high level. From DOE 13 to 22, the two treatments showed no significantly different NH_4^+-N concentrations, while the respective treatment with the higher concentration of the two showed significant differences to the remaining six treatments.

The two pH-controlled treatments of R2 (*R2_AN_pH-c*, *R2_AE_pH-c*), independent from the digestion method, showed an NH_4^+-N concentration increase from DOE 15 onwards. On DOE 28 and DOE 31, they had no significantly different NH_4^+-N concentration compared to the *R1-AN-pH_c* and *R1-AN-pH_f* treatment.

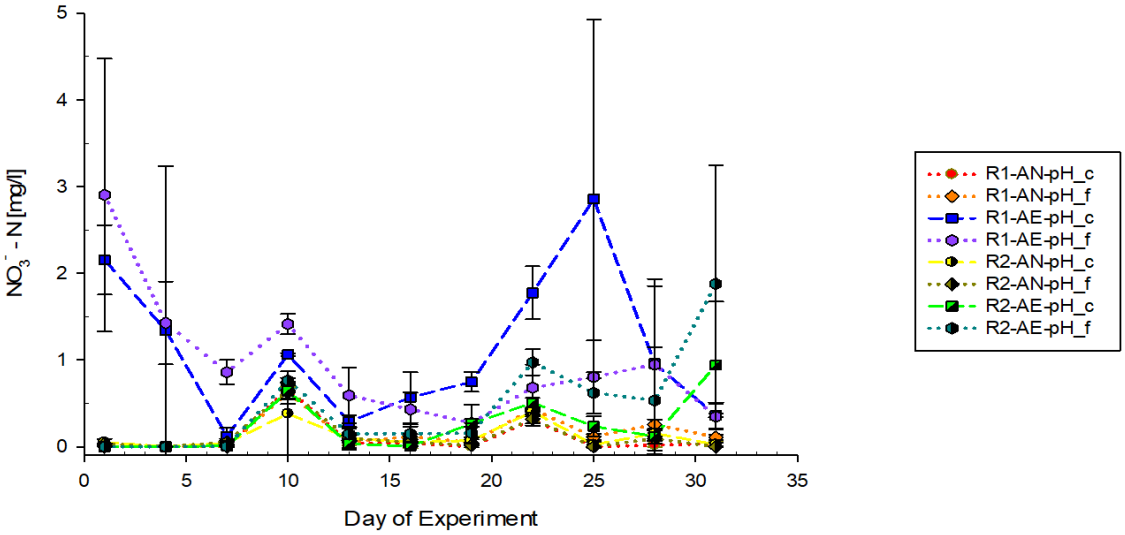
From DOE 19 onwards, the treatments with the lowest NH_4^+-N concentrations were all AE treatments. On DOE 28 and 31, the NH_4^+-N concentrations *R2_AE_pH-f*, *R1_AE_pH-f*, and *R1_AE_pH-c* differed not significantly between themselves but were significantly lower than the NH_4^+-N concentration of all other treatments.



A-Figure 5: Ammonium nitrogen (NH_4^+-N) concentration development of all treatments.

The highest concentrations measured for different treatments were 62.6 ± 7.7 mg/l NH_4^+-N for treatment R1-AN-pH_c on DOE 31, 55.3 ± 4.0 mg/l NH_4^+-N for R2-AN-pH_f on DOE 7, and 48.7 ± 8.2 mg/l NH_4^+-N for treatment R2-AN-pH_c on DOE 31 (A-Figure 5).

The measured NO_3^--N concentrations remained extremely low throughout the experimental period (A-Figure 6). The AE treatments showed fluctuations throughout the experiment, but the concentration never exceeded 3 mg/l NO_3^--N. A mixed ANOVA revealed that recipe, digestion, and DOE interaction had a significant effect (p-value <0.007) on the NO_3^--N concentration. However, the NO_3^--N data did not fit a normal distribution nor equality of variances; therefore, the result can only serve as an indicator. Due to the low, negligible concentrations for plant cultivation, no further non-parametrical statistical analyses were conducted.



A-Figure 6: Nitrate nitrogen (NO_3^--N) concentration development of all treatments.

