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Electrochemical In Situ Hydrogen Peroxide Production Can Reduce Microbial Load in Bioponic Nutrient Solutions Derived from Organic Waste

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Abstract: Technological advancement in recent decades has allowed for crop cultivation in soilless controlled environments, known as hydroponics, and this is being employed in an increasing number of factories worldwide. With continued local and regional disruptions in the supply chain to provide mineral fertilizers, new pathways to generate nutrient solutions are being developed. One potential approach is the recovery of nutrients from organic waste and wastewater using bioponics. Bioponics refers to the biological mineralization of organic residues through processes such as anaerobic and aerobic digestion and the use of such organically produced nutrient solutions in hydroponic systems. However, without disinfection of the nutrient solution, the high microbial loads increase the risk of pathogens affecting plant and consumer health. In this work, electrochemical hydrogen peroxide (H_2O_2) demonstrated success in reducing microbial loads. Different scenarios of application were considered: (1) variation in the H_2O_2 concentration in the nutrient solution by dosing H_2O_2 from ex situ electrochemical production, (2) variation in the dosing time-dependent reaction between the nutrient solution and H_2O_2 produced ex situ, and (3) the in situ production of H_2O_2 of the organic nutrient solution. The highest tested H_2O_2 concentration of 200 mg L^{-1} showed a microbial load reduction of bacteria at 93.3% and of fungi at 81.2%. However, the in situ production showed the highest reduction rate for bacteria and fungi in bioponic nutrient solutions, where longer reaction times also impact microbial concentrations in situ. Final microbial reductions of 97.8% for bacteria and of 99.1% for fungi were determined after a H_2O_2 production time of 60 min. Overall, our results show that electrochemical H_2O_2 production can be used to disinfect bioponic nutrient solutions, and the production cell can be implemented in bioponic systems in situ.

Keywords: soilless agriculture; bioponics; organic waste recycling; disinfection; electrochemical H_2O_2 production; hydrogen peroxide treatment

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1. Introduction

In recent years, hydroponic systems have emerged as a potential pioneering method of cultivating plants in a controlled environment, offering increased water efficiency [1], reduced resource consumption [2,3], and improved crop yields [4]. However, hydroponic systems are dependent on fertilizers, as the crops receive all required nutrients from a solution rather than nutrients bound in the soil. These typically mineral fertilizers have a risk of high volatile prices and, due to increased demand, reduced availability [5].

Various attempts have been made to produce hydroponic nutrient solutions from organic residues—with varying success [6]. While there is an ongoing debate about whether hydroponics can meet the criteria of organic agriculture in the absence of soil, increasing research efforts contribute to the use of organically sourced fertilizers. An emerging trend is the field of bioponics. The term bioponics refers to the production of hydroponic nutrient solutions involving microorganisms to mineralize nutrients bound in the organic residues into soluble, plant-available forms. Microorganisms in bioponic components, such as

biofilms on plant roots and digested organic waste in bioreactors, perform biological conversion of the organic nutrients in waste into readily available forms of nutrients for plants. Figure 1 illustrates the most commonly applied bioponic methods, namely, aerobic digestion, anaerobic digestion, a combination of both, and "tea"-type methods [7–9].

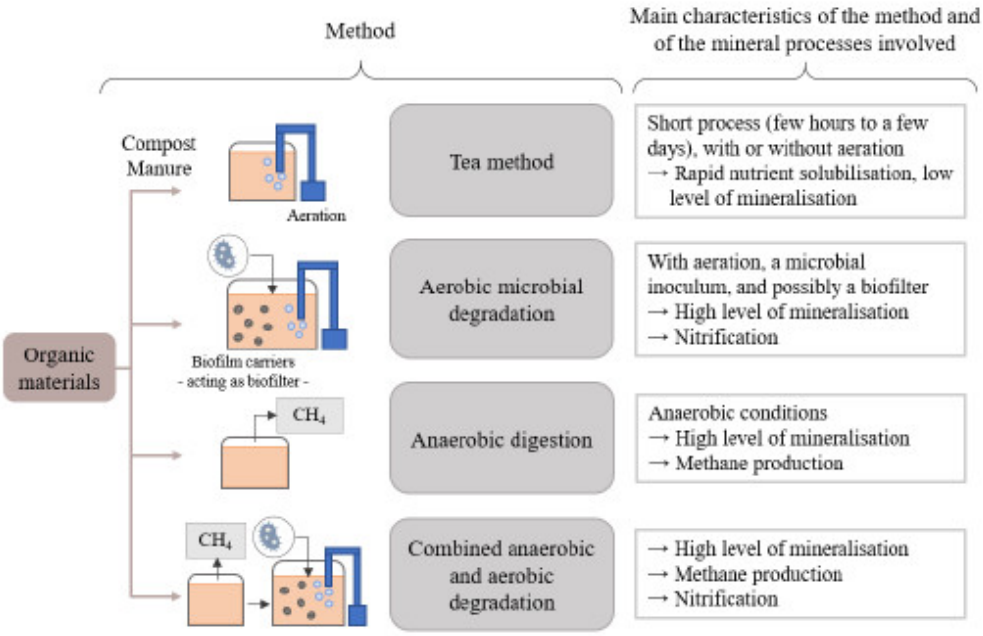

Figure 1. Overview of the most commonly used bioponic methods to produce nutrient solutions [8].

A major challenge in producing a bioponic nutrient solution (BNSL) is ensuring sufficient nitrogen availability in the solution [10]. As nitrate is required in larger quantities for vegetative growth and ammonium is toxic to plants in high concentrations, nitrification plays a crucial role. However, only low nitrification rates can be obtained by aerobic digestion itself due to the slow growth of the relevant microbial community [11,12]. Among the studied resources used to produce bioponic nutrient solutions are digestate from biodigesters [13,14], animal manures [15–17], municipal wastewater [9,18], and food waste [19]. Due to the challenge of producing an adequate and balanced nutrient solution, which is required in hydroponic cultivation, the vast majority of studies have focused on the cultivation of leafy greens, such as lettuce (*Lactuca sativa* L.), Pak Choi (*Brassica campestris v. Chinensis cv.* Joi Choi), and silverbeet (*Beta vulgaris* L). Only a few studies have investigated the bioponic production of fruiting vegetables such as tomatoes *(Solanum lycopersicum cv. "Ponderosa"*; Kobayashi Seed Co., Ltd., Kakogawa, Japan) [8]. The main reason for this is that leafy greens have a more uniform nutrient demand over their life cycle, while fruiting vegetables require adapted nutrient solutions to cater for demands during flowering and fruiting plant stages. In the majority of studies, the plants grown in bioponic nutrient solutions showed lower biomass development than the mineral control groups. Only in a few cases did the bioponic production achieve similar biomass yields when compared to their mineral controls. Commonly, yields refer to biomass development only, which is the major agronomic factor for farmers, retailers, and consumers.

