UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

EFFECT OF ALTERNATIVE FISH FEED AND ELECTRICITY INDEPENDENT OXYGENATION IN DECOUPLED AQUAPONIC SYSTEMS

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EFFECT OF ALTERNATIVE FISH FEED AND ELECTRICITY INDEPENDENT OXYGENATION IN DECOUPLED AQUAPONIC SYSTEMS

Abstract

Aquaponic systems combine Recirculating Aquaculture Systems (RAS) and hydroponic systems, combining the production of animal protein and plants. RAS wastewater enriched in nutrients is used by plants in hydroponic units. RAS rely on fishmeal and fish oil, which are finite resources as aquafeed ingredients, but alternative and more sustainable ingredients have been developed; black soldier fly (*Hermetia Illucens*) meal is one of the most promising alternatives. Therefore, the aim of experiment 1 was to investigate if using a fishmeal based diet (FIM) or, alternatively, a black solider fly meal based diet (BSF) has different effects on lettuce growth in decoupled aquaponic systems. Three different treatments were applied: one hydroponic treatment (control treatment); and two aquaponic treatments. The nutrient solution was made with fish wastewater from a RAS fed either with fishmeal based diet (FIM treatment) or black soldier fly meal based diet (BSF treatment). Abiotic parameters of the nutrient solutions were monitored (temperature, electrical conductivity, dissolved oxygen), air temperature, relative humidity, as well as micro- and macronutrients in the nutrient solutions; and fresh weight (FW), dry weight (DW), number of leaves, water consumption and SPAD-values of the lettuce. Similar lettuce yields were observed between the treatments. However, in FIM treatment, higher sodium concentrations were seen in the nutrient solution. This is the first study showing the benefits of using alternative fish diets in decoupled aquaponic systems, to avoid potentially harmful sodium levels in aquaponic nutrient solutions.

Aquaponics and hydroponics can be unsustainable in areas where electricity is unavailable, expensive or unstable; thus, the experiment 2 was carried out to test an alternative method of oxygenating nutrient solutions without electricity using H_2O_2, and its potential effects on lettuce growth in hydroponic and aquaponic systems. Three treatments were applied: hydroponic control treatment with compressed air (H air); and two other treatments with nutrient solutions provided with a passive H_2O_2-supply instead of compressed air: a hydroponic treatment (H H_2O_2) and an aquaponic treatment (RAS H_2O_2). The same parameters as in experiment 1 were examined and no significant differences in terms of growth or yield were observed. Hence, it shows that this method of oxygenation is a valid alternative for setups in areas where the electrical grid is a limitation.

Keywords: recirculating aquaculture system, hydroponics, black soldier fly meal, hydrogen peroxide, aquaponics

EFEITO DA RAÇÃO ALTERNATIVA PARA PEIXE E DA OXIGENAÇÃO INDEPENDENTE DE ELECTRICIDADE EM SISTEMAS AQUAPÓNICOS DESACOPLADOS

Resumo

Sistemas aquapónicos combinam os sistemas de recirculação em aquacultura (RAS) e sistemas hidropónicos, combinando a produção de proteína animal e plantas. A água residual do RAS rica em nutrientes é usada por plantas nas unidades hidropónicas. RAS depende da farinha e óleo de peixe, que são recursos finitos, como ingredientes para a ração dos peixes mas, ingredientes alternativos foram desenvolvidos; a farinha de mosca soldado negra (*Hermetia Illucens*) é das mais promissoras. Assim, o objetivo da experiência 1 foi investigar se o uso da ração baseada em farinha de peixe (FIM) ou alternativamente, a ração baseada em farinha de mosca soldado negra (BSF), produz diferentes efeitos no crescimento da alface em sistemas aquapónicos desacoplados. Usou-se três tratamentos, um tratamento hidropónico (controlo) e dois tratamentos aquapónicos, com solução nutritiva preparada com água residual de um RAS alimentado com ração baseada em farinha de peixe, tratamento FIM, ou baseada em farinha de mosca soldado negra, tratamento BSF. Parâmetros abióticos das soluções nutritivas foram monitorizados (temperatura, condutividade elétrica, oxigénio dissolvido), temperatura do ar, humidade relativa, tal como os micro- e macronutrientes; e massa fresca, massa seca, número de folhas, consumo de água e valores SPAD das alfaces. Observou-se semelhantes produções de alface entre os tratamentos. Porém, no tratamento FIM, maiores concentrações de sódio foram encontradas na solução nutritiva. Este é o primeiro estudo que mostra os benefícios do uso de rações alternativas em sistemas aquapónicos desacoplados. A aquaponia e hidroponia podem ser insustentáveis em áeras onde a eletricidade está indisponível, cara ou instável; assim, a experiência 2 foi realizada para testar um método alternativo de oxigenação das soluções nutritivas sem uso de electricidade usando H_2O_2. Usou-se três tratamentos: tratamento hidropónico controlo com ar comprimido (H air); e dois tratamentos com soluções nutritivas com fornecimento passivo de H_2O_2 em vez de ar comprimido: um tratamento hidropónico (H H_2O_2) e um tratamento aquapónico (RAS H_2O_2). Os mesmos parâmetros da experiência 1 foram obtidos e não se observou diferenças significativas em termos de crescimento ou rendimento. Assim, demonstra que este método de oxigenação é uma alternativa válida em áreas onde a rede elétrica é instável.

Palavras-chave: sistema de recirculação em aquacultura, hidroponia, farinha de mosca soldado negra, peróxido de hidrogénio, aquaponia

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List of Abbreviations and Symbols

% - Percentage

± - More or less

> - Higher than

< - Lower than

°C - Celsius degrees

€ - Euro

Al - Aluminum

ATP- Adenosine triphosphate

B - Boron

C - Carbon

Ca - Calcium

CFA - Continuos flow analysis

Cl - Chlorine

cm - Centimetre

CO_2 - Carbon dioxide

Cu - Copper

DFT- Deep flow technique

DHA - Docosahexaenoic acid

DLI - Daily light integral

DM - Dry matter

DO - Dissolved oxygen

DOM - Dissolved organic matter

DRAPS - Double recirculating aquaponic system

$dS\ m^{-1}$ - Decisiemens per metre

DW - Dry weight

EC - Electrical conductivity

EPA - Eicosapentaenoic acid

FCR - Feed conversion ratio

Fe - Iron

FW - Fresh weight

g - Gram

h - Hour

H - Hydrogen

HCl - Hydrochloric acid

HNO_3 - Nitric acid

H_2O_2 - Hydrogen peroxide

ICP-OES - Inductively coupled plasma-optical emission spectrometry

K - Potassium

kW - Kilowatt

L - Litre

LED - Light-emitting diode

L/kg/y - Liters per kilogram per year

m - Meter

M - Molarity

m^3 - Cubic metre

mg - Milligram

min - Minute

Mg - Magnesium

mg L^{-1} - Milligrams per liter

mg L^{-1} h^{-1} - Milligrams per liter per hour

mL - Millilitre

mm - Millimetre

mM - Millimolar

Mn - Manganese

Mo - Molybdenum

Mt - Million tons

N - Nitrogen

Na - Sodium

NaCl - Sodium chloride

NADPH - Nicotinamide adenine dinucleotide phosphate

NFT - Nutrient film technique

NH_3 - Ammonia non-ionized form

NH_4^+ - Ammonia ionized form

NH_4-N - Ammonium

Ni - Nickel

nm - Nanometer

NO_3-N - Nitrate

O_2 - Oxygen

p - P-value

P - Phosphorus

PAR - Photosynthetic active radiation

PGPR - Plant growth-promoting rhizobacteria

PGPF - Plant growth-promoting fungi

pH - Negative logarithm of the hydrogen ion concentration

PPFD - Photosynthetic photon flux density

RAS - Recirculating aquaculture system

RH% - Relative humidity

S - Sulfur

Si - Silicon

SPAD - Soil Plant Analysis Development

SRAPS - Single recirculating aquaponic system

TAN - Total ammonia nitrogen

v/v - Volume per volume

W - Watt

Zn - Zinc

µL - Microlitre

µmol m^{-2} s^{-1} - Micromole per second and square meter

1. Introduction

1.1. Aquaculture and Recirculating Aquaculture System (RAS)

Aquaculture, the farming of fish, shellfish, and aquatic plants, is the fastest growing food production sector on earth (Ahmed et al. 2019). Aquaculture fish production has been constantly increasing, reaching 82.1 million tonnes in 2018 and 46.0 percent of world fish production in 2016-2018 (FAO 2020). The global food fish consumption increased at an average annual rate of 3.1 percent from 1961 to 2017 (FAO 2020). However, a stagnation of the capture fishery production since 1980´s (FAO 2018) placed aquaculture under big pressure to increase its production to meet world fish consumption demand.

With a world population expected to reach 9.7 billion people by 2050 (FAO 2020), a continuous increase in food demand is occuring. Hence it is estimated that aquaculture fish production has to rise from 67 million tons (Mt) in 2012 to 140 Mt in 2050 (Waite et al. 2014) to meet the demand for food. However, aquaculture depends on resources such as land, freshwater, nutrients and fossil energy. The comsumption of these resources exceeds its regeneration (Conijn et al. 2018). Thus, environmental concerns have been associated with aquaculture, such as water pollution with animal waste, fertilizers and pesticides (König et al. 2016); freshwater eutrophication through nutrient release; threats to wild species by farmed-fish escapes, parasites and diseases (Klinger and Naylor 2012); emission of green house gases; and wild fish overfishing to produce fishmeal and fish oil for aquaculture feeds. All of these concerns compromise the sustainability of this food sector. Hence, it is essential to create innovative and sustainable approaches in aquaculture, to produce more while minimizing the environmental impacts.

Inland aquaculture, produces the majority of farmed aquatic animals in freshwater, so it is commonly called freshwater aquaculture (FAO 2020). Earth ponds are still the most relevant type of production, but pens, cages, raceway tanks and aboveground tanks are also largely used (FAO 2020). Marine aquaculture, can be done offshore and landbased. In offshore aquaculture, the main techniques are floating and semi-floating raft culture, net cage culture, sea ground sowing, vertical culture and pond on tidal areas (Cao et al. 2007).

Aquaculture production systems can be classified according to the water exchange as: static systems; open systems; semi-closed systems; and closed or recirculating systems (Soltan 2016). Static systems are usually ponds with no water exchange during the culture period. These systems usually have an extensive production, since it´s hard to maintain water parameters optimal with a large biomass of fish (Soltan 2016). In open systems, there´s no artificial circulation of water through or within the system, referring usually to fish farming in

natural water bodies such as lakes, ocean, bays and estuaries, where fish are confined in floating cages or net pens, enclosures etc (Lawson 1995). In semi-closed systems, water passes through the system and then is discharded, also known as flow-through systems (Soltan 2016). The closed systems or recirculating systems are those where water is recirculated within the system, more commonly called Recirculating Aquaculture Systems, RAS (Soltan 2016). RAS are land-based indoor fish farms that are able to reduce environmental negative effects comparing to traditional ways of aquaculture.

RAS are intensive fish production inland systems, based on a series of water treatments steps to filter the fish-rearing water, facilitating its reuse (Espinal and Matulić 2019). This enables a large fish production of e.g. of 500 tons of fish per year in a relatively low volume of water, in this example 4000 m^3 (Ahmed et al. 2019). The limited amount of water used is a big advantage e.g. in the context of a reduced water availability or water shortages with respect to climate change in many regions of the world (McDonnell et al. 2011). The daily water exchange with fresh water is usually in the range of 6-12% of the rearing volume (Kloas et al. 2015). RAS also enables a constant control of all the production parameters affecting the growth and well-being of the fish (Bregnballe 2015), such as oxygen and carbon dioxide levels; pH; and temperature. Here, the water has to be continuously treated to remove waste eliminated by the fish and oxygen has to be added (Bregnballe 2015). The fish release ammonium/ammonia and carbon dioxide (released by the gills), as well as uneaten feed, and feaces are responsible for a decrease in water quality. Consequently, to maintain water quality in the system, there is the need to have mechanical filters to remove solid particles, biofilters to oxidize ammonia into nitrate, devices to remove/strip the dissolved carbon dioxide and aeration to add oxygen (Espinal and Matulić 2019). The mechanical filtration, removal of organic waste products, is the first filtration process in a RAS. Feaces and uneaten feed are used by bacteria which consumes oxygen and creates ammonia. Simultaneously, the carbon dioxid levels increase and the pH drops. Hence, the removal of these organic waste products by the mechanical filtration is extremely important to maintain water quality and biofilter function. Nowadays, almost every RAS fish farm uses a microscreen fitted with a filter cloth and drum filters are the most commonly microscreens used (Bregnballe 2015). These microscreen filters remove both suspended and settleable solids (Espinal and Matulić 2019). After passing through the drum filter, the water becomes clearer with a significantly lower organic load. However, the water still has other dissolved susbtances such as phosphate and ammonium/ammonia, the latter being a product of protein metabolism (Espinal and Matulić 2019). Both of these substances are accumulating in the RAS whereas phosphate is not toxic for the fish. Ammonia exists in the system in two forms: non-ionized form (NH_3), toxic for the fish; and the ionized form (NH_4^+), with low toxicity for the fish (Espinal and Matulić 2019). These two forms together make the TAN (total ammonia nitrate). Levels of ammonia above 0.02 mg L^{-1} are generally toxic to the

fish (Bregnballe 2015). Thus, the water needs to go through a nitrifying biofilter, where ammonium is oxidized into nitrite and then to nitrate; this is done by communities of nitrifying bacteria such as *Nitrosomonas* and *Nitrosococus* (Espinal and Matulić 2019). These bacteria grow on the surface of the biofilter substrate, which provides a high surface area, where bacteria can adhere and grow, forming a biofilm. Solid susbtrates such as plastic rings and other structures, are commonly used as biofilters substrate. There are different types of biofilters, such as moving bed reactors, fluidized sand filter bioreactor and fixed-bed bioreactor (Bregnballe 2015). The non-ionized form (NH_3) fraction depends on the temperature and pH of the system. When the pH is below 7, NH_3 form is nearly absent, but when pH is above 7, the non-ionized ammonia fraction increases fast (Bregnballe 2015). However, the efficiency of the biofilter depends also on the pH of the system, since the nitrifying bacteria have an optimum pH of 7 or higher (Goddek et al. 2015); a lower pH will reduce the biofilter efficiency. But the pH increase also creates more ammonia in a non-ionized form (NH_3), which is undesired and toxic for the fish. For this reason, the recommended pH for the system should be between 7 and 7.5 (Bregnballe 2015), to have a highly efficient biofilter, without increasing the ammonia levels. Also, during the nitrifying process, bacteria release protons (H^+), lowering the pH. Thereby and to stabilize the pH into optimum levels, a base is commonly added to the RAS, such as calcium hydroxide or sodium hydroxide.

Additionally, carbon dioxide that is released from the fish and from the nitrifying bacteria needs to be removed, which is done either within the biofilter or in a separate degasser. Hence, carbon dioxide is removed from the water and oxygen is added. When fish are exposed to low levels of dissolved oxygen (DO) concentration, they can die within hours (Somerville et al. 2014). Therefore, it is essential to monitor and guarantee adequate levels of DO, which can be done with probes and aerators respectively. DO optimum levels are usually between 5-8 mg L^{-1} (Somerville et al. 2014), depending on species. Nitrifying bacterias also need an adequate oxygen level, between 4-8 mg L^{-1}.

RAS have many advantages compared to the traditional aquaculture systems, making fish production more sustainable. Water comsumption and land use in RAS is much lower. The lower water usage makes nutrient removal from wastewater easier (Bregnballe 2015), improving nutrient recycling and waste management. This type of closed system creates optimal conditions during all year, optimizing fish growth and health (Bregnballe 2015). In addition, the introduction of diseases and the possibility of fish escapes are also reduced.

However, RAS have some drawbacks, such as low energy efficiency, high initial capital investment, discharge of wastewater enriched in nutrients and the dependency of fishmeal and fish oil for aquafeed production. Fishmeal and fish oil are feed ingredients produced mainly from wild-caught fish stocks; therefore, they are a finite resource with seasonal variation and a rising cost (Adeoye et al. 2020). Consequently, to improve RAS sustainability it is essential

to identify and produce fishmeal and fish oil alternative ingredients that are economically and environmentally sustainable.

Overall, RAS are an efficient and future proof technology, which has been growing constantly in the past years in the aquaculture sector. However, to improve its sustainability and production efficiency, aquaponics systems have been developed to reuse the nutrient rich fish waste water that comes from RAS. This allows the closing of nutrient cycles, such as nitrogen and phosphorus.

1.2. Hydroponics

Hydroponics is a system to grow crops without soil. The supply of nutrients and water to the plants is done with a called nutrient solution, made with water and dissolved fertilizer salts. As mentioned earlier, population is rapidly growing and food production needs to increase by 50% globally to guarantee food security (FAO 2017). However, less than a third of the agricultural land is arable (Goddek et al. 2019) and suitable land shortage occurs mainly near population centers. Therefore, hydroponics offers a solution, where food production can be done in areas that are unsuitable for agriculture, like urban areas and arid lands. The carbon dioxid foot print of the food chain can be decreased; food production could be done closer to consumers and markets, consequently providing fresh local food to the populations.

These production systems bring many advantages compared to soil-based agriculture, such as: prevention of soil-borne diseases and pathogens; independence from the soil type and quality (Hosseinzadeh et al. 2017); more efficient water and fertilizer usage; and an improved management and monotoring of crop’s growth. The soilless-media can also be sterilized and reused for different crops (Somerville et al. 2014).

Hydroponic systems can be open or closed. In open systems, the nutrient solution not used by the plants (run-off) it is not recycled, however this wastewater should be treated before being discharged to the environment (Mielcarek et al. 2019). Plants here are always provided with fresh nutrient solution. In closed systems, the nutrient solution is recycled and recirculates within the system (Maucieri et al. 2019). Here, the nutrient solution has to be adjusted with the lacking nutrients and correct electrical conductivity to meet plant growth requeriments.

Hydroponic systems can be also classified depending on their irrigation technique: nutrient film technique (NFT); deep flow technique (DFT); drip irrigation; ebb/flow; aeroponics; and media bed technique. They can also use susbtrate (medium) or not, these substrates can be organic (e.g. coconut fibre, peat) or inorganic (e.g. stone wool and sand). Substrates provide support to the plant´s roots and moisture retention (Maucieri et al. 2019); are permeable to water and air, hence water can flow and the roots also have access to oxygen (Somerville et al. 2014). Substrate should be inert with a neutral pH. Regarding the water supply, the systems can have

a continuous water supply, e.g. in deep flow technique (DFT) or nutrient film technique (NFT); or can have a periodical water supply, e.g. drip irrigation and aeroponics (Maucieri et al. 2019). In the Deep Flow Technique (DFT), or deep water culture, plants are produced on a floating support (rafts or panels), in containers with 10-20 cm of nutrient solution (Maucieri et al. 2019), see Figure 1.The roots are always in contact with the nutrient solution. In the Nutrient Film Technique (NFT), plants grow with a thin layer of nutrient solution in the roots area, often without using any susbtrate, see Figure 2. For this, the nutrient solution is flowing and recirculates in troughs with a 1-2 cm layer of water (Maucieri et al. 2019).

With a Drip Irrigation Technique, water supply is periodical. The nutrient solution irrigation occurs in a specific location, usually near the base of the plant (Raviv et al. 2008). For the aeroponics technique, the root system is suspended in the air inside a box where nutrient solution is sprayed directly over the roots area (Maucieri et al. 2019). In the ebb and flow technique, plants are flood-irrigated on a tray or floor, where water rises to a certain level and afterwards, they are drained, allowing fresh nutrients to reach the root area and then air flow (Maucieri et al. 2019).

Another technique is the media bed technique, where substrate is used to support the roots of the plant, being the most popular technique amongst small-scale hydroponics units (Somerville et al. 2014). Here, the water supply can be achieved in many ways: continuously flow system, entering in one side and exiting in the opposite; drip irrigation system and in an ebb and flow system.