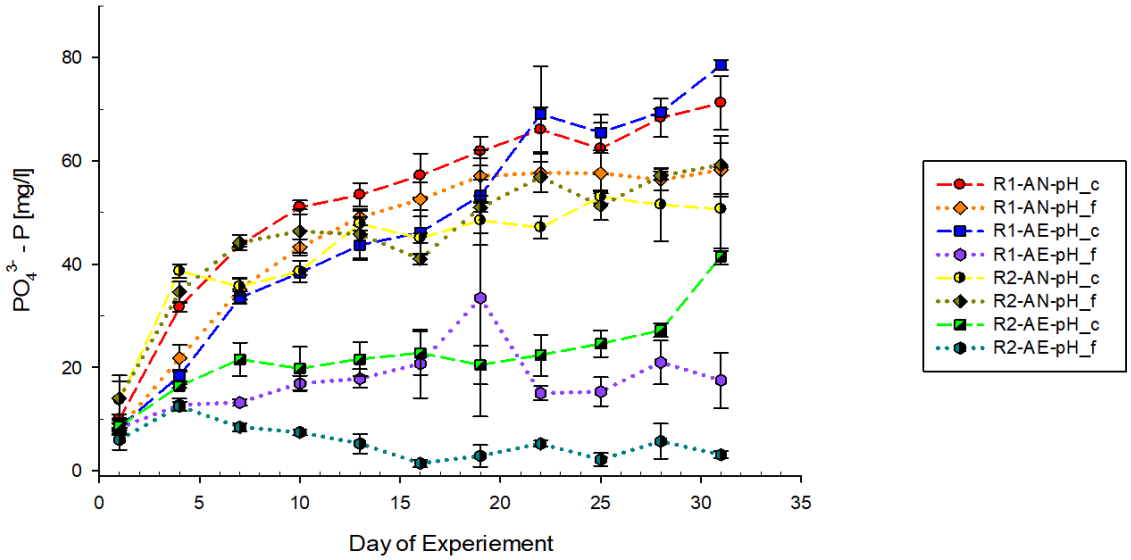
A-Table 5 presents the three highest measured NO_3^--N concentrations throughout the experimental period. All were measured for the treatment R1-AN-pH_c on different days and showed no significant differences. Due to the low NO_3^--N concentrations, only NH_4^+-N was evaluated and compared to the reference of 250 mg/l N (Sapkota et al., 2019). Apparently, the treatments with the highest measured concentrations were simultaneously the ones with the lowest deviation from the reference. A maximum of 12.5 % of the added N by the organic residues was mineralized into NH_4^+-N.

A-Table 5: Three highest measured NH_4^+-N concentrations within the experimental period of 31 days. Deviation refers to the deviation from the optimum of 250 mg/l Nitrogen (N) for hydroponic solutions (Sapkota et al., 2019) . Mineralization rate in percentage of the initially added N by organic residues.

Treatment			DOE	NH_4^+ - N [mg/l]	Deviation [%]	Mineralization rate [%]
R1	AN	pH-c	31	62.6 ± 7.7	-75.0	12.5
R1	AN	pH-c	28	57.3 ± 8.7	-77.1	11.4
R1	AN	pH-c	22	57.2 ± 8.8	-77.1	11.4

A.2.4 Phosphorus

Throughout the experimental period, the PO_4^{3-}-P concentrations increased for most treatments (A-Figure 7); only the two AE-pH_f treatments showed no larger increase or a decline (*R2-AE-pH_f*). The AN treatments showed sharp concentration increases in the first days of the experiment then the increases slowed down. *R1-AE-pH_c* showed a mostly linear increase.



A-Figure 7: Phosphate phosphorus (PO_4^{3-}-P) concentration development of all treatments.

The three highest measured PO_4^{3-}-P concentrations were all R1 treatments (A-Table 6). The highest concentration was measured for the *R1-AE-pH_c* treatment on DOE 31. A maximum of 22.8 % of the added P by organic residues was mineralized into PO_4^{3-}-P.