However, results from studies focusing on the product quality of biologically produced crops indicate improved product quality over conventionally produced hydroponic crops. Analyses of the qualitative properties and nutritional values have hardly been investigated. Mauerer [20] showed that hydroponic lettuce grown with nitrified urine resulted in increased chlorophyll content in leaves when compared to the control. Similarly, Mowa et al. [21] found that the lycopene content in tomatoes produced with manure-derived nutrient solutions was twice as high as in vegetables produced with mineral fertilizers. Lycopene is a carotenoid linked to health benefits such as blood pressure man-

agement and cancer prevention. Thus, despite the often lower biomass development in bioponic systems, this form of production could provide added value for the market, highlighting the relevance of further investigations.

Besides the nutrient composition, the microbial communities forming in bioponic systems affect the processing of organic residues, plant growth, and food safety. While microorganisms are required for biological nutrient conversion, the use of organic residues in food production bears a risk of viral, bacterial, and/or fungal pathogens, potentially harmful to plant and consumer health. In a comprehensive study on the bacterial community in bioponic nutrient solutions derived from chicken manure, the authors of [17] showed that several microbial genera were associated with organic degradation (e.g., *Nocardiopsis* spp., *Cellvibrio* spp.), nitrification (*Nitrospira* spp.), phosphorus solubilization, and plant growth promotion (for example, *WD2101_soil_group* and *Bacillus* spp.).

At the same time, hydroponic solutions and systems present a good milieu for beneficial as well as harmful microbial growth. Specifically due to high nutrient contents not only required by the plant but also by microorganisms, hydroponic nutrient solutions favor microbial growth [22]. Hence, in order to prevent microbial overpopulation, which can negatively affect plant growth and cause the growth of harmful pathogens, disinfection is necessary. Several methods have previously been described, though mostly in a hydroponic and not in a bioponic approach [23]. Non-chemical disinfection of nutrient solutions can be achieved through heat treatment, filtration, oxidation, or the most common method through UV disinfection. Examples of chemical treatments are the addition of sodium hypochlorite, ozone, or hydrogen peroxide (H_2O_2) [24,25]. Specifically, disinfection with H_2O_2 is a promising approach because oxygen radicals theoretically can be produced in situ and on demand through electrochemical processes within the nutrient solution.

H_2O_2 has long been recognized as a potent antimicrobial agent, capable of neutralizing a wide spectrum of pathogens [26]. Conventionally, H_2O_2 is introduced into hydroponic systems after being produced off-site, often leading to complexities in dosage control, cost implications, and potential disruptions to the balance of nutrient concentrations due to unpredicted reactions of chemical components within the nutrient solution. For that reason, researchers explore the viability of water-based electrochemical H_2O_2 production using new reactor concepts, components, and catalysts as a sustainable and efficient approach to combat microbial proliferation within hydroponic nutrient solutions [27].

For decades, commercial H_2O_2 has been predominantly produced via the Anthraquinone process and sold in concentrated solutions of typically 30–50% [28]. For applications where significantly lower concentrations are required, as is the case in hydroponic systems, this leads to costs and effort for the safe handling and dilution of the H_2O_2 solutions. Therefore, on-demand production, directly in the required concentration, is attractive. One promising option is the electrochemical H_2O_2 production, either by the cathodic process of oxygen reduction,

$$O_2 + 2H^+ + 2e^- \rightleftarrows H_2O_2 \quad E^0 = +0.67\ \text{V} \tag{1}$$

or by an anodic process of water oxidation [29],

$$2H_2O \rightleftarrows H_2O_2 + 2H^+ + 2e^- \quad E^0 = +1.76\ \text{V} \tag{2}$$

E^0 represents here the standard potential of the respective reaction measured against the normal hydrogen electrode. The standard potential for the anodic H_2O_2 production is significantly higher than that of the oxygen evolution reaction (E^0 = +1.23 V). Therefore, the anodic process typically uses boron-doped diamond electrodes that have a high overpotential for the competing oxygen evolution. This process has been shown to enable H_2O_2 concentrations up to 100 mM in alkaline carbonate electrolytes (corresponding to 3400 mg L^{-1}), where peroxodicarbonates are formed as intermediates [30–32].

The oxygen reduction reaction is typically realized using gas diffusion electrodes, which overcomes mass transport limitations due to the low solubility limit of oxygen in

water. A typical electrochemical reactor concept uses liquid electrolytes both on the cathode and on the anode side separated by an ion exchange membrane [33,34]. Alternatively, the gas diffusion electrode can be in direct contact with the ion exchange membrane, and even a complete polymer electrolyte membrane fuel cell configuration with a hydrogen oxidation electrode on the anode side has been described [35]. Several side and decomposition reactions typically limit the H_2O_2 concentrations achieved by both electrochemical processes. Generally, the produced H_2O_2 can chemically decompose into oxygen and water. Hydrogen evolution is one main competing reaction for the cathodic process, and the H_2O_2 can undergo further reduction into water, according to the reverse reaction in Equation (1). For the anodic process, the main competing reaction is oxygen evolution, and the produced H_2O_2 can be further oxidized according to the reverse reaction in Equation (2). These reactions typically lead to an equilibrium of production and decomposition, where the H_2O_2 concentration remains constant.

Therefore, most of the studies reported in the literature focus not only on the development of suitable catalyst materials but also on defined electrolyte compositions and process conditions where the competing reactions are suppressed. At the same time, for the disinfection of nutrient solutions, the H_2O_2 solution needs to be compatible with the hydroponic system in terms of process integration and the impact on nutrient chemistry as well as plant toxicology.

One option for H_2O_2 production is in pure water using an electrolyzer where the catalyst layer and membrane are in direct contact. One example of such an electrolyzer has been developed by the Danish company HPNow. However, the pure water supply requires an additional pre-treatment by reverse osmosis or ion exchange. In addition, the maximum H_2O_2 concentration is limited, especially considering that the H_2O_2 concentration is low after dosing to the irrigation water.

For that reason, this publication delves into the promising approach of electrochemical on-site H_2O_2 production using drinking water and bioponically produced nutrient solution for controlling the microbial load in bioponic and hydroponic systems. By leveraging electrochemistry and harnessing the innate capabilities of H_2O_2, this novel approach aims to transform disinfection in hydroponics, offering a streamlined and eco-friendly method to tackle microbial challenges. Furthermore, the incorporation of in situ H_2O_2 production in nutrient solutions not only enhances the economic feasibility of the process but also aligns with the principles of on-demand disinfection and resource sustainability.