For plant growth and reproduction, plants need 17 nutrients, named “essential elements” (Jones and Olsen-Rutz 2016), which are: Carbon (C), Hydrogen (H), Oxygen (O), Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sulfur (S), Boron (B), Chlorine (Cl), Copper (Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo), Nickel (Ni) and Zinc (Zn). Hydrogen, carbon and oxygen are captured from the air and water, hence are called non-mineral nutrients (Jones and Olson-Rutz 2016). The remaining mineral nutrients can be further devided into macro and micro nutrients, depending on its requeriments for plant growth. There are 6 macronutrients: nitrogen, phosphorus, potassium, calcium, magnesium and sulfur. However, since nitrogen, phosphorus and potassium deficiencies occur more frequently, they are called “primary macronutrients”. Calcium, magnesium and sulfur are called “secondary macronutrients”. The other 8 are the micronutrients (Jones and Olsen-Rutz 2016). The micronutrients are only needed in trace amounts, in contrast to the macronutrients that are needed in larger amounts. In hydroponics systems, these nutrients are supplied by the nutrient solution to the plant. The nutrient solution is made by adding fertilizers to the used water source. In this way, the nutrients can be added in the exactly required amounts for an optimum plant growth. The maintance of optimum water quality parameters is essential to hydroponics systems. The pH is the most important parameter; it influences plants ability to take up the

nutrients from the nutrient solution. If pH is out of the optimum range, which is between 5.5-6.5 for growing crops (Maucieri et al. 2019), nutrients can be present in the nutrient solution but the plants are unable to use them. To maintain the optimum pH, acids are used to decrease pH such as nitric acid (HNO_3) or a base such as sodium hydroxide (NaOH) to increase the pH. The DO is also important, high levels of DO above 3 mg L^{-1} are needed in the nutrient solution (Somerville et al. 2014); without it the roots will die, this is called root-rot. The optimum temperature of the nutrient solution depends on the plants produced. In winter for vegetables, such as salad, the growth is best at in temperatures between 18 and 20 ºC (Somerville et al. 2014).

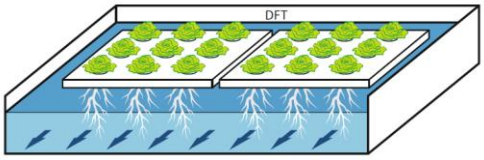

Figure 1: Illustration of a Deep Flow Technique (DFT) system with floating panels (Maucieri et al. 2019)

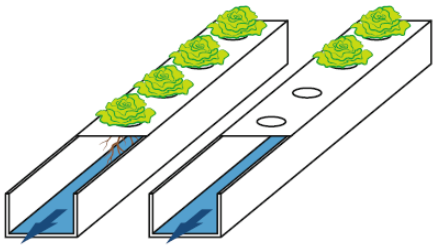

Figure 2: Illustration of a Nutrient Film Technique (NFT) system (Maucieri et al. 2019)

1.3. Aquaponics

The integration of recirculating aquaculture systems and hydroponics in one production system is called aquaponics (Somerville et al. 2014). These systems are composed of a hydroponic unit where crops are produced and a RAS unit where fish are reared. In traditional hydroponic systems, mineral fertilizers addition is needed to provide nutrients to the plants. In aquaponic systems, the fish wastewater rich in nutrients, goes to the hydroponic unit where it is used for plant growth. In this way, becomes possible to recycle nutrients and waste, closing the nutrient cycle. At the same time the water usage is reduced through recirculation, using less than 10% of the water compared to conventional agricultural (Goddek et al. 2015). Thus, aquaponics systems are a promising technique to help creating a more sustainable global and urban food production, using less resources and causing less pollution. The fish wastewater is enriched

in nutrients, mainly nitrate, which is a macronutrient essential for plant growth. Hence, this valuable waste is used by the plants, avoiding its discarge into aquatic ecosystems that could cause eutrophication (Monsees et al. 2017). Simultaneously, the use and addition of chemical fertilizers for plant growth is reduced. In this way, it is possible to produce two different outcomes, plants and fish, by mainly using one source of water and nitrogen provided from the fish feed (Somerville et al. 2014). Aquaponics need fewer nutrient inputs and create less waste outputs than hydroponic system or RAS run individually (Goddek et al. 2015). Just like hydroponics, aquaponics can be even more economically sustainable, productive and appropriate in areas where water is scarce, the soil is not arable or a small portion of land is available, such as urban and peri-urban areas.

The traditional approach of aquaponics is called SRAPS (Suhl et al. 2016), single recirculating aquaponic system, or coupled or 1-loop aquaponic system (Monsees et al. 2017). In this approach, water circulates from the fish tanks to the filtration units, afterwards fish wastewater is pumped to the hydroponic unit, where it is used and purified by the plants and bacteria present at the root zone. Then, the water returns to the RAS unit, into the rearing tanks. Hence, the water quality is the same for fish and plants, the control of water parameters is done to the whole system. However, plants and fish have different tolerance ranges for each water quality parameter. Adjusting the water quality parameters of the system as a whole will compromise growth conditions for both plants and fish, since their environmental conditions might not be within their optimum growth conditions range. A crucial point in these systems is the pH stabilization. For plants to be able to uptake nutrients in the hydroponic unit, pH should be between 5.5 and 6.5. And for fish rearing and nitrifying bacteria, the optimum pH is between 7 and 7.5 (Bregnballe 2015). Therefore, a pH compromise has to be done; usually it is kept between 6.8 and 7 (Goddek et al. 2015), which can cause suboptimal growth conditions for both plants and fish. Additionally, fish and plants have different nutrient requirements. Since the major nutrient input in these systems comes from the fish feed, some nutrients needed for plant growth would be lacking. For example, potassium is not released in enough amounts from the fish (Suhl et al. 2016). These lacking nutrients have to be added in the hydroponic unit through mineral fertilizers, which potentially have a negative impact in terms of animal welfare and health.

To solve these problems, another aquaponic approach was created by Kloas et al. (2015), called DRAPS, double recirculating aquaponic system, or now called decoupled aquaponic systems, as seen in Figure 3, which shows a schematic illustration of a coupled aquaponic system and a decoupled aquaponic system. In this approach, the hydroponic unit and RAS unit are separated and independent (Suhl et al. 2016). Hence, water quality parameters, such as pH and temperature can be adjusted in each unit independently to create optimum conditions for both plant and fish production. Fertilizers are also only added into the hydroponic

unit, without influencing the fish production. Here, the water recirculates within each unit and the water from the fish tanks goes to the hydroponic unit via a one-way valve. The water from the hydroponic unit it is not redirected to the fish tanks (Monsees et al. 2017). The hydroponic unit has a water reservoir receiving the fish water; fertilizers can be added at this point to meet specific plant requirements. Additionally, to improve water use efficiency, the lost water by evatranspiration from plants or from RAS can be captured, using a cold trap to condensate this water and introduce it back to RAS. Decoupled systems showed a higher plant production yield than coupled systems in Monsees et al. (2017) study and a reduction on the mineral fertilizer usage by 62.8% (Monsees et al. 2019).

Some limitations and weaknesses of aquaponic food production are the initial high investment to integrate these two systems, reliance on electricity and other inputs such as fish feed, fish and plant seeds (Bregnballe 2015).

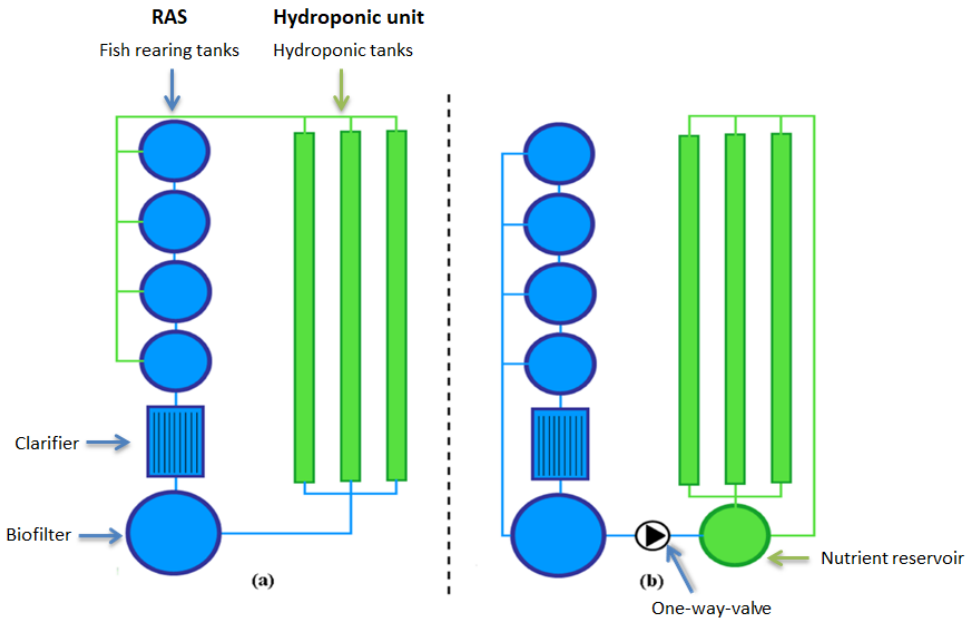

Figure 3: Coupled and decoupled aquaponic systems scheme modified and adapted from Monsees 2017. (a) Coupled aquaponic system: RAS water is always circulating from RAS unit to hydroponic unit and back to RAS. (b) Decoupled aquaponic system: RAS and hydroponic unit are separated via a one-way-vale. Water recirculates within each system independently and water goes from RAS to hydroponic unit via one-way-valve on demand only and do not return to the RAS.

1.4. Fish Feed in RAS and Aquaponics

Aquafeeds have been in the past dependent on high inclusion rates of fishmeal and fish oil as ingredients; fishmeal as a protein source and fish oil as lipid source. Fishmeal is prepared by milling and drying fish or fish parts, obtaining a proteinaceous flour-type material. Fish oil is

produced by pressing cooked fish; afterwards, the liquid obtained is centrifuged (FAO 2020). The fishmeal and fish oil can be made from wild-captured forage fish, usually small pelagic species, e.g peruvian anchoveta; but also, from fisheries and aquaculture by-products such as fish trimmings (FAO 2020). Only around 30% of fishmeal is currently made from these by-products (Hua et al. 2019). Hence fishmeal and fish oil production depend mainly on forage fish stocks. The global fish production reached 179 million tonnes in 2018, 22 million tonnes from this were used mostly to produce fishmeal and fish oil (FAO 2020); this trend has been declining over the last 20 years (Hua et al. 2019). However, while the aquaculture sector is growing, the production of fishmeal and fish oil has remained static compared to that, with a decrease on production of -1.7% and -2.6% per year, respectively (Tacon et al. 2008). These ingredients are a finite resource and prices have been increasing (Lock et al. 2016), likely to increase even more due to the increased demand. Even though the consumption of fishmeal and fish oil by aquaculture has been higher due to the growth of the sector, the inclusion of these ingredients in aquafeeds have been decreasing (Tacon et al. 2008).

As mentioned earlier, the wide use and incorporation of fishmeal and fish oil as ingredients in the majority of aquafeeds it is not sustainable. Feed is the largest production cost in aquaculture; and feed production is predicted to increase by 75% from 2015 to 2025 (Hua et al. 2019). Therefore, for the aquaculture sector to keep expanding it is essential to find environmentally friendly and economically viable alternatives for fishmeal and fish oil. Consequently, it will reduce the dependence of the aquaculture sector on marine finite resources and increase the sustainability of the feed supply chain. However, fishmeal and fish oil are extremely nutritious and digestible ingredients, and also the main source of omega-3 fatty acids (eicosapentaenoic acid, EPA, and docosahexaenoic acid, DHA) (FAO 2020). Hence, the goal might not be to completely stop including fishmeal and fish oil in aquafeeds but instead, keep decreasing its inclusion, while replacing them with alternative and more sustainable ingredients. And also keep saving its use specifically for certain production phases such as finishing diets (FAO 2020).

Hence, research and devolopment of alternative feed ingredients and substitutes for fishmeal and fish oil have been performed. Currently, alternative commercial replacements such as plant-based ingredients and animal by-products have been used. Soybean meal and corn gluten meal are examples of plant-based proteins included in aquafeeds and canola and soy oils are plant-based oils (Naylor et al. 2009). Blood-, poultry-, meat- and bone meals are examples of animal by-products used as aquafeed ingredients (Tacon et al. 2008). Plant-based protein meals however contain certain disadvantages such as an unbalanced amino-acid profile and antinutritional factors (Lock et al. 2016). Hua et al. (2019) mentions that insect meals and byproducts from fisheries and aquaculture show the higher potential as sustainable ingredients for aquafeeds in the future. The most promising insect meal is produced by the

insect black soldier fly, Hermetia illucens. Insect meals have shown high content in fats, proteins and minerals (Adeoye et al. 2020). Black soldier fly meal is easily digested, showing similar essential amino acids patterns to fishmeal, with a high protein efficiency ratio (Adeoye et al. 2020). Additionally, its production is considered ecofriendly and sustainable, with a high productivity; and the insects can be fed by a vast number of different substrates (Hua et al. 2019).

The main nutrient input in aquaponic systems is the fish feed, hence the fish feed use should be efficient and sustainable. Additionally, the fish feed composition affects the nutrient excretion by the fish which then will change the nutrient chemistry of the fish wastewater, hence influencing plant growth of the hydroponic unit.

1.5. Energy Input in Hydroponic and Aquaponic Systems

Energy is one of the main inputs needed in hydroponic and aquaponic systems. Electricity is needed for the majority of these systems, which make them potentially unsustainable in areas where electricity is expensive or not available. The same goes to areas where the electrical grid is unstable and where power outages are frequently such as in developing countries (Amadi 2015). To have a more sustainable and robust production system, it is essential to reduce finite and costly resources inputs such as land, water and fossil fuels. Conventional aerators, like air pumps need electricity, hence using hydrogen peroxide to oxygenate the nutrient solution could be a solution for the earlier mentioned scenarios; where hydroponic and aquaponic systems would rely less on electricity and would avoid production losses when electricity shortcuts occur.

2. Objectives

The experiment 1 was conducted based on the following objectives:

1. To assess if the use of an alternative fish diet, black soldier fly meal based diet (BSF), causes any negative or positive effect on lettuce growth produced in a decoupled aquaponic systems;
2. To verify if using fish wastewater as water source has any effect on lettuce growth performance in a decoupled aquaponic system comparing to a conventional hydroponic system.

The experiment 2 was developed based on the following objectives:

1. To investigate if the use of H_2O_2 solution in an oxidator to provide oxygen to the nutrient solution instead of the conventional electricity dependent aerators causes any effect on lettuce growth in hydroponic and decoupled aquaponic systems;

2. Observe if using fish wastewater as water source has any effect on lettuce growth performance in a decoupled aquaponic system comparing to a conventional hydroponic system.

In this way, both experiment 1 and 2 aim to gain more knowledge to develop more sustainable hydroponic and aquaponic systems.

3. Material and Methods

3.1. Experiment 1

3.1.1. Recirculating Aquaculture System Experimental Setup

For the experiment 1, the RAS experimental setup was conducted at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin. The RAS had a total water volume of 2560 L. The RAS unit was stocked with Nile tilapia, *Oreochromis niloticus*. The experimental RAS started on the 7th of September 2020 and was running till the 1st of November 2020. Before moving into the rearing tanks, the fish stayed two weeks in an acclimatization tank which was operated as a flowthrough system. Each rearing tank was composed of a fish section with 65 L, a sedimentation chamber section with 15.8 L, a biofilter section with 79.2 L composed of a coarse filter (1x PPI 20; Schaumstoff – Meister, Straelen, Germany dimension 50 x 40 x 10 cm), a finer filter (1x PPI 30; Schaumstoff – Meister, Straelen, Germany dimension 50 x 40 x 10 cm) and a moving bed bioreactor (MBBR) with 2 x ProSilent Aeras Micro Ball L (JBL, Germany), see Figure 4.

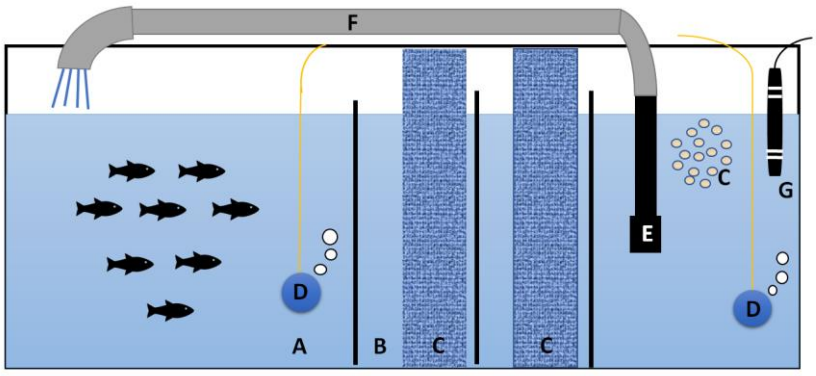

Figure 4: Scheme of experimental fish production tanks (Christopher Shaw): A-rearing part, B-sedimentation chamber, C-biofilter, D-air stone, E-water delivery part, F-air lift, G-heater.

Each different section was separated with perforated plastic separators. In total, 16 individual RAS (n= 16, 160 L each) were operated for the feeding trials, see Table 1.

Table 1. Water Volume of RAS components

RAS components	Per rearing tank	Per fish section	Per sedimentation chamber	Per biofilter section
Volume of water in L	160	65	15.8	79.2

There were 4 replicates for each different diet: FIM, fishmeal based diet; BSF, black soldier fly meal based diet; FEM, feather meal based diet; and BMF, blood meal based diet. The wastewater from the RAS from FIM and BSF diet treatments were used for this aquaponic experiment.

The mean water parameters of the RAS from FIM and BSF treatments can be seen in Table 2.

The total feed used in each BSF replicate was 881.6 g, with a mean water exchange of 8%. In BSF treatment, the mean dissolved oxygen (DO) concentration was 7.0 ± 0.05 and the mean electrical conductivity (EC) value was 0.95 ± 0.01 dS m^{-1}. The mean pH in this treatment was 7.7 ± 0.1 with a mean temperature of 26.4 ± 0.1 ºC.

The total feed used in each FIM replicate was 881.6 g with a mean water exchange of 8%. In FIM treatment, the mean DO concentration was 7.01 ± 0.3 and the mean EC value was 0.96 ± 0.01 dS m^{-1}. The mean pH in this treatment was 7.5 ± 0.1 with a mean temperature of 26.4 ± 0.1 ºC. In both treatments the mean initial body weight (g) and the mean final body weight (g) was obtained. The feed conversion ratio (FCR) was also calculated, using the formula: total feed per individual (g) / [final mean body weight (g) – initial mean body weight (g)].

Table 2. Mean water parameters of the RAS

Parameters	BSF treatment	FIM treatment
Temperature (˚C)	26.4 ± 0.1	26.4 ± 0.1
O_2 concentration	7.0 ± 0.05	7.01 ± 0.3
pH	7.7 ± 0.1	7.5 ± 0.1
Electrical Conductivity (µs cm^{-1})	951 ± 12.3	957 ± 14.1

3.1.2. Fish feed

The fish in the RAS experimental setup were fed with 2 different feed diets: Black Soldier Fly meal based diet (BSF) and fishmeal based diet (FIM). There were 4 replicates per feed treatment. The feeding method in all the replicates was hand-feeding two times per day. The feed composition and amino acid content of each fish feed is shown in Table 3 and Table 4, respectively.

Table 3. Composition and proximate analysis of experimental diets*: FIM, fishmeal based diet; and BSF, black soldier fly meal based diet.

Ingredients	Diets (% dry weight)	
	FIM	**BSF**
Fish meal[1]	52.6	-
Hermetia meal[2]	-	52.6
Feather meal[3]	-	-
Poultry blood meal[3]	-	-
Wheat bran[4]	29.5	29.5
Corn meal[5]	10.1	10.1
Fish oil[6]	6.1	6.1
Dicalcium Phosphate[7]	1.2	1.2
Vitamin and Mineral Premix[8]	0.5	0.5
Proximate analysis*		
Crude protein (%)	40.55	37.65
Crude fat (%)	12.25	8.0
Ash (%)	12.05	7.65
Crude fiber (%)	2.4	6.6
Dry mass (%)	94.05	93.8
Phosphorus (%)	1.93	1.225

[1] Bioceval GmbH & Co. KG, Cuxhaven
[2] Hermetia Baruth GmbH, Baruth/Mark
[3] GePro Geflügel-Protein Vertriebs-GmbH & Co. KG, Diepholz
[4] Höveler Pferdefutter GmbH, Münster
[5] M + M Baits, Neuenkirchen-Vörden
[6] Scheidler GmbH, Eystrup
[7] Th. Geyer GmbH & Co. KG, Berlin
[8] Bionic Nature GmbH & Co. KG, Münchweiler an der Rodalb
* Analyzed by Landesamtliche Untersuchungs- und Forschungsastalt Speyer (LUFA Speyer), Obere Langgasse 40, 67346 Speyer

Table 4. Amino acid composition of experimental diets*: FIM, fishmeal based diet; and BSF, black soldier fly meal based diet.