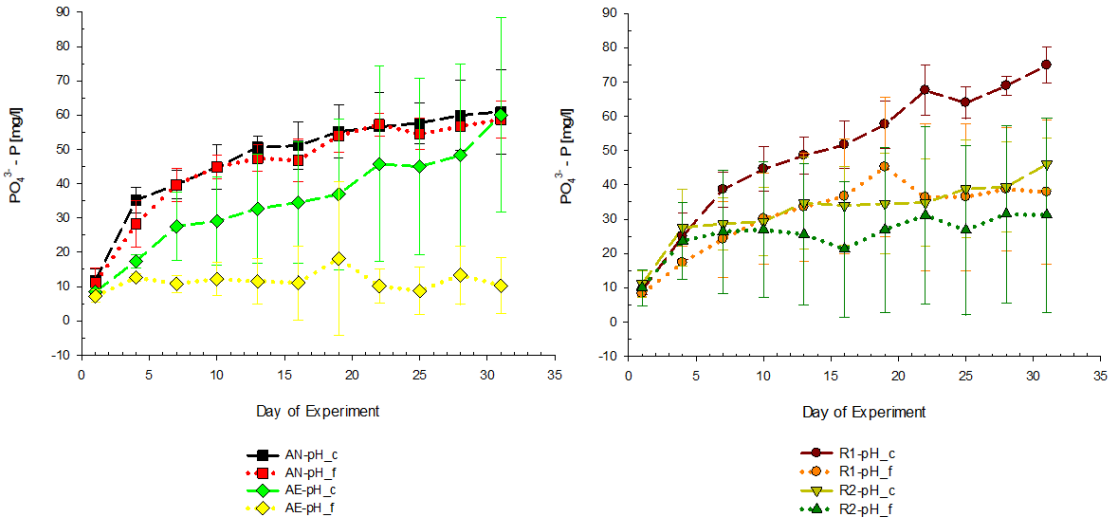
A-Table 6: Three highest measured PO_4^{3-}-P concentrations within the experimental period of 31 days. Deviation refers to the deviation from the optimum of 56 mg/l Phosphorus (P) for hydroponic solutions (Sapkota et al., 2019). Mineralization rate in percentage of the initially added P by organic residues.

Treatment			DOE	PO_4^{3-} - P [mg/l]	Deviation [%]	Mineralization rate [%]
R1	AE	pH-c	31	78.6 ± 0.9	40.3	22.8
R1	AN	pH-c	31	71.2 ± 5.2	27.2	20.7
R1	AE	pH-c	28	69.4 ± 0.7	24.0	20.2

A mixed ANOVA was also performed for the PO_4^{3-}-P concentrations. The two three-way interactions digestion, pH, and DOE and recipe, pH, and DOE proved to be highly significant (p-value < 0.001). To evaluate these effects, the PO_4^{3-}-P concentrations were averaged over the factors recipe and digestion, respectively (A-Figure 8).

The evaluation of digestion, pH, and DOE (A-Figure 8) interaction showed no significant differences between the treatments on DOE 1. The mean value of the two *AE-pH_f* treatments was lowest throughout the experiment. From DOE 22 until the end of the experiment, the PO_4^{3-}-P of *AE-pH_f* was significantly lower compared to all other treatments. In contrast, the means of the other three treatments had no significant differences from DOE 19 onwards.

The high standard deviations of *AE-pH_c* underline the large differences in PO_4^{3-}-P concentrations between *R1-AE-pH_c* and *R2-AE-pH_f* (A-Figure 8)



A-Figure 8: Plotted PO_4^{3-}-P Left: Development of PO_4^{3-}-P concentration averaged over recipe to evaluate significant interaction of digestion, pH, and days of experiment. Right: PO_4^{3-}-P concentration development averaged over digestion, to evaluate significant interaction of recipe, pH, and days of experiment.