Through the consideration of electrochemistry, microbiology, agricultural science, and waste management, this work seeks to explain the mechanisms underpinning electrochemical H_2O_2 production and its efficacy in reducing microbial loads. The research presented herein draws from both theoretical insights and practical experimentation, shedding light on the potential benefits, challenges, and implications of on-demand produced H_2O_2 within the topic of not just bioponic agriculture but also hydroponic approaches in agriculture. The focus of this study was the H_2O_2 production directly in the bioponic nutrient solution and the H_2O_2 production in tap water without any pre-treatment. Since exposure of the nutrient solution to anodic oxidation processes is expected to lead to critical side reactions such as chlorine evolution or the decomposition of organic compounds, the cathodic H_2O_2 process using liquid electrolytes on both sides was used. Hence, this work intends to serve as a first proof of concept approach towards future research where microbial management in bioponics and hydroponics is not only efficient but also aligned with on-demand disinfection using H_2O_2 of nutrient solutions. To the best of our knowledge, this is the first time that H_2O_2 has been considered for its ability to disinfect a BNSL as well as its ability to disinfect a BNSL with H_2O_2 produced ex situ and in situ.

2. Materials and Methods

2.1. Production of a Bioponic Nutrient Solution from Organic Waste

To produce a nutrient solution using organic materials, three distinct stock solutions were prepared. For the recovery of PO_4-P, bone meal (Beckmann&Brehm GmbH, Beckeln,

Germany) was selected. Kartoffelhof Sautter (Bondorf, Germany) provided potato peels from an industrial production for K recovery, and blood meal (Common Baits, Rosenfeld, Germany) was selected for NO_3-N recovery. Basak and Yigit [36] have demonstrated that blood meal can have a positive effect on plant growth of Green Bean (*Phaseolus vulgaris* L.) seedlings under salt stress. In addition, Nkoa [37] demonstrated that blood and bone meals contain significant amounts of relevant plant nutrients (e.g., N and P) with a high biodegradability and fertilizer value.

For the recovery of K, potato peels were dried for 72 h at 70 °C and ground to a powder. For the recovery, a solution consisting of de-ionized water and potato peel powder with a concentration of 320 g L^{-1} was prepared. Specifically, 8000 g of powder was dissolved in 25 L of de-ionized water and kept at room temperature for a total of 19 days under anaerobic conditions. The solution was then centrifuged at 8000 rpm for 8 min, and the pellet was discarded. Important macronutrients of the recovered solution had the following final concentrations: K = 4168 mg L^{-1}, NO_3-N $\leq$ 5 mg L^{-1}, PO_4-P = 252 mg L^{-1}, and NH_4-N = 71.9 mg L^{-1}.

For the recovery of PO_4-P, bone meal was mixed with DI water for a final volume of 50 L at a concentration of 8.05 g L^{-1} at 30 °C under anaerobic conditions for 92 days. At the start, the pH was kept below 5.5 by manually adding 10% (v/v) formic acid to the solution, which stayed below a pH of 5.5 throughout the experiment. The reactor was also manually mixed twice per week. The recovered solution had the following characteristics: PO_4-P = 109 mg L^{-1} and NH_4-N = 414 mg L^{-1} (NO_3-N concentration was not determined).

For the recovery of NO_3-N, anaerobic and aerobic processes were used to first convert organic N to NH_4-N anaerobically and then convert NH_4-N to NO_3-N aerobically. Specifically, dried blood meal was mixed with de-ionized water for a final volume of 50 L and 100 L at a concentration of 8.05 g L^{-1} at 30 °C and 20 °C, respectively. As a microbial inoculum cow manure, provided by Milchhof Blumhardt (Remseck, Germany), was turned into a stock solution (concentration: 600 g L^{-1}) and was added to the solutions for a final concentration of 2 g L^{-1}. Anaerobic digestion took place for 60 days with final NO_3-N concentrations of 444 mg L^{-1} and 178 mg L^{-1}, respectively. Subsequently, both solutions were pooled together for a total NO_3-N concentration of 267 mg L^{-1} and processed aerobically for an additional 40 days at 30 °C. The solution was then decanted after an additional 48 h to remove particles, which led to a final volume of ~100 L with the following characteristics: NO_3-N = 3.6 mgL^{-1} and NH_4-N = 71.9 mg L^{-1} (PO_4-P was not determined), suggesting inefficient ammonium conversion to NO_3-N.

Consequently, the individual stock solutions were combined for a total nutrient solution volume of 116 L composed of 8 L of potato peel solution, 45 L of bone meal solution, and 63 L of blood meal solution. The final solution then showed a concentration of macromolecules of K = 267.8 mg L^{-1}, NO_3-N = 2.07 mg L^{-1}, PO_4-P = 1.32 mg L^{-1}, and NH_4-N = 180.2 mg L^{-1}. Additionally, relevant cation and anion concentrations were determined and have the following characteristics: Ca = 96.54 mg L^{-1}, Mg = 25.86 mg L^{-1}, Na = 36.75 mg L^{-1}, Al = 1.94 mg L^{-1}, Fe = 2.01 mg L^{-1}, Mn = 0.05 mg L^{-1}, Cu = 0.02 mg L^{-1}, Zn = 0.63 mg L^{-1}, Cl = 59.48 mg L^{-1}, and SO_4 = 20.89 mg L^{-1}.

The final solution reveals inadequate levels of NO_3-N and PO_4-P, alongside elevated levels of NH_4-N. Meanwhile, K concentrations align with the target. Consequently, this situation contributes to a restricted availability of nutrients for the hydroponic cultivation of plants. Nonetheless, the procedure for generating a hydroponic solution utilizing organic materials remains unchanged. Thus, even if nutrient recovery is enhanced, it is reasonable to expect a comparable microbial presence. This aspect makes the process an illustrative example of a nutrient solution based on organic materials. Due to the centrifugation of the potato peel solution before mixing, it is expected that the microbial load from the potato solution is minimal. Conversely, the bone meal-derived solution primarily consists of anaerobic microorganisms, while the blood meal-based solutions encompass a mixture of aerobic and anaerobic microorganisms.