Amino Acids	Diets (% dry weight)	
	FIM	BSF
Aspartic acid	3.185	3.39
Serine	1.66	1.72
Glutamic acid	5.25	4.995
Proline	2.125	2.2
Glycine	3.405	2.14
Analine	2.545	2.895
Valine	1.715	2.225
Isoleucine	1.45	1.55
Leucine	2.56	2.46
Tyrosine	1.07	1.695
Phenylalanine	1.415	1.355
Histidine	0.96	1.315
Arginine	2.345	1.995
Lysine	2.355	1.99
Methionine	0.895	0.64
Cystine	0.37	0.425
Threonine	1.485	1.51

* Analyzed by Landesamtliche Untersuchungs- und Forschungsastalt Speyer (LUFA Speyer), Obere Langgasse 40, 67346 Speyer

3.1.3. Hydroponic experimental unit and Lettuce cultivation

The experiment was conducted at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin from 22nd of October 2020 till 26th of November 2020, lasting 35 days. The experiment was performed in a climate chamber, with an average room temperature of 18 ºC controlled by an integrated cooling system. In this experiment, a total of 90 lettuce heads (*Lactuca sativa*, Aquino RZ) were grown aquaponically in a deep water culture (DWC). The lettuce seedlings were randomly distributed to 9 hydroponic chambers, each chamber having 10 lettuce plants. Each chamber was composed of: a tent (Royal Room C120S 120x60x180cm); 1 LED (light-emitting diode) lamp (SANlight Q4WL S2.1 Gen2, 165W) at 40% intensity with an average distance of 90 cm to the top of the hydroponic system; light intensity with an average PPFD (Photosynthetic Photon Flux Density) value of 91 µmol m^{-2} s^{-1}, measured with a quantum sensor device (PAR 2000, Stelzner); 1 system GHE AeroFlo 10 (L-110 cm, I= 50cm, H= 50cm) with 10 plants; 1 automated fan (PK125 EC-TC Prima Klima) with a target temperature of 22 ºC, minimum speed of 10% and maximum speed of 20%; 1 HOBO for data logging of temperature and light intensity in the tent by Onset UA-002-64 HOBO Pendant Temp/Light, 64K; 1 HOBO for data logging of the nutrient´s solution temperature by Onset UA-001-64 HOBO Pendant Temp/Alarm, 64K; 1 HOBO UX100-011 data logger for air temperature and humidity inside of the grow chamber; and air supply to the nutrient solution

with compressed air and air stones. The climate chamber room also had 3 Digital time switches (LOGILINK ET0007 timer, digital, max 1800 W) automatically turning the lights on in every hydroponic chamber tent at 6am and off at 6pm, having a light period of 12 hours.

For this experiment, three different treatments were applied in three triplicates each, the treatments were: Control; BSF, black soldier fly meal based diet wastewater; and FIM, fishmeal based diet wastewater, see Figure 5. For the control treatment, the nutrient solution was prepared with tap water and destilled water (50:50, v/v, distilled: tap water) and fertilizer addition. BSF treatment nutrient solution was prepared with fish wastewater from the RAS rearing tilapia fed with black soldier fly meal based diet and fertilizer addition. And for FIM treatment, the nutrient solution was prepared with fish waste water obtained from the RAS rearing tilapia fed with fishmeal based diet and fertilizer addition.

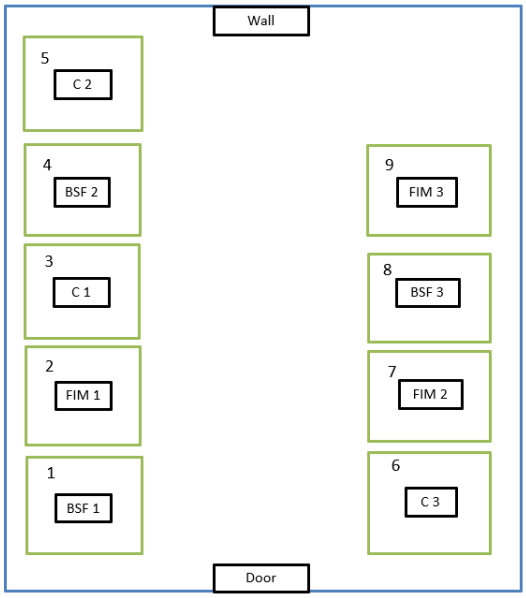

Figure 5: Scheme of the hydroponic unit in experiment 1

3.1.4. Water source for nutrient solution

The water source to prepare the nutrient solution for the control treatment was tap water and distilled water (50:50, v/v, distilled water: tap water). For the treatments BSF and FIM, the water source was fish wastewater from the RAS replicates fed with black soldier fly meal based diet and fishmeal based diet, respectively. The fish wastewater of each treatment, before being used in the hydroponic unit, was stored in tanks provided with compressed air. Each treatment tank was filled with the respective water source, with a final volume of 135 L.

3.1.5. Nutrient Solution preparation

The tap water, black soldier fly meal based diet wastewater (BSF) and fishmeal based diet wastewater (FIM) were analyzed for its nutrient concentrations by inductively coupled plasma-optical emission spectrometry (ICP-OES) and continuous flow analysis (CFA). Based on these values, the required amounts of mineral fertilizer salts that had to be added to the water source were calculated using the Hydrobuddy v1.91 Program. This program is a free and an open source program (Fernandez 2016) to design specific nutrient solutions and to support in the use of fertilizers in hydroponics or conventional crops. This program calculates the amount and the combination of salts to add by direct addition to a specific crop or by creating stock solutions composed of multiple salts. The concentration factor of the solution can also be chosen, a 100 concentration factor was chosen. The volume of the stock solution can be set; it was set to 1.35 L (for a final nutrient solution of 135 L). The water quality parameters of the water used in the hydroponic production system i.e. the nutrient concentration can be set. This data can be saved in the data base of the program and be used multiple times afterwards. The program can also alert the user when the volumes being used are too low or too high and the same to the amount of each nutrient. The chemicals available to the user can also be adjusted and added to the database. Nutrient recipes for certain crops are already pre-programmed in the program. The Lettuce General (Howard Resh) recipe was the one used in this experiment, which gives us the target concentration of nutrients needed to meet the nutrient requirements for lettuce growth. The input page from Hydrobuddy can be seen in the Figure 6.

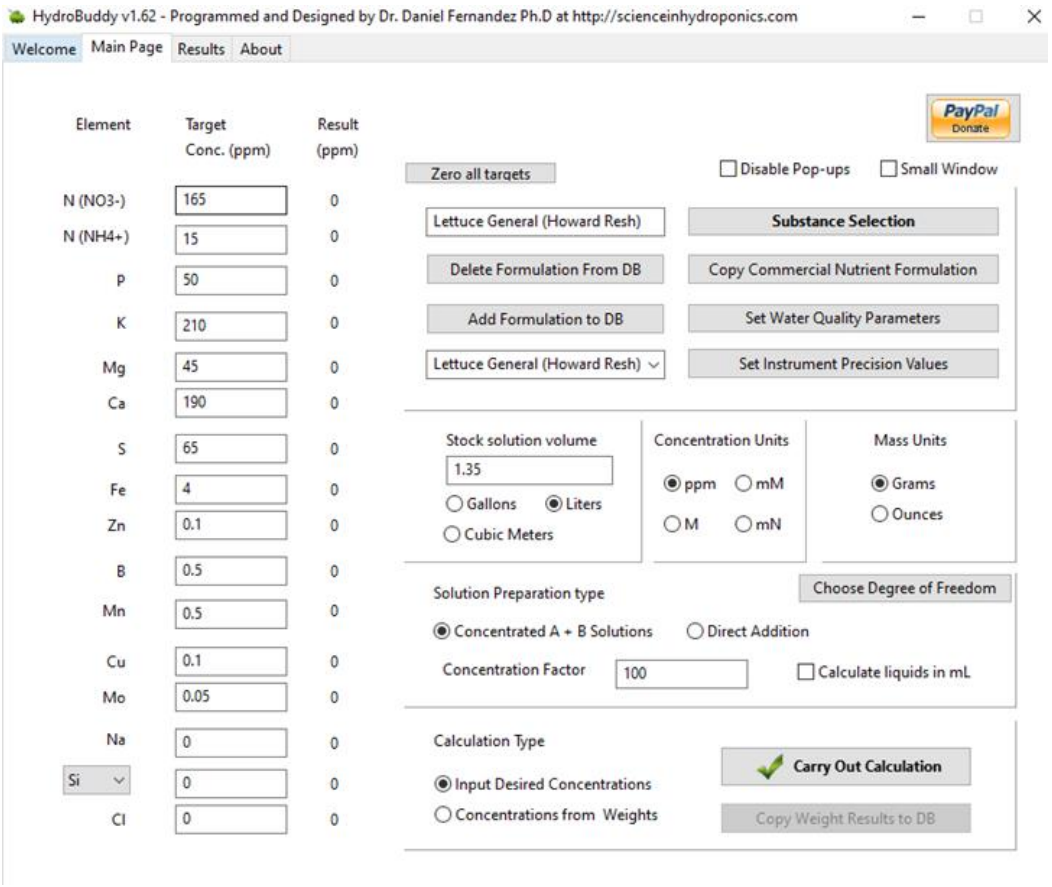

Figure 6: Input page from Hydrobuddy program: the Lettuce General (Howard Resh) recipe was selected; the target concentrations of each element are shown here. The stock solution volume was selected to 1.35 L.

The output page, as seen in Figure 7, is the results section, where is shown which salts we have to add, their amount and into which beaker (A or B), to reach the target concentrations of our nutrient solution. In this page, the expected EC of the stock solutions in mS cm^{-1} can also be seen. Hence, Hydrobuddy was used to add chemicals to the fish wastewater and control water using stock solutions.

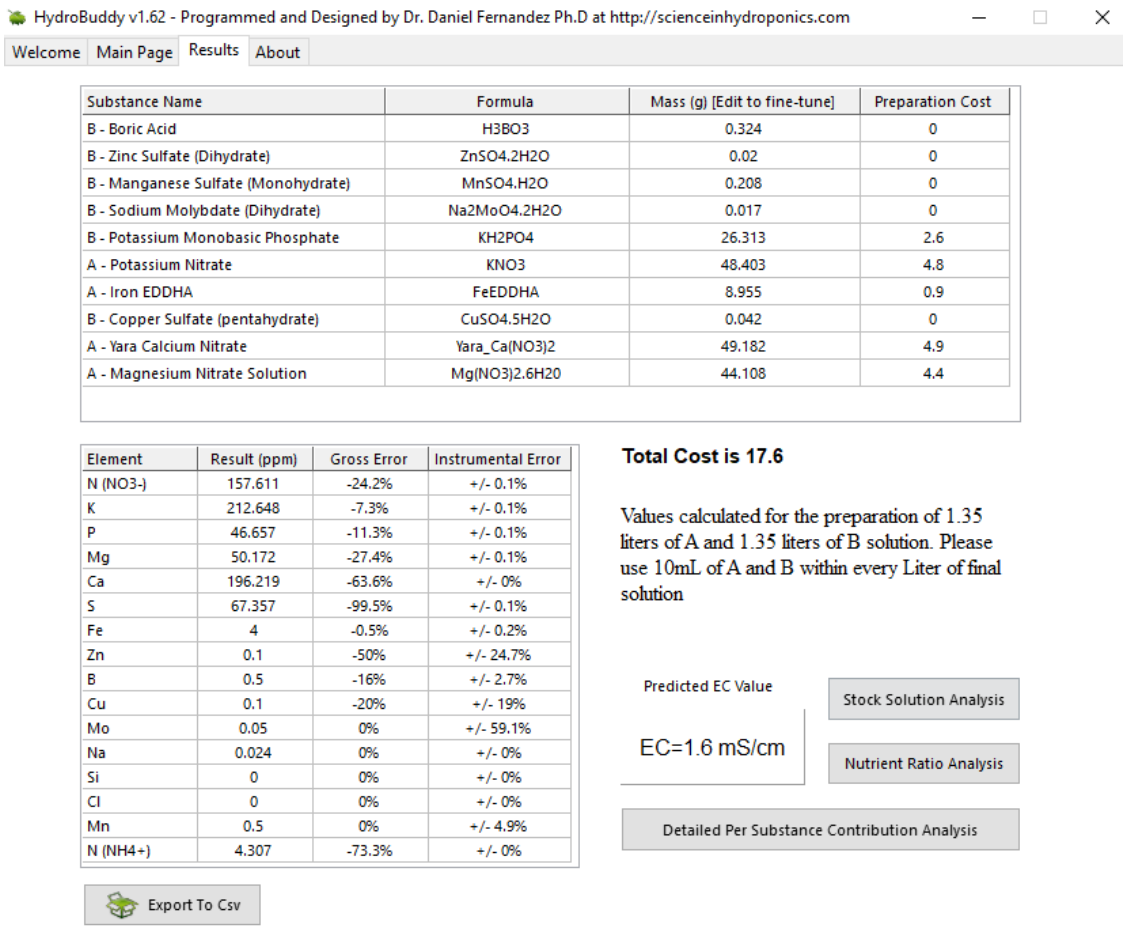

Figure 7: Output page from Hydrobuddy program: the results section shows the amounts of the substances to be used to prepare the solutions A and B

This was done by creating two stock solutions A and B. The water quality parameters section in Hydrobuddy program was filled with the nutrient concentrations from the nutrient analysis of tap water, FIM and BSF waste water, as seen in the example of the Figure 8.

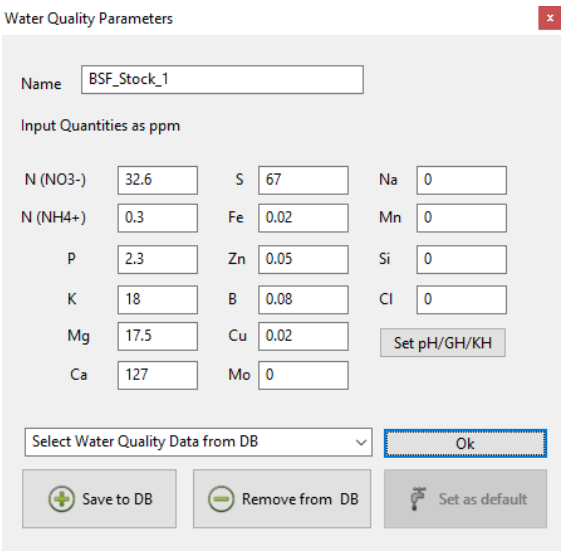

Figure 8: Water Quality Parameters window from Hydrobuddy program: BSF fish waste water nutrient concentrations were inserted and saved to the database.

The desired chemicals to use were already present in the database; they can be seen in the Figure 9. Then, the chemicals were weighted and added in the respective beaker (A and B) with distilled water and stirred until all the salts were dissolved. After salt addition each beaker was filled to a final volume of 1.35 L of distilled water, so the final volume including all salts of each stock solutions was 1.35 L. Afterwards, these stock solutions were added to the Control, BSF and FIM tanks, each tank with a volume of 135 L of the respective water source. Before adding the stock solutions to each tank, the respective pH and electrical conductivity was measured. The pH was adjusted with acid e.g. nitric acid or a base e.g. sodium hydroxide, depending if we wanted to decrease or increase the pH respectively, till a pH value close to 6.7 was reached. After this, the stocks solutions A and B were added in each tank and adjustments were done again to reach a pH value between 6.1 and 6.3. Once all the nutrient solutions were ready, they were distributed from the tanks to each respective replicate chamber with a pump, each replicate chamber with 45 L of nutrient solution.

Substance Name	Formula
B - Boric Acid	H3BO3
B - Zinc Sulfate (Dihydrate)	ZnSO4.2H2O
B - Manganese Sulfate (Monohydrate)	MnSO4.H2O
B - Sodium Molybdate (Dihydrate)	Na2MoO4.2H2O
B - Potassium Monobasic Phosphate	KH2PO4
A - Potassium Nitrate	KNO3
A - Iron EDDHA	FeEDDHA
B - Copper Sulfate (pentahydrate)	CuSO4.5H2O
A - Yara Calcium Nitrate	Yara_Ca(NO3)2
A - Magnesium Nitrate Solution	Mg(NO3)2.6H20

Figure 9: Chemicals used to prepare the stock solution A and B based on Hydrobuddy program.

3.1.6. Nutrient solution change and water consumption

The nutrient solution was changed two times during the experimental period. The first nutrient solution change was done 14 days after the beginning of the experiment, on 4th of November, named nutrient solution change 1. The next change was done 13 days after the first one, on the 17th of November, nutrient solution change 2. To change it, a pump was used to remove the “old” nutrient solution from each system; the volume of this remaining nutrient solution was measured in L, to determine the water consumption in each replicate. Afterwards, each system was filled with a pump with 45 L of the respective “fresh” nutrient solution.

On harvesting day, 26th of November 2020, 9 days after the nutrient solution change 2, the remaining nutrient solution present in each replicate chamber was also measured in L.

The actual water consumption per replicate in L was calculated, subtracting the value of the final water volume per replicate to the initial water volume per replicate in L. Additionally, to calculate the actual water consumption per plant per day in L, the actual water consumption

per replicate in L values were divided by the number of days between each nutrient solution change or between the last nutrient solution change and the harvesting day; the obtained value was then also divided by the number of plants per replicate, which is 10.

3.1.7. Sampling

From each water source, tap water for the control treatment, BSF wastewater for BSF treatment and FIM wastewater for FIM treatment, two filtered samples (0.2 µm cellulose acetate membrane filters, GE Healthcare, United Kingdom) were taken to analyze its nutrients concentrations by ICP-OES and CFA, two days before starting the experiment and every time before changing the nutrient solution preparation. These samples were named, C_stock, BSF_Stock, FIM_Stock so we have C_stock_1, C_stock_2 and C_stock_3; BSF_stock_1, BSF_stock_2 and BSF_stock_3; and FIM_stock_1, FIM_stock_2 and FIM_stock_3.
On the first day of the experiment, 22nd of October, 2 filtered samples were taken from the nutrient solution of each tank (Control tank, BSF tank, FIM tank). Consequently, till the end of the experiment, 2 filtered samples from each replicate were taken weekly to analyze its nutrients concentrations by ICP-OES and CFA.
All the samples were prepared by adding 150 µL of 2M of hydrochloric acid (HCl) to 12 mL filtered out sample, and stored in the fridge if necessary.

3.1.8. Lab analysis

The samples were analysed in the lab during the experiment. The Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) was performed using the Thermo Scientific iCAP 7400 ICP-OES (Thermo Fisher Scientific Inc., USA). ICP-OES was done to determine the concentrations of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), iron (Fe), boron (B), manganese (Mn), copper (Cu), zinc (Zn), sodium (Na), silicon (Si) and aluminum (Al). The continuous flow analysis (CFA) was performed using the FSR Seal High Resolution AA3 chemical analyzer (Seal Analytical, Germany). The CFA was done to determine nitrate (NO_3-N) and ammonium (NH_4-N) concentrations.

3.1.9. Assessment of plant growth

On the 26th of November of 2020, the lettuce heads were harvested by cutting the aboveground organ directly above the rock wool cube. Immediately after harvesting, each lettuce head was weighted to determine the fresh weight (FW) in grams. Then, 4 lettuce heads per replicate were randomly selected to count leaves and prepare subsamples for respective analyses.

These subsamples were prepared by putting one quarter of each selected plant into a plastic bag, these samples were also weighted in grams.
A total of 36 samples, were directly transferred for a few hours to a deep freezer at -80 °C. Afterwards, they were freeze-dried for at least for 72 h (Christ Alpha 1–4, Christ; Osterode, Germany). Consequently, each sample was weighted determining the dry weight (DW) of the sample in grams to further estimate the DW of the respective whole lettuce head (multiplying sample value by 4).