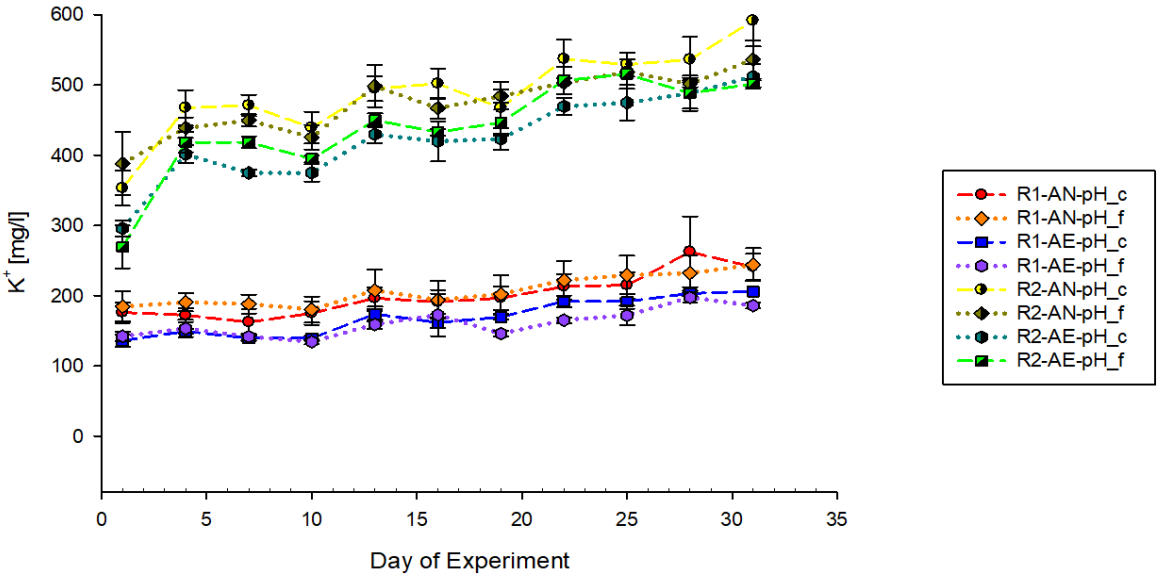
Also, evaluating the interaction of recipe, pH, and DOE averaged over the digestion method, no significant difference between the four treatments' PO_4^{3-}-P concentrations was revealed for DOE 1. From DOE 7 until the end of the experiment, *R1-pH_c* had significantly higher concentrations than the other treatments. The only expectation was on DOE 19, as no significant difference between all four treatments was measured. *R1-pH_c* also had the lowest standard deviations compared to the other treatments.

A.2.5 Potassium

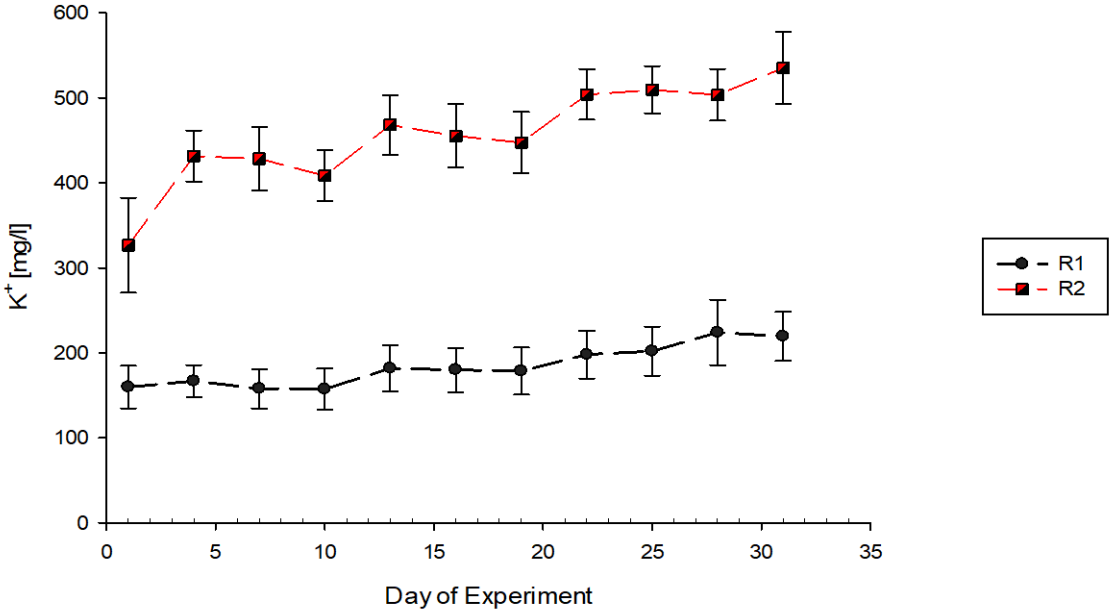
The recipe largely influenced the K^+ concentration. R2 showed higher K^+ concentrations than R1 for any treatment at any point in time (A-Figure 9, A-Figure 10) The mixed ANOVA proved this; only the interaction of the recipe and DOE was significant ($p < 0.001$). Also, the single factors DOE, recipe, and digestion had a significant effect (all $p < 0.001$).