2.2. Electrochemical H_2O_2 Production and Disinfection Tests

2.2.1. Ex Situ vs. In Situ Production

Two basic operation principles of the electrochemical H_2O_2 production for disinfection of the nutrient solution production were evaluated: the electrochemical H_2O_2 production in a separate electrolyte (ex situ) and subsequent manual dosing to the nutrient solution, and in situ production directly in the nutrient solution. For the ex situ production, the initial H_2O_2 concentration and the reaction time between the H_2O_2 and the bacteria and fungi in the nutrient solution were adjusted independently. Therefore, two series of experiments were performed for ex situ production. Scenario 1 (Experiment C1–C3 in Table 1) was designed to evaluate the effect of the H_2O_2 concentration directly after dosing to the nutrient solution on the disinfection performance, whereas the reaction time was kept constant for 30 min. The H_2O_2 concentration was set by adding the corresponding amount of H_2O_2 produced ex situ to the nutrient solution. In Scenario 2 (R1–R3 in Table 1), the initial H_2O_2 concentration was set constant, but the reaction time varied. For the in situ production directly in the nutrient solution, the H_2O_2 production time and the reaction time in the nutrient solution are identical and were varied between Experiment IS1 and IS3 as shown in Table 1, thus resulting in different H_2O_2 concentrations. As the control sample, a nutrient solution without the addition of H_2O_2 was considered with quantification before every tested scenario. Each scenario (9 scenarios in total, see Table 1) was run and tested in triplicates.

Table 1. Testing scenarios for the disinfection potential of hydrogen peroxide in a nutrient solution based on organic materials. Measured indicates that the final H_2O_2 concentration was determined after each run.

Scenario	Sample Name	Concentration [mg L^{-1}]	Reaction Time [min]	In Situ Production Time [min]	Sample Volume [mL]
	blank	0	0	–	250
1	C1	5	30	–	250
	C2	50	30	–	250
	C3	200	30	–	250
2	R1	50	15	–	250
	R2	50	30	–	250
	R3	50	60	–	250
3	IS1	measured	–	15	500
	IS2	measured	–	30	500
	IS3	measured	–	60	500

2.2.2. Electrolysis Cells and Setup

The H_2O_2 production was performed using a self-constructed electrochemical cell with one electrode pair of a geometric active area of 123 cm^2 and one gas, one liquid catholyte, and one liquid anolyte compartment, respectively (Figure 2). A commercially available gas diffusion electrode with microporous coating and carbon-based catalysts (purchased from Quintech GmbH, Sonsbeck, Germany) was used for H_2O_2 production, whereas a titanium sheet electrode coated with iridium mixed metal oxide catalysts (Magneto B.V., Delft, The Netherlands) served as an anode. The catholyte and anolyte compartments were separated by a cation exchange membrane (Fumasep F-10150 PTFE, Fumatech GmbH, Bietigheim-Bissingen, Germany) to prevent the decomposition of the H_2O_2 at the anode.

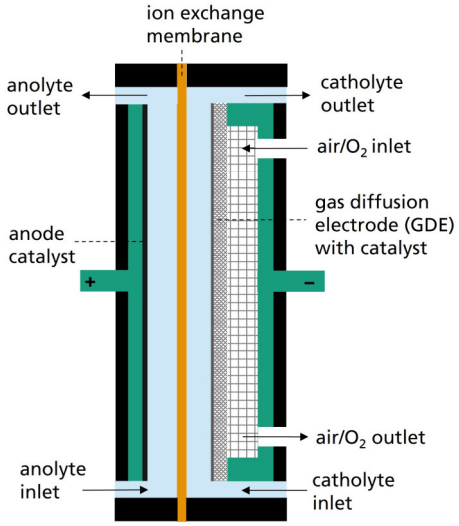

Figure 2. Schematic of the electrolysis cell for H_2O_2 production on a gas diffusion electrode.

Figure 3 shows the experimental setup for electrochemical H_2O_2 production. The electrochemical H_2O_2 production was performed in semi-batch mode; for example, the electrolytes were recirculated through the electrochemical flow cell to increase the H_2O_2 concentration compared to one single pass.

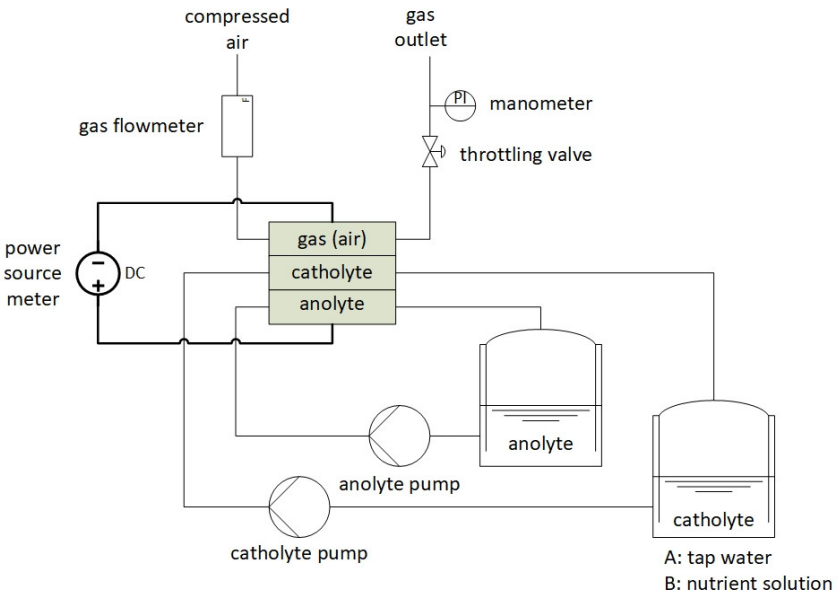

Figure 3. Setup for the electrochemical H_2O_2 production with a three-compartment (gas, catholyte, and anolyte) electrolysis cell. In the ex situ production scenario, tap water (A) was used as the catholyte, and the resulting H_2O_2 was subsequently added once to the nutrient solution. In the in situ H_2O_2 production scenario (B), the nutrient solution was directly circulated as a catholyte through the electrolysis cell.

In the case of ex situ H_2O_2 production, tap water with an electric conductivity of approximately 350 µS cm^{-1} was used both as the catholyte and the anolyte, respectively. The idea behind using tap water instead of specific salt, acid, or base electrolytes was to minimize the required pre-treatments (for example, for pure water production) and auxiliary chemicals. The consequences of using such potentially varying, low-conductivity electrolytes will be discussed in the following sections. In the case of in situ production, the nutrient solution was recirculated through the catholyte compartment of the cell. In this case, an acidified sodium sulfate (Na_2SO_4/H_2SO_4) solution was used as the anolyte.

The operation parameters air flowrate (150 L h^{-1}) and recirculation flowrates of the catholyte and anolyte (25 L h^{-1}) were kept constant for all H_2O_2 production experiments, whereas the electric cell voltage and current were adjusted depending on the electrolyte conductivity. The overpressure in the gas compartment was indicated by a manometer of 0.1–0.2 bar.