3.1.10. Preparation of lettuce for chemical analysis

To prepare for the chemical analysis, four dried selected samples per replicate were used. Samples were frozen at -80°C, freeze dried (Sublimator 3x4x5, Zirbus Technology, Germany) for 72 h and ground using a vibrating cup mill (Pulverisette 9, Fritsch, Germany). Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), iron (Fe), boron (B), manganese (Mn), copper (Cu), zinc (Zn), sodium (Na), silicon (Si) and aluminum (Al) concentrations were planned to be determined by ICP-OES (Thermo Scientific iCAP 7400 ICP-OES Thermo Fisher Scientific Inc., USA) after wet digestion (HCl 37%, HNO_3 65%, volumetric ratio 1:3) in a high pressure microwave oven (Gigatherm, Switzerland). The determination of the nitrogen and carbon content of the lettuce leaves were also planned using an elemental analyser (vario MAX, Elementar Analysensysteme GmbH; Hanau, Germany). However, due to covid-19 pandemic and delays in the lab, these analyses were not carried out in time to be included in this dissertation.

3.1.11. Abiotic parameters measurement of the nutrient solutions and in the experimental chambers

The pH value, temperature in °C, EC in dS m^{-1} and the DO concentration in mg L^{-1} of the nutrient solutions were measured and controlled every day during week days. The pH, EC and temperature of the nutrient solutions were measured with the Hach Lange HQ40d probe. The DO concentration was measured with a dissolved oxygen meter, OxyGuard Handy Polaris. The environmental conditions in each chamber were measured daily during week days. The measurement of the air temperature in °C and relative humidity (RH %) was automatically done using the HOBO UX100-011 data logger for air temperature and humidity.

3.1.12. SPAD values of lettuce leaves

One week before the harvesting, the SPAD (Soil Plant Analysis Development) value of each lettuce plant in every replicate was measured using a Chlorophyll Meter SPAD-502Plus device

(Konica Minolta, Japan). The SPAD value was taken in 5 leaves of every plant of each replicate, the device calculated automatically the mean SPAD value of the 5 leaves, resulting in the mean SPAD value of the lettuce plant.

3.1.13. Statistical evaluation

PRISM software (GraphPad Software Inc., United States of America) was used for statistical analysis. This was done to analyse the differences between the abiotic parameters of the nutrient solutions and chambers, as well as the mean FW of the lettuces. Kruskal-Wallis and Dunn-Bonferroni tests, were performed to observe if the medians varied significantly ($p < 0.05$) between the different treatments. Statistical analyses were carried out on a significance level of $p < 0.05$, where significant differences are indicated by different superscript capital letters. Where no superscript capital letters were inserted, no significant differences were detected. All data presented as mean values ± standard deviations of the respective samples.

1.2. Experiment 2

1.2.1. Recirculating Aquaculture System Setup

For the experiment 2, a RAS with total volume of 20 m^3 rearing Arapaima (*Arapaima gigas*) at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin, provided the wastewater used as water source for the aquaponic treatment, RAS H_2O_2. The fish were fed 1% of the body weight three times per week, hence 3% of the body weight per week with Aller Primo, 8 mm (Aller Aqua, Germany). The water treatment units consisted of a drum filter with a mesh size of 100 µm for solid removal, a moving bed filter as biofilter and a trickling filter for CO_2-removal.

1.2.2. Hydroponic experimental unit and Lettuce cultivation

The experiment 2 was carried out at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin from 22nd of December till 26th of January, lasting 35 days, under the same environmental conditions described in 3.1.3., except: the climate chamber room had a set average room temperature of 17 ºC; the LED lamp (SANlight Q4WL S2.1 Gen2, 165W) was set to a 40% intensity with an average distance of 60 cm to the top of the hydroponic system; light intensity with PPFD values between 120 and 130 µmol m^{-2} s^{-1} with a light period of 12 hours.

Three different treatments were applied in three replicates each, the treatments were: H air, hydroponic treatment with nutrient solution prepared with tap water and destilled water (50:50, v/v, distilled: tap water) with fertilizer addition, and provided with compressed air; H H_2O_2,

hydroponic treatment with nutrient solution prepared with tap water and destilled water (50:50, v/v, distilled: tap water) with fertilizer addition, and provided with a passive H_2O_2-supply via an Oxydator D (Dr. rer. nat. K. Söchting Biotechnik GmbH, Germany) instead of compressed air; and RAS H_2O_2, aquaponic treatment with nutrient solution prepareded with fish waste water from RAS rearing arapaima with fertilizer addition, and provided with a passive H_2O_2-supply via an Oxydator D (Dr. rer. nat. K. Söchting Biotechnik GmbH, Germany) instead of compressed air.

The distribution of the replicates in the hydroponic unit can be seen in Figure 10.

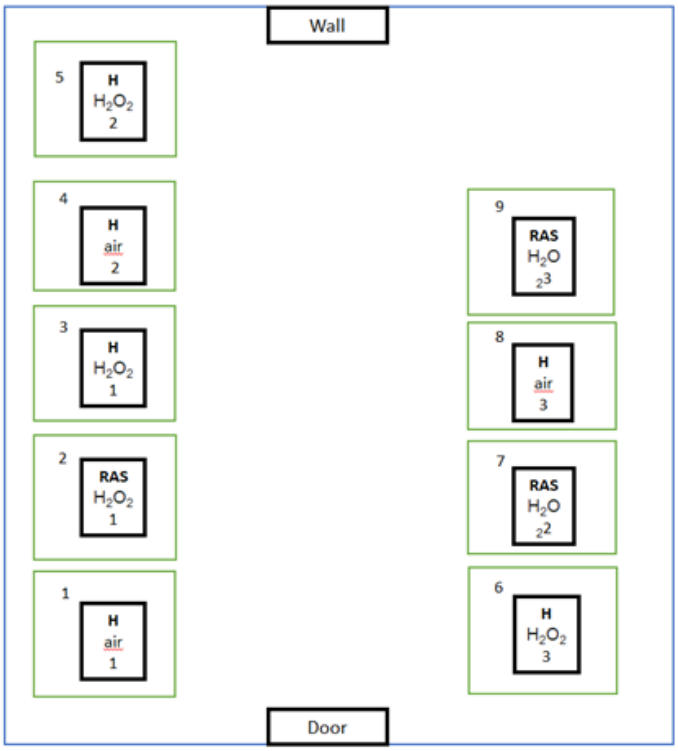

Figure 10: Scheme of the hydroponic unit in experiment 2

3.2.3. Water source for nutrient solution

In the hydroponic treatments (H air and H H_2O_2), the water source to prepare the nutrient solution was tap water and distilled water (50:50, v/v, distilled water: tap water). For the aquaponic treatment, RAS H_2O_2, fish wastewater from the RAS rearing Arapaima was used as water source to prepare the nutrient solution. The fish wastewater before being used was also stored in tanks provided with compressed air. The hydroponic treatment tank (H air and H H_2O_2) was filled with 180 L of the respective water source, since both treatments have the same nutrient solution composition. The aquaponic treatment tank (RAS H_2O_2) had a final volume of 90 L.

3.2.4. Nutrient Solution preparation

The preparation of the nutrient solutions was conducted as described in 3.1.5., the tap water and the RAS Arapaima waste water were analyzed for its nutrient concentrations by ICP-OES and CFA. Based on these values, the required amount of mineral fertilizers needed to add were calculated using the Hydrobuddy Program as described in 3.1.5. The volume of each stock solution A and B was set to 1.8 L for the H air and H H_2O_2 treatments, the same stock solutions were used in both treatments since the nutrient solution was the same. For RAS H_2O_2 treatment the volume of each stock solution A and B was set to 0.9 L. Afterwards, these stock solutions were added to the hydroponic treatments tank (H air and H H_2O_2) with 180 L of the respective water source and to the aquaponic treatment tank (RAS H_2O_2) with 90 L. The Lettuce General (Howard Resh) recipe was used but with 70% strength instead of the total strength, hence using 30% less nutrients. This was done to avoid the EC to increase over 2.0 dS m^{-1} during the experimental period, as this tendency was noticed to happen during experiment 1. Once all the nutrient solutions were ready, they were distributed from the tanks to each respective replicate chamber with a pump, each replicate chamber with 30 L of nutrient solution.

3.2.5. Nutrient solution change and water consumption

The nutrient solution was changed two times during the experimental period. The first change was done on 5th of January of 2021, 14 days after the beginning of the experiment, named nutrient solution change 1. The next one was done on 19th of January of 2021, 14 days after the previous nutrient solution change, named nutrient solution change 2. The same procedure as described on 3.1.6. was done and each system was filled with 30 L of the respective “fresh” nutrient solution from the Hydroponic (H air and H H_2O_2) and Aquaponic (RAS H_2O_2) tanks, which is the initial water volume per replicate.

At the harvesting day, 26th of January of 2021, 7 days after the previously nutrient solution change, the remaining nutrient solution present in each replicate chamber was also measured in L. The calculation of actual water consumption per replicate in L and the actual water consumption per plant per day in L was done as described in 3.1.6..

3.2.6. Sampling

From each water source, tap water for hydroponic treatments (H air and H H_2O_2) and RAS wastewater for RAS H_2O_2 treatment, two filtered samples were taken to analyze its nutrients concentrations two days before starting the experiment and every time before changing the nutrient solution preparation, as described in 3.1.7.. The samples were named RAS_stock_1,

RAS_stock_2 and RAS_stock_3 for the RAS H_2O_2 treatment and tap water for the hydroponic treatments (H air and H H_2O_2).

On the first day of the experiment, 22nd of December, 2 filtered samples were taken from the nutrient solution of each tank (Hydroponic treatment tank and RAS H_2O_2 tank). Consequently, till the end of the experiment, 2 filtered samples from each replicate were taken weekly to analyze its nutrients concentrations by ICP-OES and CFA.

All these samples were prepared as described in 3.1.7.

3.2.7. Lab analysis

The samples from the experiment were analysed in the lab during the experiment as described in 3.1.8.

3.2.8. Assessment of plant growth

On the 26th of January of 2020 the lettuce heads were harvested as described in 3.1.9. The dry weight of the selected plants was not estimated as described in 3.1.9., in this experiment, one additional entire lettuce plant was randomly selected for each replicate to be dried in a ventilated oven (Heraeus; Hanau, Germany) at 60 °C to determine the dry weight in grams of the whole lettuce head. Thus, the DW presented in the results section is not an estimate but the mean values of the DW of the additional selected lettuce plants from each replicate.

3.2.9. Preparation of lettuce for chemical analysis

To prepare for the chemical analysis, four dried selected samples per replicate were used, the procedure is described in 3.1.10.. However, due to covid-19 pandemic and delays in the lab, these analyses were not carried out in time to be included in this dissertation.

3.2.10. Abiotic parameters measurement of the nutrient solutions and in the experimental chambers

The abiotic parameters of the nutrient solutions and in the experimental chambers were measured as described in 3.1.11.

3.2.11. SPAD values of lettuce leaves

The SPAD values of the lettuce leaves were measured as described in 3.1.12.

3.2.12. H_2O_2 6% solution addition to the nutrient solution and H2O2 consumption

Here, H_2O_2 was provided to the nutrient solutions of the treatments H H_2O_2 and RAS H_2O_2. The Söchting 103 Oxidator (Söchting Oxydator, Germany) was used for this and filled initially

with 100 mL of the Söchting oxydator solution at a concentration of 6% stabilized hydrogen peroxide (Söchting Oxydator, Germany). The oxidator was checked to see if it was necessary to add more solution. More solution was added when the oxidator was only one third full. At the end of the experiment the total used volume of the solution H_2O_2 6% was measured in mL for each replicate and for treatment; the actual average H_2O_2 consumption in mL per day for the treatment H H_2O_2 and RAS H_2O_2 was also calculated.

3.2.13. Statistical evaluation

The statistical evaluation was done as described in 3.1.13.

4. Results

4.1. Experiment 1

4.1.1. Lettuce growth and lettuce yield

The use of black soldier fly meal based diet wastewater, BSF treatment, and fishmeal based diet wastewater, FIM treatment, resulted in similar yields as under conventional hydroponics (control treatment) conditions, as seen in Table 5. The lettuce heads reached an average final fresh weight of 99.1 g ± 12.3 in control treatment, 96.9 g ± 16.7 in BSF treatment and 97.1 g ± 17.6 in FIM treatment after 35 days of growth. At the time of the harvesting, the lettuce heads in control treatment formed an average of 118 ± 9 leaves, 121 ± 18 leaves in BSF treatment and 120 ± 13 leaves in FIM treatment. The lettuce heads from BSF and FIM treatment formed 3 and 2 more leaves than the control, respectively. The mean dry weight in control was the lowest with 4.24 ± 0.4 g; in BSF treatment was 4.34 ± 0.5 g; and in FIM treatment was the highest value with 4.39 ± 0.6 g.

Table 5. Growth parameters of lettuce grown in the control treatment (distilled: tap water, 50:50, v/v), BSF treatment (black soldier fly meal based diet wastewater) and FIM treatment (fishmeal based diet waste water). The data represent the mean values of thirty (mean fresh weight) or twelve (number of leaves and mean dry weight) lettuce heads per treatment. The medians of the mean fresh weight (FW) and number of leaves do not vary significantly ($p < 0.05$) using the Kruskal-Wallis test.

Treatment	Mean fresh weight (g)	Number of leaves	Mean dry weight (g)
Control	99.1 ± 12.3	118 ± 9	4.24 ± 0.4
BSF	96.9 ± 16.7	121 ± 18	4.34 ± 0.5
FIM	97.1 ± 17.6	120 ± 13	4.39 ± 0.6

4.1.2. Water source nutrient analysis

The nutrient concentrations measured in the control-, BSF- and FIM-treatment labeled from 1 to 3 each, can be seen in Table 6. The nutrient concentrations are given in mg L^{-1}.

Starting with the macronutrients (N, P, K): NO_3-N, nitrate, in the control treatment had a mean value of 1.2 ± 0.08 mg L^{-1}; in the BSF treatment the NO_3-N concentration had a mean value of 38.6 ± 5.2 mg L^{-1}; and in the FIM treatment the NO_3-N concentration had a mean value of 41.3 ± 4.5 mg L^{-1}. The P, phosphorus, concentration in the control treatment was always 0.01 mg L-1; in the BSF treatment the P concentration had mean value of 2.7 ± 0.5 mg L^{-1}; and in the FIM treatment P concentration had mean value of 3.5 ± 0.9 mg L^{-1}. K, potassium, concentration in the control treatment had mean value of 5.7 ± 0.6 mg L^{-1}; in the BSF treatment the K concentration had mean value of 20.3 ± 2.6 mg L^{-1}; and in the FIM treatment the K concentration had mean value of 13.8 ± 0.9 mg L^{-1}. The BSF treatment showed higher values of K comparing to the FIM treatment, however P concentration had higher values in the FIM treatment than in the BSF treatment. The nitrate (NO_3-N) concentration was also higher in the FIM treatment than in the BSF treatment. NO_3-N, P and K concentrations were higher in the FIM and BSF treatments than in the control treatment.

Regarding to Na, sodium, in the control treatment the mean value was 41.3 ± 0.9 mg L^{-1}; in the BSF treatment the Na concentration had mean value of 42.3 ± 1.2 mg L^{-1}; and in the FIM treatment the Na concentration had a mean value of 52 ± 2.8 mg L^{-1}.

Table 6. Nutrient concentrations in mg L^{-1} measured in water samples from the control treatment (distilled: tap water, 50:50, v/v), BSF treatment (black soldier fly meal based diet wastewater) and FIM treatment (fishmeal based diet wastewater). Labelled from 1 to 3, collected over three sampling periods (20/10/2020; 3/11/2020; 10/11/2020).

Sample	NO_3-N	NH_4-N	P	K	Ca	Mg	S	Fe	B	Mn	Cu	Zn	Na	Si	Al
Control Stock 1	1.3	0.01	0.01	5	105	11.5	51	<0.01	0.07	<0.01	0.04	0.09	42	6.4	<0.01
BSF Stock 1	32.6	0.29	2.3	18	127	17.5	67	0.02	0.08	0.02	0.02	0.05	41	7.4	<0.01
FIM Stock 1	36.0	0.39	3.0	13	126	15.7	68	0.02	0.08	<0.01	0.02	0.02	50	7.1	<0.01
Control Stock 2	1.1	0.01	0.01	5,6	123	13.2	60	<0.01	0.08	<0.01	0.31	0.52	42	7.0	<0.01
BSF Stock 2	45.3	0.29	3.4	24	118	18.0	62	0.02	0.06	<0.01	0.03	0.04	44	7.1	0.02
FIM Stock 2	47.1	0.50	4.7	15	116	16.0	64	<0.01	0.07	0.09	0.02	0.03	56	6.9	0.02
Control Stock 3	1.2	0.11	0.01	6.5	115	12.9	55	<0.01	0.08	<0.01	0.01	0.02	40	7.0	0.02
BSF Stock 3	37.8	0.20	2.4	19	117	16.9	61	0.01	0.07	<0.01	0.02	0.03	42	7.0	0.02
FIM Stock 3	40.9	0.19	2.8	13.3	115	15.2	63	<0.01	0.08	<0.01	0.01	<0.01	50	7.0	0.02

4.1.3. Nutrient analysis of the nutrient solution

The average nutrient concentrations of each treatment nutrient solution measured every week is shown in Table 7 for macronutrients and Table 8 for micronutrients and Na, Si, Al. The nutrient concentrations are in mg L^{-1}.

Starting with the macronutrients (N, P, K), as seen in Table 7: NO_3-N, nitrate, in the control treatment had a mean value of 166.7 ± 7.6 mg L^{-1}; in the BSF treatment the NO_3-N concentration had a mean value of 160.5 ± 3.8 mg L^{-1}; and in FIM treatment the NO_3-N concentration had a mean value of 157.6 ± 4.6 mg L^{-1}.

P, phosphorus, concentration in the control treatment had a mean value of 47.2 ± 2.7 mg L^{-1}; in the BSF treatment the P concentration had a mean value of 49.3 ± 4.0 mg L^{-1}; and in the FIM treatment the P concentration had mean value of 50.3 ± 3.3 mg L^{-1}.

K, potassium, concentration in the control treatment had a mean value of 210.9 ± 24.6 mg L^{-1}; in the BSF treatment the K concentration had a mean value of 206.0 ± 22.6 mg L^{-1}; and in the FIM treatment the K concentration had a mean value of 204.7 ± 22.4 mg L^{-1}.

Table 7. Macronutrient concentrations (N, P, K, Ca, Mg and S) in mg L^{-1} of the nutrient solutions from the control treatment (distilled: tap water, 50:50, v/v), BSF treatment (black soldier fly meal based diet wastewater) and FIM treatment (fishmeal based diet wastewater). Measured weekly during the experimental period. The data represent the mean nutrient concentrations of the three different replicates per treatment for each week.