If averaged over the digestion method and pH control for each recipe (A-Figure 10), the difference between R1 and R2 K^+ concentration was significant for any measurement day.

A significant K^+ concentration increase was measured from DOE 1 to DOE 31 for R1 ($p=0.005$) and R2 ($p < 0.001$). However, the percentual increase of the K^+ concentration from DOE 1 to DOE 31 was stronger for R2 with an increase of 64 %. Simultaneously, the K^+ concentration of R1 rose only by 37 %.



A-Figure 9: K^+ concentration development of all treatments.



A-Figure 10: K^+ concentration development of the two recipes averaged over digestion and pH.

A-Table 7 presents the highest measured K^+ concentration for R1 and R2. For both, the mineralization rate was over 100 % of the added K by the organic residues.

A-Table 7: Highest K^+ concentrationsfor each recipe within the experimental period of 31 days. Deviation refers to the deviation from the optimum of 300 mg/l Potassium (K) for hydroponic solutions (Sapkota et al., 2019). Mineralization rate in percentage of the initially added K by organic residues.

Treatment			DOE	K^+ [mg/l]	Deviation [%]	Mineralization rate [%]
R1	AN	pH-c	28	289.1 ± 31.2	-3.6	151.4
R2	AN	pH-c	31	551.2 ± 49.9	83.7	130.6

A.2.6 Best Treatment

To evaluate which of the eight treatments was closest to a balanced nutrient solution, the deviations from the reference of each nutrient on each DOE were calculated. Due to the low concentrations, NO_3^--N was not considered in this assessment, and only NH_4^+-N was evaluated. The ten lowest deviations from the reference value were selected and checked for overlap for each nutrient. This selection found overlaps for R1-AN-pH_f on DOE 31 and DOE 22 (A-Table 8, A-Table 9). While the treatment lacked a high proportion of N and a considerable amount of K^+, PO_4^{3-}-P was very close to the reference value at both time points.

A-Table 8: I. Treatment with lowest deviation from reference (Sapkota et al., 2019) for NPK. R1-AN-pH_f DOE 31.

Nutrient	Concentration [mg/l]	Deviation [%]
N (NH_4^+-N)	46.6 ± 9.5	- 81.4
P (PO_4^{3-}-P)	56.9 ± 4.6	1.6
K (K^+)	244.0 ± 23.4	- 18.7

A-Table 9: II. Treatment with lowest deviation from reference (Sapkota et al., 2019) for NPK. R1-AN-pH_f DOE 22.

Nutrient	Concentration [mg/l]	Deviation [%]
N (NH_4^+-N)	44.8 ± 9.2	- 82.1
P (PO_4^{3-}-P)	57.8 ± 3.4	3.3
K (K^+)	221.8 ± 28.1	- 26.1

A.3 Evaluation

The most important findings of the preliminary experiment are summarized below. The key messages learned in this experiment regarding the mineralization of the main nutrient are presented in Chapter 3 of the main part. The two different parameter values of the parameters recipe, digestion system, and pH-control are evaluated on which one is more suitable for mineralizing N, P, and K from organic residues in plant-available forms.

Overall R1 had better mineralization suitability. The highest NH_4^+-N and PO_4^{3-}-P concentrations were measured for R1 treatments on DOE 31. Only the K^+ concentrations were higher for R2, owed to the fact that more K was available in the organic residues of R2. A reason for the better mineralization of N could be the lower C:N ratios of the organic residues used in R1 (Lazicki et al., 2020).