The analysis of the H_2O_2 concentration was performed by permanganate titration, using the reduction of permanganate by H_2O_2 under acidic conditions [29]. In our study, 10 mL of the sample were supplemented with up to 50 mL of fully deionized water and acidified with 10 mL of a 5 M H_2SO_4 solution. A solution of c_{MnO4} = 0.05 M $KMnO_4$ was used for titration to the point where the solution turned purple. The H_2O_2 concentration c_{H2O2} can be calculated from the sample volumes $V_{H2O2,S}$ and the required volume of $KMnO_4$ solution V_{MnO4} using

$$c_{H2O2} = \frac{V_{MnO4}}{V_{H2O2.S}} \cdot c_{MnO4} \quad (3)$$

One parameter that quantifies the efficiency of the production process is the Faraday efficiency *FE*. The Faraday efficiency describes the amount of transferred electric charge that yields the target reaction product. For the semi-batch process used in this study, the Faraday efficiency was calculated by the following equation:

$$FE = \frac{z \cdot F \cdot c_{H2O2} \cdot V_{H2O2}}{\int I \cdot dt} \quad (4)$$

where z = 2 is the number of electrons required for one H_2O_2 molecule, the Faraday constant F = 96,485 C mol^{-1}, the total volume of the produced solution V_{H2O2} = 0.5 L, I is the electric current, and t is the time of H_2O_2 production.

2.3. Microbial Analyses

For the quantification of bacteria, trypticase soy agar was selected, and malt extract agar was used for the quantification of fungi, according to [38]. Instantly following the specific reaction times, the nutrient solution containing microorganisms and H_2O_2 was serially diluted in a 1X phosphate buffer saline solution (pH = 7.2) to reduce the cell destructive effect of H_2O_2 and was then plated. Trypticase soy agar plates were then incubated at 37 °C for 72 h, while malt extract agar plates were incubated at 25 °C for 96 h. Viable bacteria and fungi were then enumerated as colony-forming units (CFU/mL) and visualized as a Log10 reduction in comparison to the control sample of the nutrient solution that was not in contact with H_2O_2 solutions.

3. Results

3.1. Microbial Composition of the Bioponic Nutrient Solution

Before the H_2O_2 experiments were started, the total microbial load of the generated BNSL was determined. The single most occurring bacteria and fungi species were further amplified via a polymer chain reaction and then sequenced using Sanger sequencing (Eurofins Genomics, Ebersberg, Germany) similarly as described in [39]. The predominant bacterial species was identified as *Streptococcus pyogenes*, while the predominant fungal species was identified as *Cutaneotrichosporon terricola*. In terms of microbial load in the BNSL, Scenario C1–C3 averaged a bacterial load of 3.5 × 10^7 cfu mL^{-1} and a fungal load of 1.3 × 10^7 cfu mL^{-1}. Scenario R1–R3 had a bacterial load of 2.5 × 10^7 cfu mL^{-1} and a fungal load of 2.0 × 10^7 cfu mL^{-1} and thus showed a similar bacterial load as the concentration scenario. For the in situ experiments (IS1–IS3), the bacterial load was elevated compared to the other scenarios with 1.6 × 10^8 cfu mL^{-1}, while the fungal load was reduced in comparison with 7.6 × 10^6 cfu mL^{-1} (see Figure 4). Nevertheless, bacterial as well as fungal concentrations ranged within one Log10 level and thus were able to be used for comparative experiments of the three different scenarios.

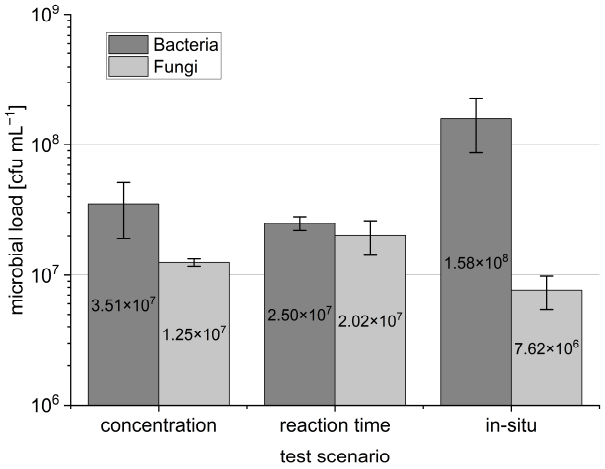

Figure 4. Bacterial and fungal load for control samples (BNSL that was not in contact with H_2O_2) for each considered scenario. Control samples were used to determine the Log10 reduction.

3.2. Electrochemical H_2O_2 Production

The performance of the electrolyzer cell was first validated using 1 M $KHCO_3/K_2CO_3$ (initial pH = 10.66; electric conductivity > 100 mS cm^{-1}) as the catholyte and the anolyte, respectively. The applied electric current density in this specific experiment was approximately 65 mA cm^{-2} (total current = 8 A), with a cell voltage V_{cell} fluctuating between 4.0 and 4.3 V.

The production process yielded an H_2O_3 concentration of approximately 32 g L^{-1} without any significant saturation effect corresponding to a Faraday efficiency *FE* of 56% (Figure 5). Due to the electrode reactions that consume or generate protons (see Equations (1) and (2) as examples), the catholyte pH increased from 10.66 to approximately 14.0, while the anolyte pH decreased to 7.6.

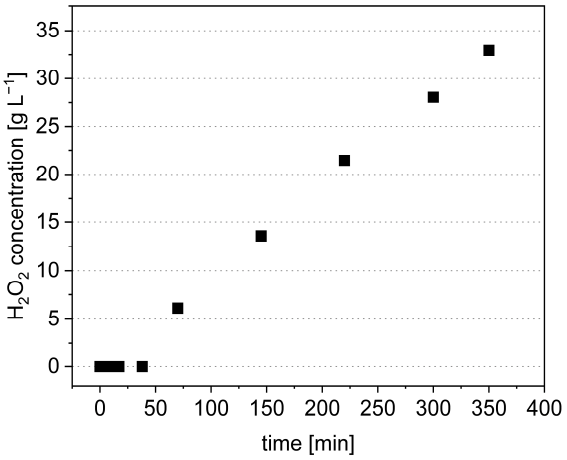

Figure 5. Electrochemical H_2O_2 production in 0.5 M $KHCO_3/K_2CO_3$ electrolyte at 81 mA cm^{-2}.