Date	Treatment	NO_3-N	NH_4-N	P	K	Ca	Mg	S
22/10/2020	Control	155.0	7.54	47	223	177	41.5	84.2
22/10/2020	BSF	153.1	4.08	46.1	220.5	166.7	39.5	67.7
22/10/2020	FIM	152.5	4.4	47.9	221.4	163.3	37.8	68.8
28/10/2020	Control	170.2 ± 2.4	7.1 ± 0.1	46.6 ± 0.3	235.9 ± 3.1	185.5 ± 1.0	43.6 ± 0.2	89.3 ± 0.4
28/10/2020	BSF	165.6 ± 1.7	3.9 ± 0.1	47.2 ± 0.3	232.3 ± 3.8	178.1 ± 1.6	41.8 ± 0.4	73.9 ± 0.6
28/10/2020	FIM	162.9 ± 3.4	3.4 ± 0.2	48.0 ± 0.5	231.2 ± 4.0	174.3 ± 2.3	39.7 ± 0.4	74.4 ± 0.1
06/11/2020	Control	165 ± 0.7	5.7 ± 0.1	49.3 ± 0.6	222.8 ± 0.8	184.5 ± 1.8	43.8 ± 0.7	80.2 ± 0.5
06/11/2020	BSF	159.5 ± 1.1	2.9 ± 0.1	53.9 ± 0.8	212.9 ± 0.8	185.9 ± 1.4	33.8 ± 0.6	66.4 ± 0.5
06/11/2020	FIM	157.6 ± 2.4	3.3 ± 0.1	53.1 ± 0.7	208.1 ± 0.5	182.2 ± 1.2	30.8 ± 0.4	67.6 ± 0.3
12/11/2020	Control	180.3 ± 3.3	0.2 ± 0.02	51.9 ± 0.2	228.5 ± 4.1	195.9 ± 1.7	48.1 ± 0.4	85.9 ± 0.3
12/11/2020	BSF	162.6 ± 2.3	0.3 ± 0.02	56.0 ± 1.4	218.5 ± 4.2	194.2 ± 4.4	36.3 ± 0.1	70.2 ± 1.8
12/11/2020	FIM	164.4 ± 0.9	0.3 ± 0.02	56.3 ± 0.3	216.4 ± 6.5	192.9 ± 1.1	33.8 ± 0.3	72.8 ± 0.5
17/11/2020	Control	164.4 ± 3.8	7.1 ± 0.03	44.8 ± 0.1	187.8 ± 1.0	181.0 ± 0.4	41.2 ± 0.2	81.9 ± 0.2
17/11/2020	BSF	161.5 ± 1.4	4.1 ± 0.02	46.6 ± 0.1	185.0 ± 0.3	184.6 ± 0.2	31.9 ± 0.04	68.2 ± 0.2
17/11/2020	FIM	155.0 ± 1.9	4.0 ± 0.1	48.3 ± 01	184.7 ± 0.8	181.6 ± 1.2	29.8 ± 0.04	69.8 ± 0.3
24/11/2020	Control	165.2 ± 1.1	0.5 ± 0.1	44.0 ± 0.3	167.6 ± 0.3	195.2 ± 0.9	43.4 ± 0.3	88.6 ± 0.5
24/11/2020	BSF	159.9 ± 0.3	0.5 ± 0.02	46.2 ± 0.4	167.0 ± 2.4	198.5 ± 2.2	33.7 ± 0.4	73.8 ± 0.8
24/11/2020	FIM	153.0 ± 3.3	0.7 ± 0.1	48.0 ± 0.4	166.1 ± 1.3	195.1 ± 0.9	31.3 ± 0.1	75.5 ± 0.3

Regarding to Na, sodium, as seen in Table 8, the control treatment had a mean value of 25.8 ± 4.2 mg L^{-1}; in the BSF treatment the Na concentration had a mean value of 46.0 ± 2.9 mg L^{-1}; and in the FIM treatment the Na concentration had a mean value of 59.4 ± 7.9 mg L^{-1}.

Table 8. Micronutrient concentrations (Fe, B, Mn, Cu, Zn), Na, Si and Al in mg L^{-1} of the nutrient solutions from the control treatment (distilled: tap water, 50:50, v/v), BSF treatment (black soldier fly meal based diet wastewater) and FIM treatment (fishmeal based diet wastewater). Measured weekly during the experimental period. The data represent the mean nutrient concentrations of the three different replicates per treatment for each week.

Date	Treatment	Fe	B	Mn	Cu	Zn	Na	Si	Al
22/10/2020	Control	3.8	0,50	0.42	0.09	0.06	24	3.2	0,01
22/10/2020	BSF	2.8	0.43	0.38	0.09	0.06	42.3	6.7	<0.01
22/10/2020	FIM	2.8	0.4	0.4	0.1	0.05	50.7	6.5	<0.01
28/10/2020	Control	2.6 ± 0.03	0.53 ± 0.00	0.43 ± 0.00	0.09 ± 0.0	0.04 ± 0.0	25.4 ± 0.2	3.8 ± 0.05	0.02 ± 0.0
28/10/2020	BSF	2.6 ± 0.01	0.45 ± 0.00	0.42 ± 0.01	0.1 ± 0.0	0.06 ± 0.0	45.4 ± 0.4	7.3 ± 0.08	0.02 ± 0.0
28/10/2020	FIM	2.5 ± 0.02	0.5 ± 0.00	0.4 ± 0.00	0.1 ± 0.0	0.04 ± 0.0	53.5 ± 0.6	7.2 ± 0.07	0.02 ± 0.0
06/11/2020	Control	2.7 ± 0.05	0.5 ± 0.0	0.5 ± 0.0	<0.1	<0.1	19.8 ± 0.3	3.1 ± 0.07	<0.1
06/11/2020	BSF	2.7 ± 0.02	0.5 ± 0.0	0.5 ± 0.0	<0.1	<0.1	44.0 ± 0.2	7.0 ± 0.09	<0.01
06/11/2020	FIM	2.6 ± 0.03	0.5 ± 0.0	0.5 ± 0.0	<0.1	<0.1	61.3 ± 0.4	6.6 ± 0.05	<0.1
12/11/2020	Control	2.7 ± 0.04	0.5 ± 0.0	0.5 ± 0.0	<0.1	<0.1	31.7 ± 0.6	3.6 ± 0.05	<0.1
12/11/2020	BSF	2.5 ± 0.06	0.5 ± 0.0	0.5 ± 0.0	<0.1	<0.1	50.2 ± 1.4	7.4 ± 0.2	<0.01
12/11/2020	FIM	2.6 ± 0.02	0.5 ± 0.0	0.5 ± 0.0	<0.1	<0.1	74.9 ± 1.8	7.2 ± 0.03	<0.1
17/11/2020	Control	2.6 ± 0.01	0.5 ± 0.0	0.5 ± 0.0	<0.1	<0.1	23.3 ± 0.1	2.3 ± 0.02	<0.1
17/11/2020	BSF	2.6 ± 0.01	0.5 ± 0.0	0.5 ± 0.0	<0.1	<0.1	44.5 ± 0.1	5.8 ± 0.04	<0.01
17/11/2020	FIM	2.6 ± 0.01	0.5 ± 0.0	0.5 ± 0.0	<0.1	<0.1	54.9 ± 0.2	5.8 ± 0.04	<0.1
24/11/2020	Control	2.6 ± 0.01	0.3 ± 0.0	0.5 ± 0.0	<0.1	<0.1	31.0 ± 0.5	2.8 ± 0.1	<0.1
24/11/2020	BSF	2.6 ± 0.03	0.5 ± 0.0	0.5 ± 0.0	<0.1	<0.1	49.5 ± 0.3	6.6 ± 0.2	<0.01
24/11/2020	FIM	2.6 ± 0.02	0.5 ± 0.0	0.4 ± 0.0	<0.1	<0.1	61.1 ± 0.9	6.6 ± 0.0	<0.1

4.1.4. Abiotic Parameters of the nutrient solution

Regarding to pH, the mean value was 6.2 ± 0.1 in all treatments. The medians did not vary significantly ($p < 0.05$) between the three different treatments using the Kruskal-Wallis test and Dunn-Bonferroni test, Figure 11.

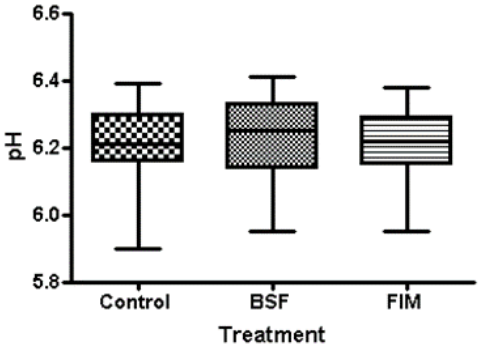

Figure 11: pH values of the nutrient solutions from the control treatment (distilled: tap water, 50:50, v/v), BSF treatment (black soldier fly meal based diet wastewater) and FIM treatment (fishmeal based diet wastewater). The medians do not vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test.

The EC measured in dS m^{-1} had a mean value of 2.1 ± 0.1 in all treatments. The medians varied significantly ($p < 0.05$) between the three treatments, using the Kruskal-Wallis test and Dunn-Bonferroni test, Figure 12.

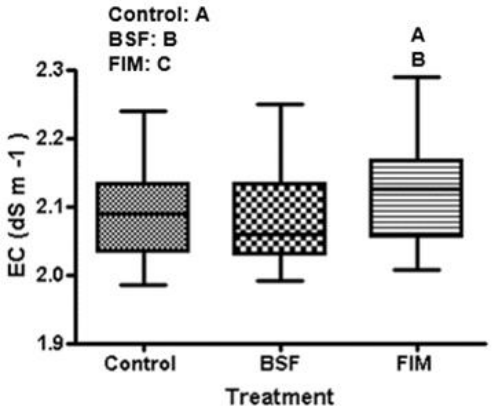

Figure 12: Electrical conductivity values in dS m^{-1} of the nutrient solutions from the control treatment (distilled: tap water, 50:50, v/v), BSF treatment (black soldier fly meal based diet wastewater) and FIM treatment (fishmeal based diet wastewater). The medians vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test. Different capital letters indicate significant differences between the three different nutrient solutions and are listed from top to bottom: control, BSF, FIM.

Regarding to the temperature, the mean value of control treatment was 20.9 ± 0.3 °C, of the BSF treatment was 20.7 ± 0.2 °C and of the FIM treatment was 20.7 ± 0.3 °C. The temperature values varied significantly ($p < 0.05$) between the three treatments, using the Kruskal-Wallis test and Dunn-Bonferroni test, Figure 13.

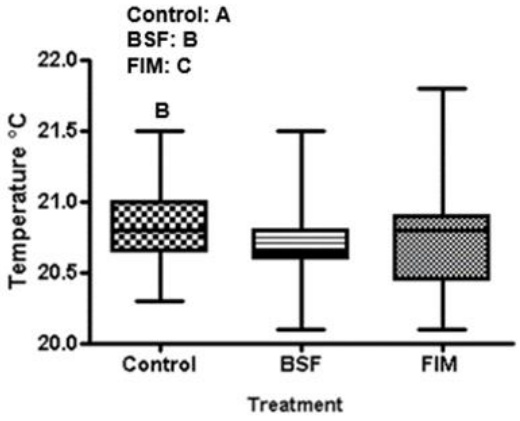

Figure 13: Temperature of the nutrient solutions from the control treatment (distilled: tap water, 50:50, v/v), BSF treatment (black soldier fly meal based diet wastewater) and FIM treatment (fishmeal based diet wastewater). The medians vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test. Different capital letters indicate significant differences between the three different nutrient solutions and are listed from top to bottom: control, BSF, FIM.

The mean dissolved oxygen (DO) concentration in the three treatments was 8.0 ± 0.1 mg L^{-1}. The medians did not vary significantly ($p < 0.05$) between the three different treatments, using the Kruskal-Wallis test and Dunn-Bonferroni test, Figure 14.

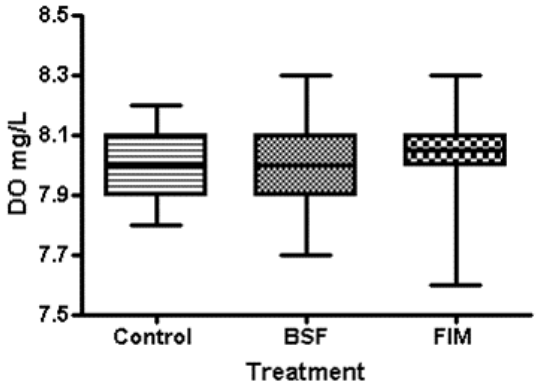

Figure 14: Dissolved oxygen concentrations in mg L^{-1} of the nutrient solutions from the control treatment (distilled: tap water, 50:50, v/v), BSF treatment (black soldier fly meal based diet wastewater) and FIM treatment (fishmeal based diet wastewater). The medians do not vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test.

4.1.5. Abiotic Parameters in the experimental chambers

Regarding the control treatment, the mean air temperature was 22.2 ± 1.1 °C and the mean relative humidity was 63.9 ± 6.6 %. In BSF treatment, the mean air temperature was 22.1 ± 1.0 °C and the mean relative humidity was 62.1 ± 5.7 %, Figure 15.

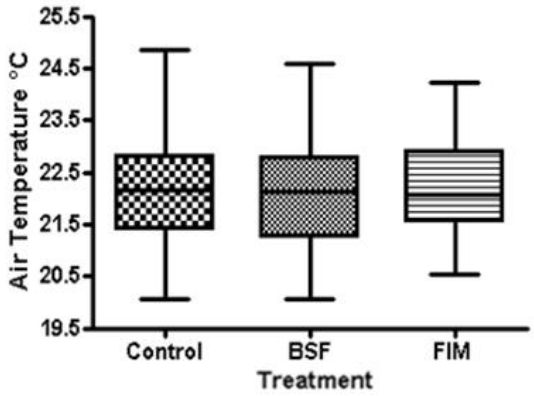

Figure 15: Air temperature values in the experimental chambers from the control treatment (distilled: tap water, 50:50, v/v), BSF treatment (black soldier fly meal based diet wastewater) and FIM treatment (fishmeal based diet wastewater). The medians do not vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test.

In FIM treatment, the mean air temperature was 22.1 ± 0.9 °C and the mean relative humidity was 62.2 ± 5.7 %. Both air temperature and mean relative humidity medians did not vary significantly ($p < 0.05$) between the three different treatment groups using the Kruskal-Wallis test and Dunn-Bonferroni test, as seen in Figure 15 and Figure 16.

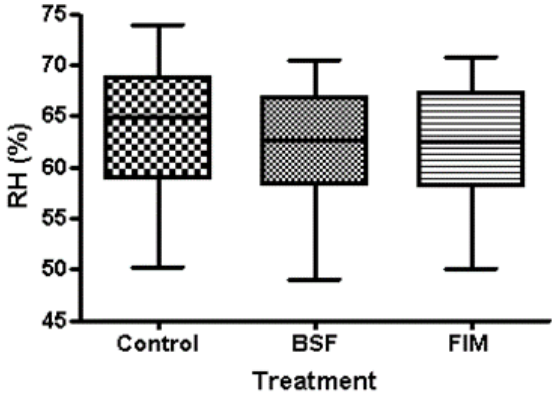

Figure 16: Relative humidity (RH %) values in the experimental chambers from the control treatment (distilled: tap water, 50:50, v/v), BSF treatment (black soldier fly meal based diet wastewater) and FIM treatment (fishmeal based diet wastewater). The medians do not vary significantly ($p < 0.05$) done with Kruskal-Wallis test and Dunn-Bonferroni test.

4.1.6. Water Consumption

As seen in Table 9, for the nutrient solution change 1, the actual mean water consumption per plant per day since the start of the experiment till the nutrient solution change 1, was 0.036 L in control, 0.034 L in BSF treatment and 0.033 L in FIM treatment.

In the nutrient solution change 2 on 17th of November 2020, the actual mean water consumption per plant per day since nutrient solution change 1 till nutrient solution change 2, was 0.045 L in control, 0.025 L in BSF treatment and 0.033 L in FIM treatment.

In the day of the harvesting on 26th of November 2020, the actual mean water consumption per plant per day since the nutrient solution change 2 till harvesting, was 0.047 L in control treatment, 0.042 L in BSF treatment and 0.037 L in FIM treatment.

Table 9. Initial water volume per replicate (L); final water volume per replicate (L); actual water consumption per replicate (L); days of growth; actual water consumption per plant per day (L) from the control treatment (distilled: tap water, 50:50, v/v), BSF treatment (black soldier fly meal based diet wastewater) and FIM treatment (fishmeal based diet wastewater). The data in final water volume per replicate, in the actual water consumption per replicate and in the actual water consumption per plant per day represent the mean values of the three replicates for each treatment.

	Treatment	Initial water volume per replicate (L)	Final water volume per replicate (L)	Actual water consumption per replicate (L)	Days	Actual water consumption per plant per day (L)
Nutrient	Control	45	39.9 ± 1.7	5.1	14	0.036
Solution	BSF	45	40.3 ± 3.0	4.7	14	0.034
Change 1	FIM	45	40.4 ± 1.0	4.6	14	0.033
Nutrient	Control	45	39.1 ± 0.5	5.9	13	0.045
Solution	BSF	45	41.7 ± 2.2	3.3	13	0.025
Change 2	FIM	45	40.7 ± 1.7	4.3	13	0.033
	Control	45	40.8 ± 1.4	4.2	9	0.047
Harvesting	BSF	45	41.2 ± 2.0	3.8	9	0.042
	FIM	45	41.7 ± 0.7	3.3	9	0.037

4.1.7. SPAD values of lettuce leaves

In Table 10, the mean SPAD value of the 30 plants of each treatment is shown. The values of the control treatment ranged from 25.8 to 30.6 with a mean value of 28.5 ± 1.2, being the highest mean value. The values of the BSF treatment ranged from 25.0 to 32.7 with a mean value of 27.8 ± 1.5, being the lowest mean value. The values of the FIM treatment ranged from 25.2 to 31.40 with a mean value of 28.1 ± 1.3.

Table 10. Mean SPAD values in lettuce grown in the control treatment (distilled: tap water, 50:50, v/v), BSF treatment (black soldier fly meal based diet wastewater) and FIM treatment (fishmeal based diet wastewater).The data represent the mean value of the 30 plants in each treatment.

Treatment	SPAD value
Control	28.5 ± 1.2
BSF	27.8 ± 1.5
FIM	28.1 ± 1.3

4.1.8. Fish growth

As seen in Table 11 (unpublished data, Christopher Shaw, 2021) the fish of the BSF replicates had a mean initial body weight of 24.3 ± 0.4 g and a mean final body weight of 59.9 ± 1.6 g. The BSF treatment had a mean FCR (feed conversion ratio) of 1.23.

The fish of the FIM replicates had a mean start weight of 23.7 ± 0.6 g and a mean end weight of 70.2 ± 1.5 g. The FIM treatment had a mean FCR of 0.94.

Table 11. Mean initial body weight (g), mean final body weight (g), FCR (feed conversion ratio) of the RAS for BSF treatment (black soldier fly meal based diet) and FIM treatment (fishmeal based diet). Values represent means ± standard deviations. Unpublished data from Christopher Shaw.

Treatment	Mean initial body weight (g)	Mean final body weight (g)	FCR
BSF	24.3 ± 0.4	59.9 ± 1.6	1.23
FIM	23.7 ± 0.6	70.2 ± 1.5	0.94

4.2. Experiment 2

4.2.1. Lettuce growth and lettuce yield

The use of H_2O_2 in the treatments H H_2O_2 and RAS H_2O_2, resulted in similar yields as under conventional hydroponics, H air, conditions, as seen in Table 12. The lettuce heads reached an average final fresh weight of 153.5 g ± 26.5 in H air treatment, 148.9 g ± 31.8 in H H_2O_2 treatment and 146.7 g ± 24.8 in treatment RAS H_2O_2 after 35 days of growth. At the time of the harvest, the lettuce heads in H air treatment formed an average of 181 ± 24 leaves, 188 ± 25 leaves in H H_2O_2 treatment and 171 ± 29 leaves in RAS H_2O_2 treatment. The lettuce heads from H H_2O_2 treatment formed 7 and 17 more leaves than the H air and RAS H_2O_2 treatments, respectively. The mean dry weight in H air was 6.1 ± 0.2 g; in H H_2O_2 treatment was the lowest with 5.9 ± 0.7; and in RAS H_2O_2 treatment was the highest value with 6.5 ± 0.6 g.

Table 12. Growth parameters of lettuce grown in the H air treatment (distilled: tap water, 50:50, v/v provided with air), H H_2O_2 treatment (distilled: tap water, 50:50, v/v provided with H_2O_2) and RAS H_2O_2 treatment (RAS arapaima wastewater provided with H_2O_2). The data represent the mean values of thirty (mean fresh weight), twelve (number of leaves) and three (mean dry weight) lettuce heads per treatment. The medians of the mean fresh weight (FW) and number of leaves do not vary significantly ($p < 0.05$) using the Kruskal-Wallis test.

Treatment	Mean Fresh weight (g)	Number of leaves	Mean Dry weight (g)
Hair	153.5 ± 26.5	181 ± 24	6.1 ± 0.2
H H_2O_2	148.9 ± 31.8	188 ± 25	5.9 ± 0.7
RAS H_2O_2	146.7 ± 24.8	171 ± 29	6.5 ± 0.6

4.2.2. Water source nutrient analysis

The nutrient concentrations measured in the tap water sample for H air treatment and H H_2O_2 treatment, and RAS arapaima wastewater labeled from 1 to 3 for RAS H_2O_2 treatment can be seen in Table 13. The nutrient concentrations are given in mg L^{-1}.