Anaerobic digestion was more suitable for the mineralization of nutrients. In total, this was the case for all three nutrients, where the digestion method significantly affected the concentration. Another factor that should not be disregarded is the higher temperature of the AN treatments. Although the temperature difference was only between 1-2 °C, it can increase the velocity of chemical and enzymatic reactions. Only two AE treatments reached NH_4^+-N and PO_4^{3-}-P concentrations comparable to the concentrations reached with AN digestion on DOE 31 (*R2-AE-pH_c* for NH_4^+, *R1-AE-pH_c* for PO_4^{3-}).

The high K mineralization rates of over 100 % for all treatments of the added K were attributed to inaccuracies in the extraction method or inhomogeneous organic material.

pH control significantly affected the concentrations of NH_4^+-N and PO_4^{3-}-P. Especially for AE digestion, pH adjustment to 6.5 had a significant effect; higher nutrient concentrations were measured for the AE-pH_c treatments. Overall, pH seems to be very important for high mineralization rates. Especially for P mineralization, a lower pH was beneficial, which becomes apparent when A-Figure 3 is compared with A-Figure 7.

None of the different treatments produced a nutrient solution that can be stated as balanced. One problem were the different mineralization rates of the main nutrients. The best approach, which had the lowest deviations for each of the main nutrients from the reference, was *R1-AN-pH_f* on DOE 31 (A-Table 9). While PO_4^{3-}-P concentration was very close to the suggested concentration and the 18.7 % lower K^+ concentration should not impede plant growth significantly, the low N concentration would hinder plant growth for certain. Moreover, most of the N was in NH_4^+-N form and only a vanishingly small proportion in NO_3^--N form. High concentrations of NH_4^+-N can be toxic for plants (Britto & Kronzucker, 2002), so it is doubtful that lettuce can grow well in the prepared bioponic nutrient solution.
No balanced nutrient solution could be created by the approach of mixing organic residues and

subsequent digestion, neither with Recipe 1 nor with Recipe 2 with the tested parameters of anaerobic or aerobic digestion and adjusted or unadjusted pH.

This preliminary experiment provides, however, relevant information on the mineralization of the main plant nutrients, the role of the pH, and digestion methods. These findings served as the basis for the main experiment.

Appendix II - Mixed ANOVA - Preliminary Experiment

A-Table 10: Preliminary experiement mixed ANOVA output tables conducted for NH_4^+-N, NO_3^--N, PO_4^{3-}-P, and K^+ concentrations. With Greenhouse–Geisser correction method. significance level p=0.05.

Anova Table (Type 3 tests)

Response: NH4

	Effect	df	MSE	F	ges	p.value
1	(Intercept)	1, 16	0.61	7167.23 ***	.990	<.001
2	Digestion	1, 16	0.61	1190.32 ***	.942	<.001
3	Recipe	1, 16	0.61	72.11 ***	.496	<.001
4	pH	1, 16	0.61	81.74 ***	.527	<.001
5	Digestion:Recipe	1, 16	0.61	43.12 ***	.370	<.001
6	Digestion:pH	1, 16	0.61	41.92 ***	.364	<.001
7	Recipe:pH	1, 16	0.61	4.10 +	.053	.060
8	Digestion:Recipe:pH	1, 16	0.61	9.88 **	.119	.006
9	DOE	5.71, 91.35	0.38	36.24 ***	.639	<.001
10	Digestion:DOE	5.71, 91.35	0.38	15.46 ***	.430	<.001
11	Recipe:DOE	5.71, 91.35	0.38	29.68 ***	.592	<.001
12	pH:DOE	5.71, 91.35	0.38	18.00 ***	.468	<.001
13	Digestion:Recipe:DOE	5.71, 91.35	0.38	32.74 ***	.615	<.001
14	Digestion:pH:DOE	5.71, 91.35	0.38	5.53 ***	.213	<.001
15	Recipe:pH:DOE	5.71, 91.35	0.38	28.07 ***	.578	<.001
16	Digestion:Recipe:pH:DOE	5.71, 91.35	0.38	18.63 ***	.476	<.001

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘+’ 0.1 ‘ ’ 1

Sphericity correction method: GG

Anova Table (Type 3 tests)