In the case of tap water as the catholyte and anolyte, the current density was limited by the low conductivity of the tap water. Figure 6 (open symbols) shows the H_2O_2 production during an experiment where the cell voltage was fixed to 12 V and the current density was more than 10 times smaller compared to the case of $KHCO_3/K_2CO_3$. Within 60 min, an H_2O_2 concentration in tap water of 630 mg L^{-1} was reached and used for the experiments of Scenario 1 and 2. This concentration still corresponds to a Faraday efficiency of 64%. The electrolysis process induced pH changes in the electrolytes where the final pH of the H_2O_2-containing catholyte solutions varied between 9.4 and 9.9 and that of the anolyte solutions varied between 2.93 and 2.98.

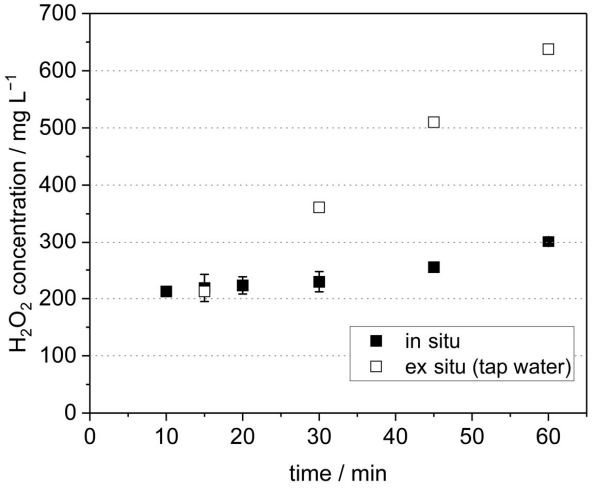

Figure 6. Ex situ H_2O_2 production in tap water for subsequent dosing to the nutrient solution (open symbols) and in situ production directly in the nutrient solution (full symbols). The current density was approximately 6 mA cm^{-2} for the ex situ and 16 mA cm^{-2} for the in situ experiment.

The nutrient solution with its electric conductivity of approximately 2.9 mS cm^{-1} enabled current densities between the two extreme cases of >100 mS cm^{-1} and 350 µS cm^{-1} (tap water). For the in situ H_2O_2 production, a value of 16 mA cm^{-1} was chosen, corresponding to cell voltages varying with time between 6.1 and 7.2 V. The resulting H_2O_2 concentrations are shown in Figure 6 (full symbols). Interestingly, the H_2O_2 concentration increased only slightly with time and even less compared to tap water, despite the higher current density. Within the first 10 min, the production was still efficient (calculated *FE* = 50%), but for longer production times, the calculated Faraday efficiency decreased significantly. Nevertheless, an H_2O_2 concentration of approximately 300 mg L^{-1} was reached.

The pH increase in the catholyte was significantly reduced compared to the case of tap water, due to the acidic anolyte used in this experiment (see discussion below).

In summary, the experiments resulted in H_2O_2 concentrations suitable for the subsequent disinfection experiments. However, the relatively low electrolyte conductivities, especially for tap water, remain a challenge for future development.

3.3. Disinfection of Bioponically Produced Nutrient Solutions Using H_2O_2 Produced under Different Conditions

To consider the effect of H_2O_2 on the reduction of the microbial load, both bacteria and fungi were considered in all scenarios, as H_2O_2 can affect microorganisms differently [40]. To determine the reduction effect of H_2O_2 the final microbial load was compared to the initial microbial load before H_2O_2 was introduced. Here, a Log10 reduction of 1 refers to a microbial reduction of microorganisms by 90%, a Log10 reduction of 2 refers to a reduction of 99%, while Log10 reductions of 0.301 and 0.602 refer to reductions of 50% and 75%, respectively.

In the first scenario, the disinfection potential of different concentrations of H_2O_2 produced ex situ was considered (Figure 7). Here, only a concentration of 200 mg L^{-1} showed a Log10 reduction above 1 for bacteria (1.18), while the reduction of fungi with the same H_2O_2 concentration only showed a reduction of 0.73. Additionally, a concentration of 5 mg L^{-1} did not have any effect on the reduction of fungi (0), while it showed some success in the reduction of bacteria with a Log10 value of 0.33. For an H_2O_2 concentration of 50 mg L^{-1}, bacteria (0.61) and fungi (0.46) were reduced compared to the original microbial load of the BNSL. Overall, the H_2O_2 showed a better reduction of bacterial load than of fungal load when incubation time was set to 30 min.

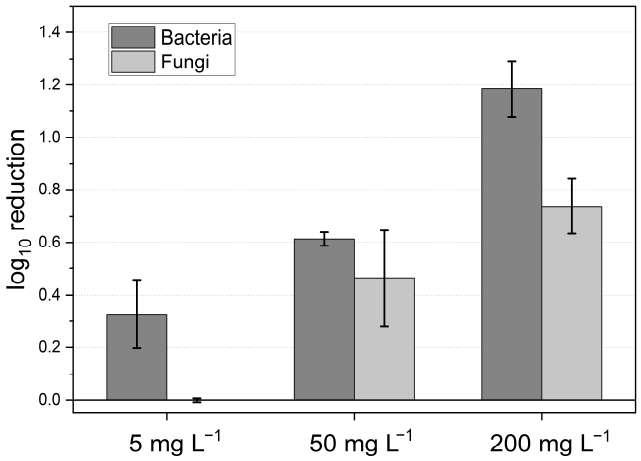

Figure 7. Log10 reduction of bacteria and fungi for different concentrations of H_2O_2 from ex situ electrochemical production compared to the control samples (no active H_2O_2 ingredient) after an incubation time of 30 min under stirring conditions (Scenario C1–C3).

In the second scenario, 50 mg L^{-1} of H_2O_2 as the active ingredient was considered, and reaction times with the microbial load were adjusted. As seen in Figure 8, an increased reaction time of H_2O_2 with microorganisms does not result in increased microbial reduction. In fact, bacterial reduction was lower after 60 min (0.54) when compared with the reduction after 15 min (0.59) and 30 min (0.61). Fungal reduction, however, is highest after 60 min (0.71) and lowest after 30 min (0.46), while the fungal reduction after 15 min is 0.63. In total, the range of reaction time-dependent microbial reduction ranges between 0.46 after 30 min and 0.71 after 60 min, which indicates reductions of 62.6% and 80.5%, respectively. Whether or not fungal or microbial reduction is improved at longer reaction times could not be observed.