Starting with the macronutrients (N, P, K, Ca, Mg and S): NO_3-N, nitrate, in the RAS H_2O_2 treatment samples had mean value of 56.2 ± 8.4 mg L^{-1}. The P, phosphorus, concentration in the RAS H_2O_2 treatment had a mean value of 7.8 ± 0.14 mg L^{-1}. The K, potassium, concentration in the RAS H_2O_2 treatment had a mean value of 15.8 ± 0.96 mg L^{-1}. Ca, calcium, concentration in the RAS H_2O_2 treatment had a mean value of 125.6 ± 8.54 mg L^{-1}. Mg, magnesium, concentration in the RAS H_2O_2 treatment had a mean value of 16.1± 0.41 mg L^{-1}. S, sulfur, concentration in the RAS H_2O_2 treatment had a mean value of 66.9 ± 4.76 mg L^{-1}. Regarding to Na, sodium, the concentration in the RAS H_2O_2 treatment had a mean value of 47.2 ± 1.4 mg L^{-1}.

Table 13. Nutrient concentrations in mg L^{-1} measured in water samples from tap water for H air treatment (distilled: tap water, 50:50, v/v provided with air) and H H_2O_2 treatment (distilled: tap water, 50:50, v/v provided with H_2O_2), and from RAS H_2O_2 treatment (RAS arapaima wastewater provided with H_2O_2). Labelled from 1 to 3, collected over 1 sampling period (Tap water) and three sampling periods for RAS Stock (2/12/2020;4/01/2021;12/01/2021).

Sample	NO_3-N	NH_4-N	P	K	Ca	Mg	S	Fe	B	Mn	Cu	Zn	Na	Si	Al
Tap Water	1.2	0.11	0.01	6.5	115	12.9	55	<0.01	0.08	<0.01	0.01	0.02	40	7.0	0.02
RAS Stock 1	47.1	0.5	7.8	15.4	121	16.4	64	<0.01	0.06	0.05	<0.01	0.03	46	6.9	0.02
RAS Stock 2	54.1	<0.1	8.0	17.1	138	16.4	74	0.02	0.07	<0.01	0.07	0.07	49	9.7	0.01
RAS Stock 3	67.4	0.3	7.6	14.9	118	15.5	63	0.01	0.09	<0.01	<0.01	0.03	47	7.0	0.01

4.2.3. Nutrient analysis of the nutrient solution

The average nutrient concentrations of each treatment nutrient solution measured every week is shown in Table 14 for macronutrients and Table 15 for micronutrients and Na, Si, Al. The nutrient concentrations are in mg L^{-1}.

Starting with the macronutrients (N, P, K, Ca, Mg and S), as seen in Table 14Table 14: NO_3-N, nitrate, in the H air treatment had a mean value of 140.3 ± 11.9 mg L^{-1}; in the H H_2O_2 treatment the NO_3-N concentration had a mean value of 143.0 ± 15.4 mg L^{-1}; and in the RAS H_2O_2 treatment the NO_3-N concentration had a mean value of 114.6 ± 19.7 mg L^{-1}.

P, phosphorus, concentration in H air treatment had a mean value of 30.2 ± 1.6 mg L^{-1}; in the H H_2O_2 treatment the P concentration had a mean value of 30.0 ± 1.8 mg L^{-1}; and in RAS H_2O_2 treatment the P concentration had a mean value of 31.7 ± 2.1 mg L^{-1}. K, potassium, concentration in the H air treatment had a mean value of 137.4 ± 27.2 mg L^{-1}; in the H H_2O_2

treatment the K concentration had a mean value of 137.2 ± 26.5 mg L^{-1}; and in the RAS H_2O_2 treatment the K concentration had a mean value of 135.3 ± 26.8 mg L^{-1}.

Ca, calcium, concentration in the H air treatment had a mean value of 121.6 ± 9.6 mg L^{-1}; in the H H_2O_2 treatment the Ca concentration had a mean value of 120.7 ± 8.8 mg L^{-1}; and in the RAS H_2O_2 treatment the Ca concentration had a mean value of 134.7 ± 8.6 mg L^{-1}. Mg, magnesium, concentration in the H air treatment had a mean value of 22.6 ± 0.8 mg L^{-1}; in the H H_2O_2 treatment the Mg concentration had a mean value of 22.4 ± 0.8 mg L^{-1}; and in the RAS H_2O_2 treatment the Mg concentration had a mean value of 26.6 ± 1.8 mg L^{-1}. S, sulfur, concentration in the H air treatment had a mean value of 32.7 ± 1.9 mg L^{-1}; in the H H_2O_2 treatment the S concentration had a mean value of 32.5 ± 1.9 mg L^{-1}; and in the RAS H_2O_2 treatment the S concentration had a mean value of 69.7 ± 4.5 mg L^{-1}.

Table 14. Macronutrient concentrations (N, P, K, Ca, Mg and S) in mg L^{-1} of the nutrient solutions from the H air treatment (distilled: tap water, 50:50, v/v provided with air), H H_2O_2 treatment (distilled: tap water, 50:50, v/v provided with H_2O_2) and RAS H_2O_2 treatment (RAS arapaima wastewater provided with H_2O_2). Measured weekly during the experimental period. The data represent the mean nutrient concentrations of the three different replicates per treatment for each week.

Date	Treatment	NO_3-N	NH_4-N	P	K	Ca	Mg	S
22/12/2020	H air	131.3 ± 0.8	4.4 ± 0.03	29.8 ± 0.1	148.0 ± 0.3	110.4 ± 0.4	21.3 ± 0.1	30.5 ± 0.1
22/12/2020	H H_2O_2	130.4 ± 0.7	4.5 ± 0.1	29.8 ± 0.1	148.2 ± 0.7	110.5 ± 0.3	21.3 ± 0.1	30.5 ± 0.1
22/12/2020	RAS H_2O_2	115.6 ± 1.0	1.2 ± 0.02	31.1 ± 0.1	143.2 ± 1.4	122.5 ± 0.4	25.6 ± 0.1	63.8 ± 0.4
30/12/2020	H air	159.3 ± 3.8	2.0 ± 0.4	31.3 ± 0.4	163.6 ± 2.9	121.5 ± 2.1	23.6 ± 0.4	34.6 ± 0.5
30/12/2020	H H_2O_2	164.6 ± 3.0	2.7 ± 0.7	30.9 ± 0.4	164.1 ± 1.8	121.3 ± 1.3	23.7 ± 0.3	35.1 ± 0.5
30/12/2020	RAS H_2O_2	131.7 ± 0.6	0.5 ± 0.2	32.2 ± 0.2	155.4 ± 2.0	135.7 ± 2.3	28.1 ± 0.2	70.4 ± 0.3
05/01/2020	H air	148.0 ± 3.2	4.1 ± 0.05	29.7 ± 0.1	154.8 ± 0.04	111.1 ± 0.8	22.0 ± 0.1	30.3 ± 0.1
05/01/2020	H H_2O_2	161.2 ± 0.9	4.2 ± 0.1	29.6 ± 0.1	153.6 ± 0.4	110.2 ± 0.2	21.8 ± 0.1	30.0 ± 0.3
05/01/2020	RAS H_2O_2	138.0 ± 1.7	1.2 ± 0.01	30.2 ± 0.1	155.0 ± 0.8	124.7 ± 0.8	27.1 ± 0.2	65.3 ± 0.3
12/01/2021	H air	144.3 ± 3.0	0.5 ± 0.04	28.6 ± 0.5	140.5 ± 4.8	119.8 ± 2.2	23.5 ± 0.5	33.0 ± 0.6
12/01/2021	H H_2O_2	141.1 ± 0.8	0.5 ± 0.02	27.9 ± 0.2	138.9 ± 3.4	119.3 ± 0.8	23.3 ± 0.2	32.7 ± 0.2
12/01/2021	RAS H_2O_2	120.0 ± 0.3	0.6 ± 0.02	29.1 ± 0.1	146.9 ± 1.2	142.1 ± 0.5	29.3 ± 0.1	71.7 ± 0.2
19/01/2021	H air	136.5 ± 3.3	4.1 ± 0.3	33.1 ± 0.5	137.5 ± 2.5	129.1 ± 1.2	22.2 ± 0.3	32.4 ± 0.5
19/01/2021	H H_2O_2	138.9 ± 0.6	4.3 ± 0.1	33.4 ± 0.2	136.8 ± 3.6	128.7 ± 1.2	22.0 ± 0.1	32.1 ± 0.8
19/01/2021	RAS H_2O_2	104.3 ± 1.3	1.0 ± 0.03	36.0 ± 0.3	133.6 ± 2.2	137.1 ± 0.6	24.1 ± 0.2	69.5 ± 0.4
25/01/2021	H air	122.3 ± 3.6	0.6 ± 0.02	28.7 ± 0.6	79.8 ± 4.3	137.8 ± 2.6	22.9 ± 0.6	35.4 ± 0.9
25/01/2021	H H_2O_2	122.0 ± 0.7	0.5 ± 0.1	28.6 ± 0.2	81.4 ± 1.6	134.3 ± 1.4	22.5 ± 0.3	34.6 ± 0.5
25/01/2021	RAS H_2O_2	77.9 ± 1.2	0.5 ± 0.05	31.6 ± 0.3	77.7 ± 2.7	146.3 ± 1.2	25.1 ± 0.2	77.4 ± 0.3

Regarding to Na, sodium, as seen in Table 15Table 15, the concentration in the H air treatment had a mean value of 28.8 ± 3.7 mg L^{-1}; in the H H_2O_2 treatment the Na concentration had mean value of 27.6 ± 3.2 mg L^{-1}; and in the RAS H_2O_2 treatment the Na concentration had a mean value of 54.3 ± 3.7 mg L^{-1}.

Table 15. Micronutrient concentrations (Fe, B, Mn, Cu, Zn), Na, Si and Al in mg L^{-1} of the nutrient solutions from the H air treatment (distilled: tap water, 50:50, v/v provided with air), H H_2O_2 treatment (distilled: tap water, 50:50, v/v provided with H_2O_2) and RAS H_2O_2 treatment (RAS arapaima wastewater provided with H_2O_2). Measured weekly during the experimental period. The data represent the mean nutrient concentrations of the three different replicates per treatment for each week.

Date	Treatment	Fe	B	Mn	Cu	Zn	Na	Si	Al
22/12/2020	H air	2.6 ± 0.03	0.5 ± 0.0	0.3 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	26.6 ± 0.2	2.6 ± 0.00	<0.01
22/12/2020	H H_2O_2	2.6 ± 0.01	0.5 ± 0.0	0.3 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	26.3 ± 0.2	2.6 ± 0.03	<0.01
22/12/2020	RAS H_2O_2	2.3 ± 0.02	0.5 ± 0.0	0.4 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	51.2 ± 0.5	6.1 ± 0.05	<0.01
30/12/2020	H air	2.6 ± 0.04	0.5 ± 0.01	0.3 ± 0.01	0.1 ± 0.1	0.1 ± 0.0	33.3 ± 1.0	3.5 ± 0.1	<0.01
30/12/2020	H H_2O_2	2.5 ± 0.03	0.5 ± 0.01	0.8 ± 0.02	0.1 ± 0.02	0.1 ± 0.02	32.8 ± 1.6	3.9 ± 0.1	<0.01
30/12/2020	RAS H_2O_2	2.3 ± 0.1	0.5 ± 0.01	0.9 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	57.2 ± 0.7	7.6 ± 0.1	<0.01
05/01/2020	H air	2.6 ± 0.03	0.5 ± 0.0	0.3 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	26.7 ± 0.1	2.7 ± 0.01	<0.01
05/01/2020	H H_2O_2	2.6 ± 0.01	0.5 ± 0.0	0.4 ± 0.02	0.1 ± 0.00	<0,1	26.1 ± 0.4	2.7 ± 0.1	<0.01
05/01/2020	RAS H_2O_2	2.7 ± 0.03	0.4 ± 0.00	0.5 ± 0.00	0.2 ± 0.00	0.1 ± 0.01	53.5 ± 0.2	6.4 ± 0.1	<0.01
12/01/2021	H air	2.8 ± 0.02	0.5 ± 0.01	0.3 ± 0.02	0.1 ± 0.01	0.1 ± 0.00	34.2 ± 1.0	3.3 ± 0.1	<0.01
12/01/2021	H H_2O_2	2.7 ± 0.01	0.5 ± 0.0	0.6 ± 0.04	0.1 ± 0.01	<0,1	30.7 ± 0.8	3.8 ± 0.02	<0.01
12/01/2021	RAS H_2O_2	2.8 ± 0.03	0.5 ± 0.00	0.7 ± 0.01	0.2 ± 0.00	0.1 ± 0.00	60.1 ± 0.3	7.7 ± 0.04	<0.01
19/01/2021	H air	2.9 ± 0.01	0.5 ± 0.01	0.4 ± 0.02	0.1 ± 0.00	<0,1	24.3 ± 1.0	3.3 ± 0.1	<0.01
19/01/2021	H H_2O_2	2.9 ± 0.02	0.5 ± 0.0	0.5 ± 0.01	0.1 ± 0.00	<0,1	23.3 ± 0.1	3.2 ± 0.1	<0.01
19/01/2021	RAS H_2O_2	2.9 ± 0.01	0.4 ± 0.00	0.5 ± 0.01	0.1 ± 0.01	0.1 ± 0.00	48.8 ± 1.2	7.1 ± 0.1	<0.01
25/01/2021	H air	3.2 ± 0.1	0.6 ± 0.02	0.2 ± 0.1	0.1 ± 0.00	<0,1	27.5 ± 1.1	4.1 ± 0.2	<0.01
25/01/2021	H H_2O_2	3.0 ± 0.03	0.6 ± 0.01	0.5 ± 0.01	0.1 ± 0.00	<0,1	26.1 ± 0.7	4.4 ± 0.1	<0.01
25/01/2021	RAS H_2O_2	3.0 ± 0.03	0.5 ± 0.00	0.5 ± 0.02	0.1 ± 0.00	<0,1	54.7 ± 0.3	8.7 ± 0.1	<0.01

4.2.4. Abiotic Parameters of the nutrient solution

Regarding the H air treatment, pH mean value was 6.3 ± 0.2, in the H H_2O_2 treatment 6.3 ± 0.1 and in the RAS H_2O_2 treatment 6.3 ± 0.1. The medians did not vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test, Figure 17.

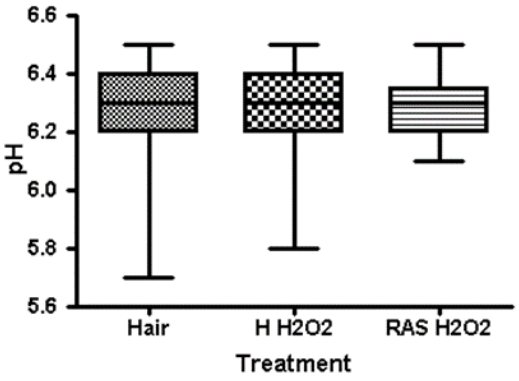

Figure 17: pH values of the nutrient solutions from the H air treatment (distilled: tap water, 50:50, v/v provided with air), H H_2O_2 treatment (distilled: tap water, 50:50, v/v provided with H_2O_2) and RAS H_2O_2 treatment (RAS arapaima wastewater provided with H_2O_2). The medians do not vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test.

The electrical conductivity measured in dS m^{-1} had mean value of 1.5 ± 0.1 in the H air treatment, 1.5 ± 0.1 in the H H_2O_2 treatment and 1.7 ± 0.1 in RAS H_2O_2. The medians did vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test, Figure 18.

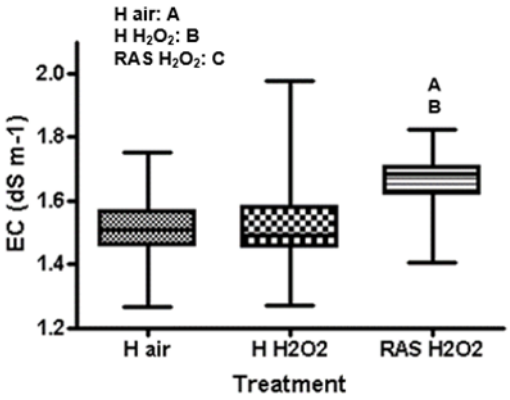

Figure 18: Electrical conductivity values in dS m^{-1} of the nutrient solutions from the H air treatment (distilled: tap water, 50:50, v/v provided with air), H H_2O_2 treatment (distilled: tap water, 50:50, v/v provided with H_2O_2) and RAS H_2O_2 treatment (RAS arapaima wastewater provided with H_2O_2). The medians do vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test. Different capital letters indicate significant differences between the three different nutrient solutions and are listed from top to bottom: H air, H H_2O_2, RAS H_2O_2.

Regarding to the temperature, the mean value of the H air treatment was 20.9 ± 0.5 °C, in the H H_2O_2 treatment 21.1 ± 0.4 °C and in the RAS H_2O_2 treatment 21.0 ± 0.4 °C. The medians did vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test, Figure 19.

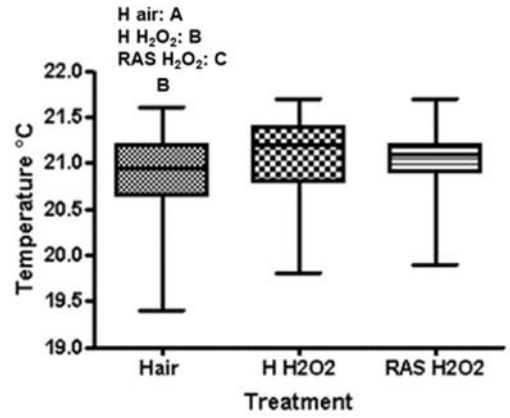

Figure 19: Temperature of the nutrient solutions from the H air treatment (distilled: tap water, 50:50, v/v provided with air), H H_2O_2 treatment (distilled: tap water, 50:50, v/v provided with H_2O_2) and RAS H_2O_2 treatment (RAS arapaima wastewater provided with H_2O_2). The medians do vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test. Different capital letters indicate significant differences between the three different nutrient solutions and are listed from top to bottom: H air, H H_2O_2, RAS H_2O_2.

The mean dissolved oxygen (DO) concentration in the H air treatment and in the RAS H_2O_2 treatment was 7.8 ± 0.1 mg L^{-1}, and in the H H_2O_2 treatment 7.7 ± 0.1 mg L^{-1}. The medians did vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test, Figure 20.

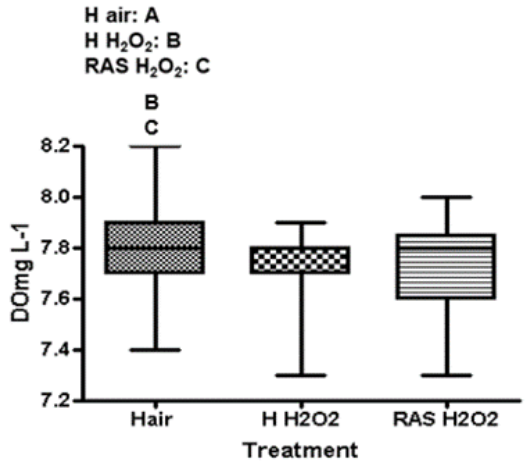

Figure 20: Dissolved oxygen concentrations in mg L^{-1} of the nutrient solutions from the H air treatment (distilled: tap water, 50:50, v/v provided with air), H H_2O_2 treatment (distilled: tap water, 50:50, v/v provided with H_2O_2) and RAS H_2O_2 treatment (RAS arapaima wastewater provided with H_2O_2). The medians do vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test. Different capital letters indicate significant differences between the three different nutrient solutions and are listed from top to bottom: H air, H H_2O_2, RAS H_2O_2.

4.2.5. Abiotic Parameters in the experimental chambers

Regarding to the H air treatment, the mean air temperature was 21.2 ± 0.7°C and the mean relative humidity was 64.9 ± 5.5 %.