Response: NO3

	Effect	df	MSE	F	ges	p.value
1	(Intercept)	1, 16	0.24	188.79 ***	.477	<.001
2	Recipe	1, 16	0.24	32.97 ***	.137	<.001
3	Digestion	1, 16	0.24	92.11 ***	.308	<.001
4	pH	1, 16	0.24	0.36	.002	.558
5	Recipe:Digestion	1, 16	0.24	30.69 ***	.129	<.001
6	Recipe:pH	1, 16	0.24	1.65	.008	.217
7	Digestion:pH	1, 16	0.24	0.01	<.001	.922
8	Recipe:Digestion:pH	1, 16	0.24	3.08 +	.015	.098
9	DOE	3.34, 53.51	0.85	4.45 **	.204	.006
10	Recipe:DOE	3.34, 53.51	0.85	4.00 **	.188	.010
11	Digestion:DOE	3.34, 53.51	0.85	2.72 *	.136	.048
12	pH:DOE	3.34, 53.51	0.85	0.79	.044	.516
13	Recipe:Digestion:DOE	3.34, 53.51	0.85	4.31 **	.199	.007
14	Recipe:pH:DOE	3.34, 53.51	0.85	1.07	.058	.374
15	Digestion:pH:DOE	3.34, 53.51	0.85	0.79	.044	.515
16	Recipe:Digestion:pH:DOE	3.34, 53.51	0.85	1.47	.078	.229

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘+’ 0.1 ‘ ’ 1

Sphericity correction method: GG

Anova Table (Type 3 tests)

Response: P

	Effect	df	MSE	F	ges	p.value
1	(Intercept)	1, 16	65.25	4921.96 ***	.987	<.001
2	Digestion	1, 16	65.25	546.61 ***	.891	<.001
3	Recipe	1, 16	65.25	136.47 ***	.670	<.001
4	pH	1, 16	65.25	168.39 ***	.715	<.001
5	Digestion:Recipe	1, 16	65.25	49.36 ***	.424	<.001
6	Digestion:pH	1, 16	65.25	115.69 ***	.633	<.001
7	Recipe:pH	1, 16	65.25	34.48 ***	.339	<.001
8	Digestion:Recipe:pH	1, 16	65.25	0.62	.009	.441
9	DOE	3.17, 50.70	65.87	137.82 ***	.868	<.001
10	Digestion:DOE	3.17, 50.70	65.87	16.15 ***	.435	<.001
11	Recipe:DOE	3.17, 50.70	65.87	22.51 ***	.517	<.001
12	pH:DOE	3.17, 50.70	65.87	14.91 ***	.415	<.001
13	Digestion:Recipe:DOE	3.17, 50.70	65.87	2.17 +	.094	.099
14	Digestion:pH:DOE	3.17, 50.70	65.87	15.89 ***	.431	<.001
15	Recipe:pH:DOE	3.17, 50.70	65.87	6.16 ***	.227	<.001
16	Digestion:Recipe:pH:DOE	3.17, 50.70	65.87	2.18 +	.094	.098

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘+’ 0.1 ‘ ’ 1

Sphericity correction method: GG

Anova Table (Type 3 tests)

Response: K

	Effect	df	MSE	F	ges	p.value
1	(Intercept)	1, 16	2927.33	9293.17 ***	.993	<.001
2	Digestion	1, 16	2927.33	41.86 ***	.390	<.001
3	Recipe	1, 16	2927.33	1595.70 ***	.961	<.001
4	pH	1, 16	2927.33	0.02	<.001	.883
5	Digestion:Recipe	1, 16	2927.33	3.49 +	.051	.080
6	Digestion:pH	1, 16	2927.33	0.30	.005	.590
7	Recipe:pH	1, 16	2927.33	0.03	<.001	.868
8	Digestion:Recipe:pH	1, 16	2927.33	3.57 +	.052	.077
9	DOE	3.09, 49.48	2929.97	43.14 ***	.671	<.001
10	Digestion:DOE	3.09, 49.48	2929.97	1.77	.077	.163
11	Recipe:DOE	3.09, 49.48	2929.97	11.25 ***	.347	<.001
12	pH:DOE	3.09, 49.48	2929.97	1.21	.054	.316
13	Digestion:Recipe:DOE	3.09, 49.48	2929.97	1.54	.068	.214
14	Digestion:pH:DOE	3.09, 49.48	2929.97	1.46	.064	.237
15	Recipe:pH:DOE	3.09, 49.48	2929.97	1.48	.065	.230
16	Digestion:Recipe:pH:DOE	3.09, 49.48	2929.97	1.34	.059	.273