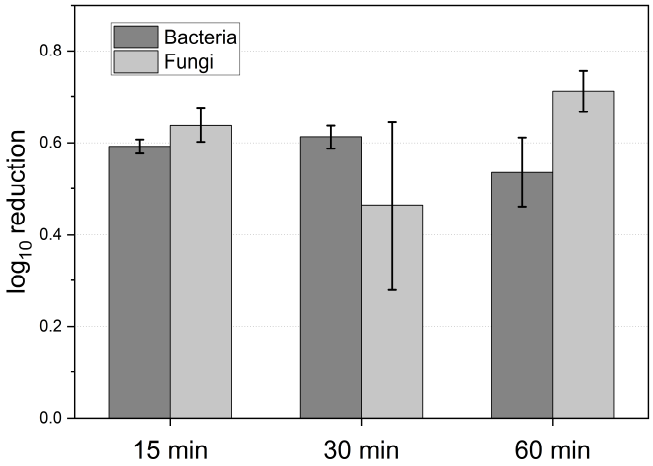

Figure 8. Log10 reductions of bacteria and fungi for different reaction times of microorganisms with H_2O_2 from ex situ electrochemical production compared to the control samples (no incubation time with H_2O_2), with an active ingredient concentration of 50 mg L^{-1} under stirring conditions (Scenario R1–R3).

Finally, the production of H_2O_2 was considered in situ through electrochemical production as described above to determine the potential of producing H_2O_2 on demand directly in a BNSL (Figure 9). In comparison to the results from the ex situ production of H_2O_2, the in situ experiments showed higher reduction rates for almost every considered production time. Additionally, increased production time also increased fungal and bacterial reduction, with reduction rates being the highest after 60 min, with a bacterial

reduction of 1.80 and a fungal reduction of 2.10. The final H_2O_2 was determined to be 301 mg L^{-1}. At every time interval of production, the bacterial reduction was lower than the fungal reduction. After 15 min of H_2O_2 production, bacterial and fungal reductions were determined to be 0.31 and 0.96, respectively, with a final H_2O_2 concentration in the BNSL of 220 mg L^{-1}. A similar H_2O_2 concentration (248 mg L^{-1}) was measured after a 30 min production time; however, bacterial and fungal reduction levels were increased by 1.25 and 1.69, respectively. With a pump circulation speed of 25 L h^{-1}, the solution was circulated through the electrochemical cell approximately 12.5 times in 15 min, 25 times in 30 min, and 50 times in 60 min.

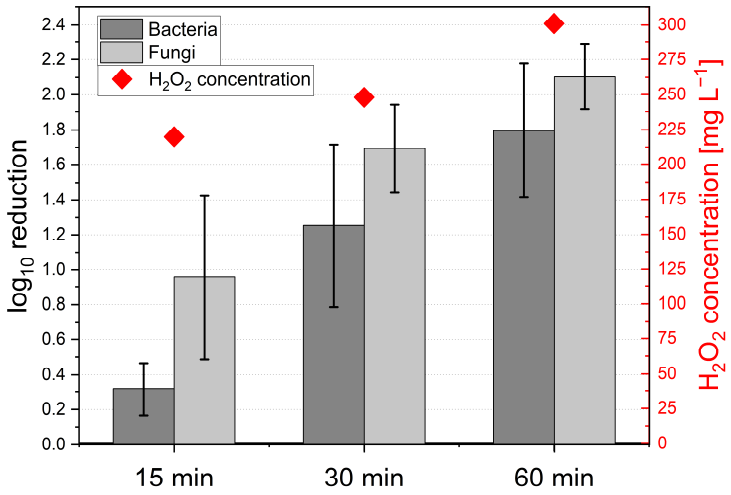

Figure 9. Log10 reductions of bacteria and fungi for different in situ H_2O_2 production times compared to the control samples (no in situ H_2O_2 production) at a pump speed of 25 L h^{-1} and for a total nutrient solution of 500 mL. The red diamonds indicate the average final hydrogen peroxide concentrations after each production time interval (Scenario IS1–IS3).

When comparing all three scenarios, the highest reduction levels were achieved through in situ H_2O_2 production (Figure 9), followed by high reaction concentrations of 200 mg L^{-1} (Figure 7), and the least reduction was found with longer reaction times using a 50 mg L^{-1} concentration of H_2O_2 produced ex situ (Figure 8). It also needs to be noted that no visible complications were determined when circulating a BNSL through the electrochemical cell. Further, no deposition of materials or microorganisms was found on the cell, thus limiting the success of H_2O_2 production.

4. Discussion

The results show that electrochemically produced H_2O_2 can be used for disinfection of organically derived bioponic nutrient solutions. While the analyzed approach did not achieve the same results as state-of-the-art disinfection methods in terms of Log10 reduction, the findings indicate successful microbial reduction in a hydroponic nutrient solution produced from organic waste.

In the scenarios of ex situ H_2O_2 production and subsequent dosing to the nutrient solution, the main parameter that determined the disinfection performance was the H_2O_2 concentration, but not the reaction time (see Figures 7 and 8). The results indicate that an H_2O_2 concentration of 200 mg L^{-1} or higher is necessary for the efficient disinfection of this type of nutrient solution, which leads to the question of whether this might also inhibit plant growth. In addition, when producing the H_2O_2 ex situ in tap water, the low electric conductivity leads to low electric current densities and therefore low production rates per cm^2 of electrode area. An even more critical effect is the dilution of the nutrient concentration by the H_2O_2 electrolyte. For an H_2O_2 concentration of 200 mg L^{-1} in the nutrient solution, one would need to add approximately 470 mL of the H_2O_2 electrolyte produced ex situ if the concentration of this electrolyte is 630 mg L^{-1} (as achieved in

Scenario 1). This would lead to a dilution of the nutrient concentration by 32%. Conversely, the in situ production circumvents this problem, since no additional liquid volume is added to the BNSL.

Nevertheless, the H_2O_2 concentration increases almost linearly with time and current input during production in tap water, and optimization of the electrolysis cell design for lower ohmic resistance is expected to improve the process performance.

The slight increase in H_2O_2 concentration for production times above 15 min in the in situ experiment led to a significant disinfection improvement. This means that the disinfection performance depends not only on the H_2O_2 concentration in the nutrient solution but also on the process time, which is not the case for external production (see Figure 8). Both results indicate that there is an equilibrium of H_2O_2 decomposition that triggers the disinfection and parallel electrochemical re-production of H_2O_2. Following this assumption, a longer in situ production time corresponds to a larger amount of produced H_2O_2, although the measured H_2O_2 concentrations do not differ significantly.

Furthermore, taking into account the H_2O_2 concentration and process time, the disinfection performance for fungi seems to be more efficient for the H_2O_2 produced in situ when compared to the disinfectant produced ex situ. For example, the Log10 reduction in the in situ experiment for t = 30 min is more than double (1.68 compared to 0.73, see Figures 7 and 9) than for the scenario of external production with the same process time, although the concentrations differ only slightly (236 mg L^{-1} compared to 200 mg L^{-1}). However, a direct comparison between ex situ and in situ production, taking only H_2O_2 concentration and process time into account, is difficult, since both parameters cannot be directly compared between both scenarios.