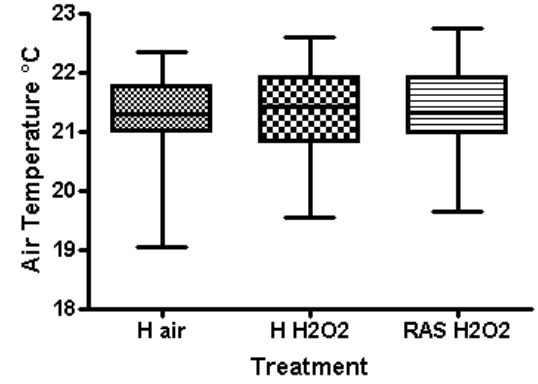

Figure 21: Air temperature values in the experimental chambers from the H air treatment (distilled: tap water, 50:50, v/v provided with air), H H_2O_2 treatment (distilled: tap water, 50:50, v/v provided with H_2O_2) and RAS H_2O_2 treatment (RAS arapaima wastewater provided with H_2O_2). The medians do not vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test.

In H H_2O_2 treatment, the mean air temperature was 21.3 ± 0.7 and the mean relative humidity was 64.7 ± 5.8 %. In RAS H_2O_2 treatment, the mean air temperature was 21.4 ± 0.7°C and the

mean relative humidity was 65.6 ± 5.2 %. Both air temperature and mean relative humidity medians did not vary significantly ($p < 0.05$) between the three different treatment groups, using the Kruskal-Wallis test and Dunn-Bonferroni test, Figure 21 and Figure 22 respectively.

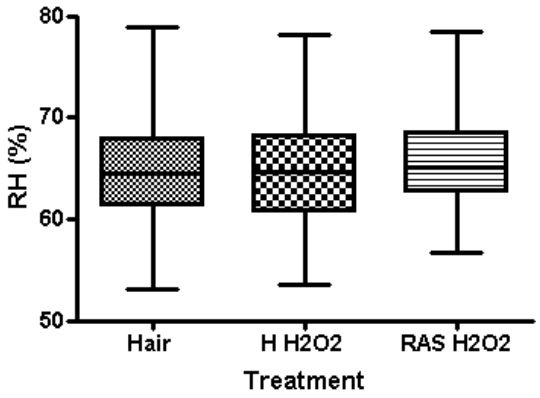

Figure 22: Relative humidity (RH %) values in the experimental chambers from the H air treatment (distilled: tap water, 50:50, v/v provided with air), H H_2O_2 treatment (distilled: tap water, 50:50, v/v provided with H_2O_2) and RAS H_2O_2 treatment (RAS arapaima wastewater provided with H_2O_2). The medians do not vary significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test.

4.2.6. Water Consumption

As seen in Table 16Table 16, in the first nutrient solution change 1 on 5th of January 2021, the actual mean water consumption per plant per day since the start of the experiment till nutrient solution change 1, was 0.063 L in the H air treatment, 0.051 L in the H H_2O_2 treatment and 0.059 L in the RAS H_2O_2 treatment. All replicates had an initial water volume of 30 L.

In the second nutrient solution change 2 on 19th of January 2021, the actual mean water consumption per plant per day since the nutrient solution change 1 till the nutrient solution change 2, was 0.063 L in the H air treatment, 0.067 L in the H H_2O_2 treatment and 0.046 L in the RAS H_2O_2 treatment.

On the day of the harvesting on 26th of January 2021, the actual mean water consumption per plant per day since the nutrient solution change 2 till harvesting, was 0.039 L in the H air treatment, 0.043 L in the H H_2O_2 treatment and 0.069 L in RAS H_2O_2 treatment.

In the H air treatment and the H H_2O_2 treatment the actual water consumption per plant per day in L, decreased. In the last week of the experiment to 0.039 L in the H air treatment and to 0.043 L in the H H_2O_2 treatment; but in the RAS H_2O_2 treatment, it increased to 0.069 L.

Table 16. Initial water volume per replicate (L); final water volume per replicate (L); actual water consumption per replicate (L); days of growth; actual water consumption per plant per day (L) from the H air treatment (distilled: tap water, 50:50, v/v provided with air), H H_2O_2 treatment (distilled: tap water, 50:50, v/v provided with H_2O_2) and RAS H_2O_2 treatment (RAS arapaima wastewater provided with H_2O_2). The data in final water volume per replicate, in the actual water consumption per replictate and in the actual water consumption per plant per day represent the mean values of the three replicates for each treatment.

	Treatment	Initial water volume per replicate (L)	Final water volume per replicate (L)	Actual water consumption per replicate (L)	Days	Actual water consumption per plant per day (L)
Nutrient Solution Change 1	H air	30	21.2 ± 1.6	8.8	14	0.063
	H H_2O_2	30	22.8 ± 0.7	7.2	14	0.051
	RAS H_2O_2	30	21.7 ± 0.6	8.3	14	0.059
Nutrient Solution Change 2	H air	30	21.2 ± 0.2	8.8	14	0.063
	H H_2O_2	30	20.6 ± 1.1	9.4	14	0.067
	RAS H_2O_2	30	23.6 ± 1.7	6.4	14	0.046
Harvesting	H air	30	27.3 ± 2.0	2.7	7	0.039
	H H_2O_2	30	27.0 ± 3.7	3.0	7	0.043
	RAS H_2O_2	30	25.2 ± 3.0	4.8	7	0.069

4.2.7. SPAD values of lettuce leaves

The average SPAD value in each plant of every replicate was measured. In Table 17Table 17, the mean SPAD value of the 30 plants of each treatment is shown. The values of the H air treatment ranged from 26.0 to 32.1, with a mean value of 28.7 ± 1.6. The values of the H H_2O_2 treatment ranged from 25.0 to 30.8, with a mean value of 28.8 ± 1.4, being the highest mean value. The values of the RAS H_2O_2 treatment ranged from 25.8 to 34.5, with a mean value of 28.4 ± 1.9, being the lowest mean value.

Table 17. Mean SPAD values in lettuce grown in the H air treatment (distilled: tap water, 50:50, v/v provided with air), H H_2O_2 treatment (distilled: tap water, 50:50, v/v provided with H_2O_2) and RAS H_2O_2 treatment (RAS arapaima wastewater provided with H_2O_2). The data represent the mean value of the 30 plants in each treatment.

Treatment	SPAD value
H air	28.7 ± 1.6
H H_2O_2	28.8 ± 1.4
RAS H_2O_2	28.4 ± 1.9

4.2.8. H_2O_2 6% solution addition to the nutrient solution and H_2O_2 consumption

The mean total volume of the solution H_2O_2 6% used per replicate in the treatment H H_2O_2 was 317 ± 20.7 mL. In the treatment RAS H_2O_2 it was a 300 ± 14.1 mL, as seen in Table 18. The

average of the actual H_2O_2 consumption per day per replicate was 0.49 ± 0.07 mL in the H H_2O_2 treatment and 0.48 ± 0.01 mL in the RAS H_2O_2 treatment. Additionally, the total used volume of the solution H_2O_2 6% for the treatment H H_2O_2, calculated by the addition of the total used volume of each replicate was 952 mL; and in treatment RAS H_2O_2 was 900 mL.

Table 18. The mean total used volume of the solution H_2O_2 6% in mL per replicate from H H_2O_2 treatment (distilled: tap water, 50:50, v/v provided with H_2O_2) and RAS H_2O_2 treatment (RAS arapaima wastewater provided with H_2O_2).

Treatment	Total volume solution H_2O_2 per replicate
H H_2O_2	317.3 ± 20.7
RAS H_2O_2	300.0 ± 14.1

5. Discussion

Due to the unsustainability of the wide use of fishmeal and fish oil as aquafeed ingredients, alternative ingredients have been developed, such as black soldier fly meal. Therefore, the aim of experiment 1 was to test the influence of an alternative fish feed based on black soldier fly meal on lettuce growth in a decoupled aquaponic systems. Experiment 2 aimed to test the potential of an alternative oxygen source, H_2O_2, to potentially develop robust, electrical independent oxygen supply systems for future aquaponic applications.

5.1. Experiment 1

5.1.1. Plant Yield and Plant Growth

The mean FW of the lettuces heads, were not significantly different between the hydroponic treatment (control) and the aquaponic treatments (BSF and FIM), as seen in Table 5. This can be a first indicator for the assumption, that alternative feed ingredients such as black soldier fly meal has no negative effects on aquaponic lettuce production. Consequently, the number of leaves per lettuce were not significantly different; and the mean DW was similar between the three treatments (Table 5). Nonethless, even if the water source was different, the nutrient concentrations of the nutrient solutions were similar in the three treatments (Table 7 and Table 8), which could lead to the assumption of similar plant growth. Also, the feeds composition of black soldier fly meal based diet for BSF treatment and fishmeal based diet for FIM treatment are very similar, with only a different protein source.

These results, showing similar growth in hydroponic and aquaponic treatments, are supported by different aquaponic studies. In Delaide et al. (2019) the total yield of tomato fruits grown in hydroponic and aquaponic treatments were not significantly different. The same was reported by Suhl et al. (2016), in which sumplemented decoupled aquaponic system and conventional

hydroponic tomato production resulted in similar yields. Monsees et al. (2019) reported the same, where the FW of lettuce produced in decoupled aquaponic systems was similar to those produced in conventional hydroponic treatments. Nevertheless, also significantly increased growth of aquaponic lettuce production has been reported. Delaide et al. (2016) described that supplementing RAS water with fertilizers increased by almost 40% the FW of lettuce, comparing to a conventional hydroponic and a non complemented aquaponic treatment. In accordance to the latter, Goddek et al. (2018) observed that using RAS based water increased lettuce growth by almost 8% when compared to hydroponic control. In Delaide et al. (2016) study, the root weight in both aquaponic and complemented aquaponic treatments was higher than in hydroponic treatment; the authors assumed that RAS wastewater must have certain substances responsible to increase root growth. They assumed two factors present in RAS water with a promoting growth effect: DOM (dissolved organic matter) and plant growth-promoting rhizobacteria and/or fungi (PGPR and/or PGPF). It is known that an accumulation of DOM components such as humic-like and protein-like occurs in RAS water (Hambly et al. 2015). Humic substances are highly complex mixtures with high molecular heterogenecity, composed of three main fractions which are: humic acid, fulvic acid and humin (Maccarthy 2001). In Adani et al. (1998), the addition of humic acids to the hydroponic solution stimulated the growth of tomato plants. In the same line, Haghighi et al. (2012) also observed benefits on lettuce growth by adding humic acid, which was explained by a stimulation of N uptake by the plants, consequently increasing chlorophyll content which leads to higher photosynthesis rate and plant growth. However, the assumption by Delaide et al. (2016) would have resulted in a higher lettuce growth in BSF and FIM treatments comparing to the Control treatment, which was not seen. It is also stated that, depending on feed intake and/ or water exchange rate, RAS will accumulate DOM differently (Leonard et al. 2002; Hambly et al. 2015).

In a study of Goddek et al. (2018), the nutrient solution from the RAS-based hydroponic system showed a significantly higher Na concentration than the nutrient solution of the hydroponic system. This finding was justified by the authors by the addition of sodium chloride (NaCl) to the RAS system to prevent stress and diseases and to restore fish osmoregulation. Same was described in Delaide et al. (2019), where the aquaponic treatment nutrient solution showed higher EC since NaCl was periodically added to the RAS water with the aim to prevent fish health problems as explained by the authors. However, sodium chloride is sometimes added into the systems to inhibit nitrite toxicity. Nitrite is an intermediate product of the nitrification process that converts ammonia to nitrate. High concentrations of nitrite can cause brown blood disease, where nitrite oxidizes hemoglobin to methemoglobin, losing its ability to bind and transport oxygen (Lewis and Morris 1986). Nitrite uptake in fish occurs in the chloride cells located in the gills (Lewis and Morris 1986). These cells also uptake other ions, such as chloride; hence nitrite and chloride have showed very similar uptake rates (Lewis and Morris

1986). Therefore, since chloride competes with nitrite for transportation across the gills, sodium chloride (NaCl) can be added to prevent nitrite toxicity. In this way, the reason for the addition of NaCl in the previous mentioned studies by Goddek et al. (2018) and Delaide et al. (2019) could be a reaction to an incomplete nitrification process which can lead to nitrite accumulation. Nevertheless, the fish feed itself, especially based in fishmeal can also contribute to the increase of NaCl in the RAS water. In RAS to have an optimal system management, it is essential to guarantee an adequate solid removal technique that minimizes the suspended solids in the water and a biofilter working effectively. If the amount of suspended solids is increased, this could lead to heterotrophic bacteria proliferation which multipliy faster than nitrifying bacterias. This will result in oxygen depletion and negatively effect the nitrifying bacteria, thus biofiltration. Carbon dioxid will also increase and the pH will drop. Additionally, ammonia can also be produced by these heterotrophic bacteria. All of this, compromises the biofilter function causing ammonia and nitrite accumulation within the system, which can be toxic to fish even at low concentrations; ammonia has a mean acute toxicity value of 2.79 mg L^{-1} for many freshwater fish species (Randall and Tsui 2002) and, nitrite when in concentrations of 50 mg L^{-1} or above can cause fish mortality (Kroupova et al. 2005). This accumulation and therefore the use of NaCl can be avoided and prevented when a correct RAS management is done, making sure all the components of the system are operating efficiently to guarantee an optimal water quality and fish health and growth.

Overall, we can assume by this study that BSF waste water do not have any potential substances that could have a positive or negative effect on lettuce growth. However, further studies should be performed to understand better the influence of this alternative feed on the fish waste water chemistry and consequently on plant growth in decoupled aquaponic systems.

5.1.2. Water Source Nutrient Analysis

The nutrient that brought more attention within the overall nutrient concentrations of the different water sources (control water, black soldier fly meal based diet wastewater and fishmeal based diet wastewater) was the sodium (Na) concentration. Fishmeal based diet wastewater, FIM treatment, showed higher Na concentrations than black soldier fly meal based diet wastewater, BSF treatment, and control water (tap:destilled water, 50:50, v:v + fertilizer), as seen in Table 6. This also resulted in higher Na concentrations in the nutrient solution of this treatment during the experiment, as seen in Table 8. It is known that the majority of crop plants such as lettuce are not able to survive with salt concentrations higher than 100-200 mM (Adhikari et al. 2019). Salinity reduces the water uptake of the plant, reducing mineral uptake and photosynthesis (Adhikari et al. 2019). However different lettuce genotypes show different responses to salinity. This leads to several different findings regarding the effect of salinity on

lettuce plant yield. Adding to the differences created by the genotype, environmental conditions (light intensity, temperature and relative humidity) and different cropping systems are also responsible for influencing plant response to salinity stress (Freitas et al. 2019). However, Fernandez (2017) describes that Na can block K uptake and becomes toxic to the lettuce when concentrations are > 100 mg L^{-1}.

In Fernandez et al. (2016) study, no effects on fresh weight were seen in lettuce yield when applying three different salinity treatments (2.5; 5; 10 dS m^{-1}) by adding NaCl to a hydroponic system. However, Andriolo et al. (2005) estimated for hydroponically grown lettuce, a threshold value of 2.0 dS m^{-1} with a linear slope of 14.9% when increasing one unit of electrical conductivity. Al Maskri et al. (2010) also showed that lettuce growth was affected by an increasing salinity of the nutrient solution; a decrease on plant growth was seen while increasing the salinity levels of the nutrient solution from 0 mM salt to 50 mM and to 100 mM, by adding NaCl.

Nonetheless, in this study, in FIM treatment, the mean Na concentration in the fishmeal based wastewater was 52 ± 2.8 mg L^{-1} and in the nutrient solution was 59.4 ± 7.9 mg L^{-1}; which is below 100 mg L^{-1}, hence not considered toxic to the lettuce plants. This was confirmed because no negative effect on lettuce growth was seen and also similar EC values between the treatments were observed. Delaide et al. (2016) study goes in line with this finding, where aquaponic treatments showed substantial higher Na concentrations, however any negative impact on lettuce growth was not reported. They also observed higher Na content in lettuce leaves of the aquaponic treatments which can indicate the ability to perform some Na uptake from the nutrient solution. In Goddek et al. (2018) study, higher sodium concentrations were also seen in RAS water and in the nutrient solution of the RAS-based hydroponic system. Yet this didn´t create any negative effect on lettuce growth, the growth was actually higher compared to the control group when using the RAS water despite the high content of Na.

However, for this study, in terms of feed design, perhaps black soldier fly meal based diet, could be more benefitial for the hydroponic unit, since the Na content in the fish wastewater is lower. As mentioned, even though Na levels were below the toxicity threshold (>100 mg L^{-1}) and no lettuce growth differences were seen between BSF and FIM treatment; it is something that in further experiments could be a problem if the nutrient solution is reused and recirculated for a longer period. Due to the fact that plants do not absorb Na so easily and do not need it necessarily (Fernandez 2017); it can eventually result in the accumulation of this ion in the hydroponic unit and become toxic to the plants.

As seen in Table 3, the ingredients compostion of BSF diet and FIM diet only vary by the use of fishmeal in FIM diet and black soldier fly meal in BSF diet. Hence, only the protein fraction is different in these diets. The same fish oil content is seen in both diets. The fishmeal Na content as described by FAO (1986) in % of DM (dry matter) varies between 0.70 and 1.30,

depending on the fishmeal type. However, the Na content of black soldier fly meal can vary between 0.13 and 0.27 %, depending on the feed source given to the insect (Barragan-Fonseca et al. 2017). Additionally, fishmeal is known to be one of the main inputs of salt to the diet (Salman 2009), being richer in salt than other protein sources like black soldier fly meal. The majority of the dietary salt that is absorbed into the blood by the fish is then excreted by the gills; this Na efflux is made by the chloride cells and Na+/K+-ATPase activity, which are abundant in the gills (Salman 2009). Hence the higher dietary salt present in the fishmeal will lead to higher excretion of Na to the water by the gills of the fish fed with this diet, as well as to leaching effects during feeding or in the case of uneaten feed. Therefore, this could explain the higher Na concentrations found in the FIM wastewater and consequently on FIM nutrient solutions.

5.1.3. Abiotic parameters of the nutrient solutions and in experimental chambers and nutrient concentrations of the nutrient solutions

The abiotic parameters of the nutrient solution in all treatment groups were all kept very similar during the experiment to avoid variations that could lead to any growth differences between treatments. Even though the medians of the EC and temperature of the nutrient solution, varied significantly ($p < 0.05$) using the Kruskal-Wallis and Dunn-Bonferroni tests, as seen in Figure 12 and Figure 13 respectively, the mean values of the abiotic parameters were very similar between the treatments. This occurs since it is not for example possible to have the exact same EC in all treatments, due to the properties of the BSF treatment and FIM treatment wastewaters and also the differences within the recipes of the nutrient solutions. For instance, as mentioned above, fishmeal based diet wastewater for FIM treatment contains more sodium than control water and black soldier fly meal based diet wastewater for BSF treatment, which causes an increase in EC. Furthermore, all of them were within the recommended and usual used ranges for lettuce hydroponic production. pH was within the reccomended range of 5.5-6.5; EC was within the range of 0.9 to 2.5 dS m^{-1} observed in lettuce growth (Genuncio et al. 2012); the temperature of the nutrient solution was below 25°C and the DO concentration was greater than 4.0 as it is recommended by Brechner and Both (2013). The air temperature and RH% found were also similar to the recommended values by Brechner and Both (2013), of having 24°C in the day and 19°C in the night, and a minimum RH% of 50% and below 70%.
Just as the abiotic parameters were kept in a very similar range for the three treatments, the same was done and obtained for the nutrient solutions macro, micronutrients, Na, Si and Al concentrations, as seen in Table 7 and Table 8. The difference seen in Na concentrations between FIM treatment and BSF treatment was already discussed above. It is technically very hard to have exactly the same initial nutrient concentrations; the nutrient solutions are prepared

with salts; hence one nutrient concentration can be a limitation to add other nutrients and reach their respective target value. It is also pratically impossible to have the same values during the experiment, for example due to the complex and varied interactions that happen between nutrients in the root zone (Monsees et al. 2019). Here, we can see that especially small differences on nutrient concentrations as seen in this study did not cause any difference on lettuce growth. Thus, the water source origin is mainly the only difference between the treatments.