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘+’ 0.1 ‘ ’ 1

Sphericity correction method: GG

Appendix III - Three-Way ANOVA - Plant Masses

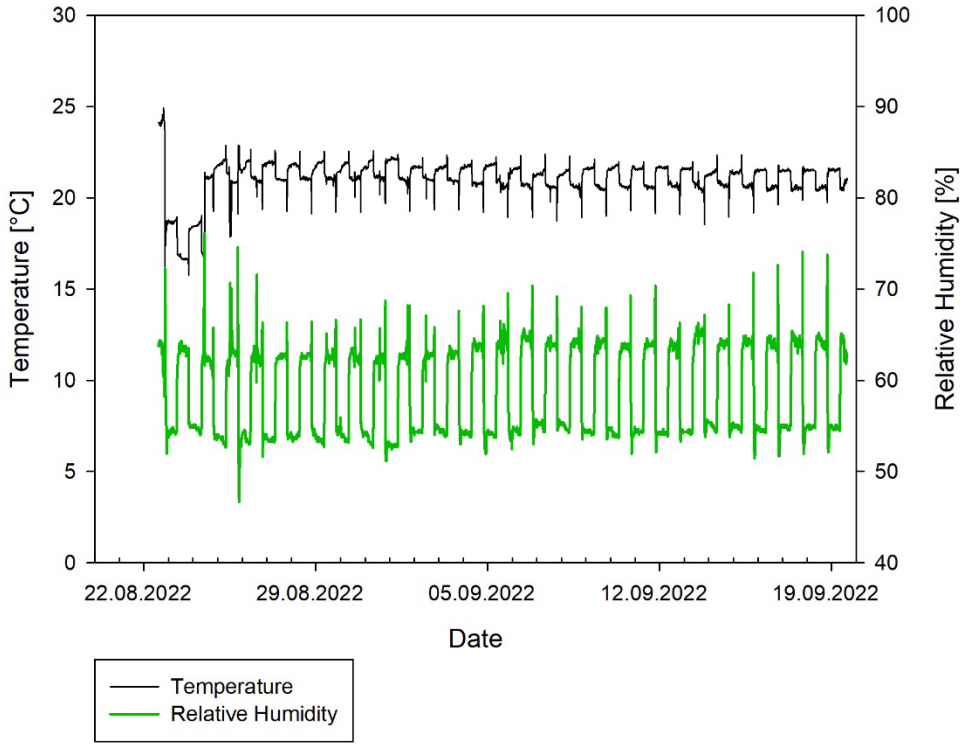
A-Table 11: Three-way ANOVA output table conducted for plant masses produced with organic, spiked-organic, and mineral nutrient solutions in the hydroponic experiment .n=four plants With Greenhouse–Geisser correction method. significance level p=0.05.

```
Anova Table (Type 3 tests)


Response: Mass
                  Effect          df    MSE          F  ges p.value
1           (Intercept)       1, 12 629.28 955.37 *** .967   <.001
2     Nutrient.solution       2, 12 629.28  36.32 *** .693   <.001
3                   DOE 2.05, 24.54 516.76 216.65 *** .919   <.001
4 Nutrient.solution:DOE 4.09, 24.54 516.76  31.19 *** .765   <.001
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘+’ 0.1 ‘ ’ 1


Sphericity correction method: GG
```

Appendix IV - Temperature and Relative Humidity



A-Figure 11: Temperature and relative humidity measured in the climate chamber during the hydroponic experiment using the tinytag tv-4505

Declaration

Declaration*

I,

Surname, First name Heintze, Sebastian

Matriculation number 629973

declare that I have followed the Principles of Good Scientific Practice while writing the present

☐ Bachelor's thesis, ☒ Master's thesis,
☐ seminar paper.

I have written the paper/thesis independently and have used no other sources or aids than those given and have marked the passages taken from other works word-for-word or paraphrased.

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Topic of the paper/thesis

Evaluation of a low-tech Approach to Mobilize Nutrients from Organic Residues for the Production of a Hydroponic Nutrient Solution

Semester 8

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