Longer reaction times of H_2O_2 produced ex situ did not result in improved bacterial reductions (Figure 8). As expected, the in situ production of H_2O_2 led to improved disinfection results over time.

Several studies on the most common pathogens in hydroponic systems, *Fusarium*, *Phytophthora*, and *Pythium* [22], have shown that, in order to completely remove unwanted microorganisms in hydroponic systems by H_2O_2, high doses, long contact times, or both are required (Table 2).

Table 2. Performance of hydrogen peroxide for inactivation of plant pathogens in closed hydroponic systems (adapted from [23]).

Type of Organism	H_2O_2 Dosage [mg L^{-1}]	Time [min]	Efficiency [%]	Reference
Phytophtora	185	1	100%	[41]
Bacillus subtilis	N/A	360	100%	[42]
Pythium spp.	12.03	N/A	100%	[43]
Fusarium foetens	135	15	100%	[44]
Fusarium foetens	34	N/A	100%	[45]
Tomato mosaic virus	400	N/A	99.97%	[46]
Fusarium oxysporum f.	100	5	100%	[46]

At the same time, the disinfection treatments can also affect beneficial microbial populations. Especially root-attached microbial communities have a high impact on plant health but can be lost due to disinfection procedures, which then impact plant development. Bioponic nutrient solutions are characterized by a great microbial diversity that fulfills a series of functions [17,22]. For example, some bacteria and fungi might even be beneficial and involved in processes keeping pathogens at low concentrations, as has been observed in conventional hydroponic systems [22]. Lau and Mattson [47] showed that the addition of H_2O_2 to a commercial fish-based organic fertilizer led to an improved growth of lettuce plants at a concentration of 37.5 mg L^{-1} when compared to no addition of H_2O_2. In contrast, plants grown in commercially available mineral fertilizers had reduced performance with increasing H_2O_2 concentrations in terms of fresh weight, root length, leaf width, and

plant height. Here, the reaction of produced hydroxyl free radicals does not only attack membrane lipids, DNA, and other cell components of bacteria and fungi to cause their death, they can also remain in the solution, which increases the dissolved oxygen content similar to the application of O_3 for disinfection [9]. The in situ experiments (Figure 9) suggest concentrations of H_2O_2 in the BNSL that can dissociate into free radicals, thus increasing dissolved oxygen content to promote nutrient uptake by the plant. This should be considered in future applications to monitor dissolved oxygen concentrations for the optimization of nutrient uptake and microbial load reduction.

In addition, organic residues in bioponic systems might not only cause the risk of pathogens in the cultivation but also increase root exudates as a reaction to adjust the nutrient uptake around the root. Hosseinsadeh et al. (2019) showed that an H_2O_2 application of approximately 200 mg L^{-1} led to the best results in removing root exudates, thus improving the growth environment for the plant [23]. This indicates that the presence and application of H_2O_2 as a disinfectant for the BNSL not only reduces microbial concentrations but also reduces plant exudates and thus improves plant health and growth. This concept needs to be further evaluated.

The balance between using microorganisms to moderate nutrient management and recovery as well as nutrient availability in hydroponics systems remains a challenge when optimizing H_2O_2 dosing for bioponic nutrient solutions. However, from a commercial perspective, pathogenic removal continues to be a high priority. Nonetheless, previous studies have shown attempts to optimize the application of H_2O_2 in hydroponic systems. Phytotoxic effects have been observed in H_2O_2 concentrations ranging from 8 mg L^{-1} for lettuce to 125 mg L^{-1} for cucumber cultivation. As there is a wide range of phytotoxic effects based on cultivated plants, suggestions have been made to apply H_2O_2 to source water in combination with conventional mineral fertilizers [23]. At the same time, our research has shown that microbial reduction takes place when H_2O_2 is applied to organic-based NSLs and, for this reason, should not only be considered for the disinfection of source water but also for prepared nutrient solutions. Greater value may lay in the disinfection of nutrient solutions, as the half-life in aqueous solutions (5–10 h), compared to other disinfection agents such as O_3 (8–30 min), is prolonged and thus can have longer reaction times with microorganisms. Further research is necessary on this, as the decomposition is strongly dependent on the amount of metal impurities, which can be high in a BNSL.

One challenge for the integration of electrochemical H_2O_2 production by oxygen reduction (cathodic process) in bioponic cultivation is the fact that H_2O_2 solutions tend to be alkaline, since the cathodic process consumes protons or generates hydroxide anions (see Equation (1)). This is independent of the question of whether the H_2O_2 solution is produced outside the nutrient solution or in situ. Hydroponic systems usually operate under slightly acidic conditions (pH 5.5–6) for optimal nutrient uptake in the root zone [8]. One possible way to prevent this pH increase is to compensate for this effect by H^+ transport from the anode side of the electrolysis cell, for example, by using an acidic anolyte, as is done in in situ production experiments. It is important to note that the acid on the anode side is not consumed, since the anode reaction produces H^+ as a side product. This stabilization of the pH could also prevent a decrease in nutrient uptake.

5. Conclusions

The results demonstrated the potential of using electrochemically produced H_2O_2 for the disinfection of a BNSL in situ by showing a maximum microbial reduction of 97.8% for bacteria and of 99.1% for fungi. Further, H_2O_2 produced ex situ showed the highest reduction of bacteria (93.3%) and fungi (81.2%) after a 30 min reaction time at an H_2O_2 concentration of 200 mg L^{-1}. Specifically, in situ experiments demonstrated the potential for the technology to be used for the disinfection of bioponic and generally hydroponic solutions. Future work should focus on two areas. First, the optimization of the electrochemical cell for lower cell resistance needs to consider the increased risk of blocking cell channels with deposits. Further, the long-term stability of the process is

unclear, and solutions may need to be filtered beforehand to remove particles. Additionally, pH stability through modified cell configuration or using only acid on the anode side may further improve H_2O_2 production. Second, this proof-of-concept approach of in situ H_2O_2 production needs to be included in plant-producing hydroponic and bioponic systems to analyze the effect of such production on plants. As part of this, the optimization of on-demand production as well as H_2O_2 concentration needs to be evaluated and compared to conventional disinfection methods. While the first evidence on the feasibility of applying H_2O_2 generated in situ in bioponic systems has been demonstrated, more research is needed to understand the impact on nutrient chemistry and subsequent plant growth.

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