5.1.4. Water consumption

In terms of water consumption and water management, the nutrient solution was changed frequently in order to prevent the accumulation of certain nutrients (e.g. sodium and chlorine) and root exudates that could have a toxic effect on plants. Since we have used in this experiment a closed hydroponic system, the nutrient solution is recycled for a certain period. It is known that toxic exudates released from the roots of the plants can accumulate within the recycled nutrient solution, leading to autotoxicity. Autotoxicity happens when a plant produces and releases toxic susbtances that inhibit the germination and growth in the same species of plants (Miller 1996). The root exudates known to be released in greater quantities are organic acids, sugars and aminoacids (Richa et al. 2020). In lettuce, the main root exudates are organic acids, such as benzoic acid, phenylacetic acid, cinnamic, p-hydroxybenzoic acid, lauric acid, phthalic acid, vanillic acid, palmitic acid, and stearic acid (Hosseinzadeh et al. 2017). A great growth reduction on lettuce was seen when produced with a reused nutrient solution containing these organic acids (Hosseinzadeh et al. 2017), so in order to prevent this, the nutrient solution was changed frequently.

The actual water consumption per replicate in L that occurred between each nutrient solution change and the harvesting in the end, only ranged between 3.3 and 5.9 L per replicate as seen in Table 9. This indicates that the sustainability of this system can be improved by recycling the nutrient solution for a longer period. However, to recycle the nutrient solution for a longer period, it is recommended to treat it, to remove root exudates that could potentially be accumulated in it. Several tecniques have been recommended to do so, such as granular activated carbon, slow sand filtration, electrical degradation and UV/H_2O_2 (Hosseinzadeh et al. 2017). Additionally, the EC should be kept at optimum levels, a nutrient solution with high EC and not changed frequently can lead to an increase of the EC to toxic levels (Hosseinzadeh et al. 2017). Another option to reduce the water waste in this system, is using a lower initial volume of nutrient solution, however management wise, we don´t recommend decreasing too much since it becomes harder to make the daily measurements and adjustments of pH and EC.

The actual water consumption per plant per day (L) in our study was between 0.025 L and 0.047 L. Barbosa et al. (2015) study estimated a water demand of 20 ± 3.8 L/kg/y for hydroponic lettuce production, 2.892 L per lettuce with a weight of 144.6 g with 30 days of growth, resulting in a water consumption per plant per day in L of approximately 0.096 L. This indicates us that the water consumption per plant per day seen in this study seems to be within the normal water consumption range. Additionally, the water consumption could also be influenced by a higher daily light integral (DLI) e.g. through the prolongation of the light period from 12 to 18 h of light per day or the use of stronger light sources. However, the water consumption per plant per day (L) was slightly higher in the control treatment as see in Table 9.

5.1.5. SPAD values of lettuce leaves

SPAD values were measured to obtain an estimate of the chlorophyll content in the plants. The average values seen in control treatment, BSF treatment and FIM treatment were respectively, 28.5 ± 1.2, 27.8 ± 1.5 and 28.1 ± 1.3, as seen in Table 10, with a light intensity of approximately 91 µmol m^{-2} s^{-1} and 12 hours of light period. These values are similar to the ones found in previous studies. In Wenke et al. (2009) they ranged from 28.3 to 35.0 with a light intensity of 84 ± 14 µmol m^{-2} s^{-1} with a light period of 13 hours; Yang and Kim (2020) reported values from 24.6 and 25.7, with a light intensity of 168 µmol m^{-2} s^{-1} and 14 hours of light period; and Cho et al. (2018) observed values of 25.0, 28.13, 30 with the respective light intensity of 100, 150 and 200 µmol m^{-2} s^{-1} with 16 hours of light period.

SPAD-502 meters measure the chlorophyll content of leaf tissues in vivo (León et al. 2007); based on the absorbance of radiation by chlorophyll, it calculates a relative SPAD value that should be correspondent to the leaf chlorophyll content (Uddling et al. 2007). Nitrogen is one of the utmost components of the chlorophyll molecule, hence N leaf content is proportional to the chlorophyll content (Bojović and Marković 2009); a loss of green color in the leaves is seen if N is in deficit (Maleki et al. 2012). In this study, the lettuce showed a healthy green color, not suggesting any N nutrient deficit. However, the SPAD value is only a prediction of the leaf chlorophyll content (Limantara et al. 2015), so an elemental analyser is needed to determine the accurate N content. The leaf samples were prepared to perform this anaylsis, however due to covid-19 pandemic and delays in the lab; this was not possible to obtain for this study.

5.1.6 Fish growth

The fish fed with the black soldier fly meal based diet (BSF) showed a lower mean final body weight, 59.9 g, comparing to the mean final body weight of the fish fed with fishmeal based diet, 70.2 g, as seen in Table 11. Also, the FCR value in the BSF treatment, 1.23, was higher than in the FIM treatment, 0.94. In this preliminary experiment, the black soldier fly meal based

diet resulted in lower fish growth and performance. However, many studies have shown opposite results, Muin et al. (2017) observed that replacing 50% of the fishmeal with black soldier fly meal did not cause any negative effects on fish growth and feed utilization parameters such as FCR. Nevertheless, Muin et al. 2017 also mentioned, that while increasing the fishmeal replacement, the fish weight gain also decreases and the FCR increases; considering then 50% inclusion of BSF as an optimum level. But in disagreement with the previous study, Agbohessou et al. 2021 showed that the total replacement of fishmeal by black soldier fly meal did not cause any substancial effects on growth rate in Nile tilapia. Tippayadara et al. 2021 results also showed that the growth indexes and feed utilization efficiency indices were not significantly different between the fish fed with fishmeal and with black soldier fly meal, with no adverse effects seen with the total replacement (100%) of the fishmeal with blacksoldier fly meal. High chitin levels of the black soldier fly meal is known to be one of the main causes of the adverse effects seen when the replacement of fishmeal is above 50% (Priyadarshana et al. 2021), because of its lower digestibility; hence in this study, the lower fish growth of the BSF treatment could possibily be due to this. Adding to this, this feed experiment was a preliminary experiment not yet optimized, hence further experiments to study the use of this very promising alternative ingredient in aquafeeds, black soldier fly meal, should be and are being performed.

5.2. Experiment 2

Likewise the experiment 1, the mean fresh weight (FW) of the lettuce heads is one of the most important results. The aim of this experiment 2, was to perform a first approach on using H_2O_2 solution (6% hydrogen peroxide stabilized, Söchting Oxydator, Germany) in an oxidator (Söchting Oxydator, Germany) to passively provide O_2 to the nutrient solution instead of the conventional aerators e.g. air pump that require electricity; and to answer the question if this application consequently effects lettuce growth. However, this study was just a primal approach and with further potential for improvement of the experimental setup, since the pump present in each chamber creates some aeration in the nutrient solution of the treatments without air (H H_2O_2 and RAS H_2O_2).

H H_2O_2 and RAS H_2O_2 treatments showed not significantly different mean fresh weight (FW) of the lettuce heads compared to the conventional hydroponic treatment H air, as seen in Table 12. Consequently, aquaponic treatment, RAS H_2O_2 had similar plant growth to the hydroponic treatments, H air and H H_2O_2. In this way, the same was observed for the mean number of leaves, they were also not significantly different. And the mean dry weight (DW) was similar between the three different treatments.

The nutrient solution of the hydroponic treatments, H air and H H_2O_2, was the same, hence the only difference was the method of oxygen supply. It could be observed that using the oxydator with H_2O_2 to provide oxygen to the nutrient solution showed to be as effective as the conventional aeration method. The oxydator breaks down the H_2O_2 into a molecule of water and oxygen (O·- radical), this oxygen released is pure and activated. The activated oxygen increases the redox potential of the water and makes H_2O_2 an oxidizing agent, with a microbicidal activity (McDonnell 2014). Therefore, H_2O_2 in the range of 3-6% can provide oxygen to the nutrient solution and act as a disinfectant at the same time (McDonnell 2014). In Bögner et al. (2021) study, applying H_2O_2 in the RAS was effective to guarantee the oxygenation of the system and decrease the microbial load, using low concentrations of H_2O_2 (from 2.4 mg L^{-1} h^{-1} to 15.8 mg L^{-1} h^{-1}). However, to our knowledge, no study was previously done using H_2O_2 to provide oxygen to the nutrient solution in hydroponic production systems. Concerns that can possibly arise from this usage are: potential phytotoxic effect on the plants; and, or the potential oxidation of some nutrients present in the nutrient solution. Eicher-Sodo et al. (2019) applied water with different H_2O_2 concentrations (ranging from 0 to 200 mg L^{-1}) daily by foliar spraying in three lettuces cultivars; concentrations equal or above 25 mg L^{-1} increased the percentage of damage leaves, and the recommended maximum concentrations of H_2O_2 in the irrigation water were 75 mg L^{-1} or 125 mg L^{-1}, depending on the lettuce cultivar. In our study we used an oxydator with passive H_2O_2-supply, hence passively releasing oxygen by concentration gradient. Safe levels of DO concentration were always kept during the experiment; the H air and RAS H_2O_2 treatments had a mean DO concentration of 7.8 ± 0.1 mg L^{-1} and the H H_2O_2 treatment of 7.7 ± 0.1 mg L^{-1}. Thus, no adverse effects on lettuce growth or leaves health were seen and also not expected due to this application method using the oxydator. However, it is known that excessive H_2O_2 concentrations can cause deleterious effect in plants, such as oxidative stress (Khan et al. 2018). Anyhow, hydrogen peroxide is extremely important for plant health when in low concentrations in the plant; it takes part in several growth processes, working as a signalling molecule. It is also responsible and important to attenuate several biotic and abiotic stresses that plants may face (Khan et al. 2018). Hence, applying low exogenous doses of H_2O_2 has been proved to improve plant growth and aid plant´s tolerance to various stresses (Khan et al. 2018). Nonetheless, no effect on lettuce growth was seen when using H_2O_2 in the nutrient solution in this study; further studies have to be performed to gain more knowledge about the effect of H_2O_2 on lettuce when used for the oxygenation of the nutrient solution in hydroponic units. The other rising concern regarding to using H_2O_2, was the potential oxidation of some nutrients present in the nutrient solution and consequently becoming less or not available for plant´s uptake; here, the lettuces of H H_2O_2 and RAS H_2O_2 treatments showed no significant differences in growth compared to the control group (H air treatment), hence no obvious negative effects were seen. Therefore,

more experiments have to be performed to gain more knowledge about the effect of H_2O_2 in the nutrient availability of the nutrient solutions.

In terms of the nutrient concentrations of the nutrient solutions, reproducable higher Na concentrations were found in RAS H_2O_2 treatment; this event was already explained in the discussion of the experiment 1, where it´s known that fish waste water has higher Na content due to the fishmeal based diet. Further findings were the higher sulfur concentration, as well in the nutrient solution of the RAS H_2O_2 treatment. This can be explained by higher S concentrations in RAS fish wastewater than in hydroponic water (distilled: tap water, 50:50, v:v). The tap water has similar S concentrations to the RAS wastewater as seen in Table 13. However to prepare the hydroponic treatments (H air and H H_2O_2) nutrient solution, the tap water is diluted (50:50, v:v) with distilled water, hence the S concentration is going to be much lower. To prepare the stock solutions, salts are used, hence sulphur is added when we use the salts: zinc sulphate (dehydrate), manganese sulfate (monohydrate) and copper sulfate (pentahydrate). Since the RAS waste water almost does not have any zinc, manganese and copper, these salts have to be added to reach the target concentrations for these nutrients. However, while doing it, more S is added to the stock solutions along with the nutrients in lack. And the RAS waste water already has a higher S concentration than the target value, this will result in higher S concentrations in the nutrient solution. In hydroponic treatments, the same does not occur, since the S concentration of the water (distilled: tap water, 50:50, v: v) is much lower than the target value, resulting in a sulfur concentration in the nutrient solution slightly lower than this value. More S could not be added in this case, because then zinc, manganese and copper concentrations could exceed the target values. Hence, we can understand why is difficult to reach exactly the target values when producing the stock solutions and consequently in the nutrient solutions. One nutrient concentration can influence others since they are added together as salts. The nitrate (NO_3-N) concentration in nutrient solutions of the hydroponic treatments (H air and H H_2O_2) was always higher than in RAS H_2O_2, as seen in Table 14. To prepare the stock solutions, nitrate is added using the salts: potassium nitrate, yara calcium nitrate and magnesium nitrate. The RAS fish wastewater already has nitrate present in it, while the hydroponic water (distilled: tap water, 50:50, v:v) almost does not have any nitrate. Additionally, the RAS fish wastewater also has higher Ca concentrations close to the target value and higher K and Mg concentrations than hydroponic water. In this way, the addition of nitrate to the RAS fish wastewater is limited, mainly to not allow Ca concentrations to overshoot even more the target value. This also explains why the Ca concentration in RAS H_2O_2 nutrient solution was higher than in H air and H H_2O_2 treatments. In hydroponic treatments (H air and H H_2O_2), this limitation it is not present, since the hydroponic water has lower Ca, K and Mg concentrations; which allows to add more nitrate to the stock solutions to reach the target values.

However, like in Experiment 1, the rest of the macro and micronutrients were kept very similar between the three different treatments as seen in Table 14 and Table 15; and the small differences observed in the nutrient concentrations of the nutrient solutions did not cause any difference on lettuce growth between treatments.

Concerning to the abiotic parameters of the nutrient solution and in the experimental chambers, they were all kept very similar between the different treatments, to not potentially influence the lettuce growth. Even though the medians of the EC, temperature and DO of the nutrient solutions varied significantly ($p < 0.05$) using the Kruskal-Wallis test and Dunn-Bonferroni test, as seen in Figure 18, Figure 19, Figure 20, respectively; the mean values were very similar within the treatments. In addition, all the parameters were also within recommended and usual ranges for hydroponic lettuce production, as mentioned in the discussion of experiment 1.

Regarding to the water consumption, the actual water consumption per plant per day (L) in this experiment showed higher values ranging from 0.039 to 0.069 L, compared to experiment 1; however, the consumption was still lower than the approximate value of 0.096 L found in Barbosa et al. (2015) study. The higher water consumption could be due to the fact that lettuces were bigger, with higher fresh weights as seen in Table 12, than in experiment 1. Then, they will consequently have a more developed and extent root system, increasing the water absorption; water absorption occurs in the roots of the plants by a passive or active mechanism (Kramer 1945). The roots uptake of water is also influenced by the temperature and relative humidity; for example, lower temperature decreases water uptake. However, this might not be the cause of the higher water consumption since the temperature of the nutrient solutions and the air temperature was very similar between this experiment 2 and experiment 1.

Even though less than 30% nutrients were used for this experiment, the plants grew more than in experiment 1, reaching higher fresh weights. This can be explained by the fact that they were provided with a higher light intensity. Using a quantum meter device, the PAR (photosynthetically active radiation) value is expressed in PPFD given in µmol m^{-2} s^{-1}. PAR light is the wavelengths of light ranging from 400 to 700 nanometers (nm), being the only energy source used by plants to perform phytosynthesis (Ge et al. 2011). PPFD characterizes the number of photons in the 400 to 700 nm wavelength, that fall per unit time per unit surface in µmol m^{-2} s^{-1}. The values reported were between 120 and 130 µmol m^{-2} s^{-1}. Providing the plants with a higher PPFD value, results in more phytosynthesis and consequently plant growth. This was observed in several studies. In Kang et al. (2013) study, the higher growth in lettuce plants was observed when they were provided with the higher PPFD of 290 µmol m^{-2} s^{-1}, with a short photoperiod of 6 h/ 2h (light/dark). Zhou et al. (2019) reported highest fresh weight of lettuce plants produced under medium temperatures (23 °C / 18 °C), when the light

intensity was 500 µmol m^{-2} s^{-1}. Fu et al. (2012) recommended a range of light intensity between 400 and 600 µmol m^{-2} s^{-1} to produce lettuce, the highest fresh weight was observed when using 600 µmol m^{-2} s^{-1}. Xin et al. (2018) mentions that using a PPFD of 150 µmol m^{-2} s^{-1} causes lower yield; this is because a higher light intensity (under the light saturation point) has a growth-promoting effect on lettuce. Plants need light energy to perform photosynthesis, where light energy is transformed into chemical energy. ATP (adenosine triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate) are produced in this process, CO_2 is fixed to produce carbohydrates and O_2 is released (Zhou et al. 2019). Hence, more light will result in more photosynthesis, and consequently more plant growth. Adding to this, the light period is also very important for plant growth, with a longer light period is possible to increase lettuce growth; Xin et al. (2018) mentions that increasing the light period from 16 hours to 20 hours, increases lettuce growth by 20%. Hence, to reach higher fresh weights in this experiment, we could have increased the light period, however we were not able to do it, in order to not increase too much the air temperature of the chamber room. The same can be done to the light intensity, we used LED lamps with 165 watts with 40% intensity, however we could have used lamps with higher power and intensity to increase lettuce growth, but which would also have increased temperature and humidity. Therefore, was also a decision in the experimental design to choose lower intensites, but establishing similar conditions in all chambers without negative effecting temperature and humidity.

The SPAD values of lettuce leaves observed here, showed mean values of 28.7 ± 1.6 in H air treatment, 28.8 ± 1.4 in H H_2O_2 treatment, and 28.4 ± 1.9 in RAS H_2O_2 treatment, as seen in Table 17. These values just as described for the experiment 1, are similar to the values found in Wenke et al. (2009) study, where they ranged from 28.3 to 35.0 with light intensity of 84 ± 14 µmol m^{-2} s^{-1} in a light period of 13 hours; and Cho et al. (2018) reported similar values of 25.0, 28.1, and 30.0, with the respective light intensity of 100, 150 and 200 µmol m^{-2} s^{-1} with 16 hours of light period.

The total volume of H_2O_2 6% solution used for the treatment H H_2O_2 was 952 mL and 900 mL for RAS H_2O_2 treatment. The price of the Söchting Oxydator solution 12% of 5 L is 32.4 € with tax included. However, since we diluted the solution to 6% H_2O_2 with distilled water, the price is the half, hence 16.2 €. In this way, the cost of the total H_2O_2 6% solution used during the whole experiment, for the H H_2O_2 treatment was 3.1 € and 2.9 € for RAS H_2O_2 treatment. The price of the used distilled water also has to be added to this. The price of 5L of distilled water in Dm is 1.45 €. Then for the H H_2O_2 treatment the cost was 0.14 € and 0.13 € for RAS H_2O_2 treatment. Therefore, the total cost for the H H_2O_2 treatment was 3.24 € and 3.03 € for RAS H_2O_2 treatment, which per replicate is 1.08 € for H H_2O_2 and 1.01 € for RAS H_2O_2. In order to compare the cost of using H_2O_2 to provide oxygen instead of air, we used the example of an EHEIM air100 pump with a pump output of 100 L/hour with a consumption of 3.5 watts per

hour. Since the experiment lasted 36 days, this results in 3024 watts per replicate or 3.02 kilowatts (kW). In Germany, the price of electricity in 2020, was 0.3 € per kilowatt per hour. Which is 0.9 € per replicate, thus 2.7 € for one treatment group, for the 36 days of the experimental period. Hence, we can see that adding H_2O_2 to the nutrient solution is slightly more expensive than air. However, in small-scale hydroponic or aquaponic systems using H_2O_2 can be interesting to remain independent of electrical supply; the same applies if an energy shutdown occurs. This enables the oxygen levels to remain optimum within the system and consequently maintain the production yield. The same can be applied in rural areas with an unstable electrical grid and or more expensive.

6. Conclusion

6.1. Experiment 1

In the experiment 1, we were able to show that the use of black soldier fly meal as an alternative protein source for aquafeed does not negatively influence the growth of lettuce in decoupled aquaponic systems. Fishmeal based diet wastewater showed higher Na concentrations and thereby higher Na concentrations regarding to the nutrient solution of the FIM treatment were also seen. This implies that this problem with regards to reaching too high sodium concentration in the nutrient solution when using a fishmeal based diet could be effectively addressed by using the alternative protein source of black solider fly meal in fish feed. Nevertheless, more research is required in order to better understand the influence of the black soldier fly meal based diet on the nutrient concentrations and water chemistry of the fish wastewater and then its consequence on plant growth in hydroponic units.

6.2. Experiment 2

The experiment 2 was a first approach of using H_2O_2 as an alternative to the conventional electrical dependent oxygen supply systems for the nutrient solutions in hydroponic and aquaponic systems. This first pilot study was very promising, using this alternative source of O_2, H_2O_2, resulted similar lettuce growth as when using conventional aerators. Therefore, negative effects regarding plant growth and nutrient solution properties are not present when using H_2O_2 as an alternative method of oxygenation. However, further studies should be performed to improve the experimental setup and optimize H_2O_2 usage.

7. References

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