
A study on the mineral elements available in aquaponics, their impact on lettuce productivity and the potential improvement of their availability.

Etude des éléments minéraux disponibles en aquaponie, de leur impact sur la productivité des laitues et de la potentielle amélioration de leur disponibilité.

Boris DELAIDE

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Dissertation originale présentée en vue de l'obtention du grade de docteur en sciences agronomiques et ingénierie biologique

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Summary

Delaide Boris (2017): A study on the mineral elements available in aquaponics, their impact on lettuce productivity and the potential improvement of their availability (thèse de doctorat). Université de Liège, Gembloux Agro-Bio Tech, Belgique, 100 pages, 15 tables, 15 figures.

Aquaponics is an integrated farming concept that combines fish and hydroponic plant production in a recirculating water system. This innovative technique has the potential to reduce the impact of fish and plant production on the environment by namely closing the nutrient loop. Indeed, the nutrients leaving the fish part are used to grow hydroponic plants.

This thesis focused on the mineral elements available in aquaponics to grow plants. The thesis started by deepening the aquaponic concept. It was identified that the mineral elements available for plants growth in solution were lower concentrated than in hydroponics. It was assumed that an important parts of the nutrients input were unavailable and lost out of the aquaponic system via sludge spillage. This led to the necessity to determine the consistency of the plant growth and the proportion of mineral elements that were recycled in aquaponic systems. A solution to improve the recycling of these elements and increase their availability was also studied. Therefore, the performances of a one loop aquaponic system named the plant and fish farming box (PAFF Box), in terms of yields of fish and plant, energy and water consumption, and mineral elements mass balances were studied. The mineral nutritive elements were also characterised. For experimentation convenience, lettuce was taken as a model plant. To determine if aquaponics can assure consistent plant growth compared to conventional systems, lettuce growth has been compared between a one loop aquaponic solution, a hydroponic solution and a complemented aquaponic solution in deep water systems in controlled conditions. The latest allowed studying also the growth when nutrient concentrations are increased in the aquaponic solution. The potential of improvement of nutrient recycling for increasing their availability to plant by sludge digestion onsite was studied. Therefore, the mineralisation performance of sludge has been explored in simple aerobic and anaerobic reactors and in up-flow anaerobic sludge blanket reactors (UASB).

In the term of this work, it appeared that aquaponics consumed and discharged less water to produce fish and plant but required more energy than conventional farming systems. The lettuce showed similar growth performance between aquaponic and hydroponic solution but significantly higher growth (i.e. 39% fresh mass increase) in complemented aquaponic solution. This indicated that lower mineral elements concentrations did not impact negatively plant growth and that an increase of concentrations improved growth compared to conventional hydroponics. Also the microorganisms and dissolved organic matter may play an important role for promoting plant roots and shoots growth in aquaponics. Mineral elements mass balances analysis showed that an important part of the elements were accumulating in sludge and lost by water and sludge spillage. However, the sludge digestion onsite showed promising results to recover these elements in available form for plants. It would allow reducing environmental footprints by limiting the nutrients loss and recycle even more water. Regarding these results an improvement of the one loop aquaponic system was suggested as a hybrid decoupled aquaponic system that would limit water and nutrients discharge and improve plant growth.

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Author's Note

When my thesis started only a few papers had been published on the aquaponic subject. They were mostly research done at the University of the Virgin Islands (UVI) by Prof James E. Rakocy. Most of the other papers at that time were based on imitations of his aquaponic system and corroborated his results (J. E. Rakocy personal communication). We could however feel on the scientific side a growing interest and the beginning of a new research area where everything was possible and everything had to be done. Indeed, European commission started to fund a COST network dedicated to aquaponics. We took part into it and this allowed us to greatly improve our research by cooperation and sharing ideas. Dr Simon Goddek whom I published most of my papers was a member of this EU COST (Action FA1305). This fascination for aquaponics from the scientific community but also from the general public in the noble aim of better feeding people while preserving the environment, gave sense to our work and extremely motivated us. Throughout my thesis, I have seen the number of papers published about aquaponics increasing months after months validating aquaponics as a new growing research field. In parallel, the general public interest increased the same and many aquaponic farms, associations, projects and business emerged.

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List of abbreviations

AE	Aerobic
AER	Aerobic reactor
AN	Anaerobic
ANOVA	Analysis of variance
ANR	Anaerobic reactor
AP	Aquaponics
CAP	Complemented aquaponics
COD	Chemical oxygen demand
CHP	Combined heat and power
DAPS	Decoupled aquaponic system
DO	Dissolved oxygen
DOM	Dissolved organic matter
DRAPS	Double recirculation aquaponic systems
DWC	Deep water culture
EAF	Ebb and flow
EC	Electro-conductivity
EGSB	Expended granular sludge bed reactor
FCR	Feed conversion ratio
HP	Hydroponics
HRT	Hydraulic retention time
IAA	Indole-3-acetic acid
ICP-OES	Inductively coupled plasma optical emission spectrometry
NFT	Nutrient film technique
NSPs	Non starch polysaccharides
ORP	Oxidation reduction potential
RAS	Recirculating aquaculture system
RH	Relative humidity
SD	Standard deviation
SRT	Sludge retention time
T°	Temperature
TAN	Total ammoniacal nitrogen
TSS	Total suspended solids
TS	Total solids
UA	Urban agriculture
UASB	Up-flow anaerobic sludge blanket reactor
VFA	Volatile fatty acid
WUR	Wageningen University and Research

1. General introduction

Human world population has grown continuously through history and in 2015 reached 7.3 billion [1]. The United Nations association is clear “global population is virtually certain to rise in the short-to-medium term future”. With approximately 83 million more people annually, world population is projected to reach 8.5 billion by 2030 and increase further to 9.7 billion by 2050 [1]. More than two third of this population will reside in cities [1]. Rural populations are currently literally rushing to urban areas. Thirty-five years ago, more than 60 percent lived in rural areas and nowadays slightly more than the half of the global population is urban [2].

Humanity, during its development, has modified the Earth environment like no other species before. According to marked shifts in Earth’s state geologists suggest that Earth may have entered a new human-dominated geological epoch, the Anthropocene[3].

Humanity now faces the double challenge: preserving its environment to avoid the increase in unliveable space, whilst also sustainably feeding itself, especially its soilless growing urban population.

Indeed, it has been seriously estimated that several important planetary boundaries (i.e. by order of importance biosphere integrity, N and P cycle, land system change, Fig 1.1) are currently overtaken and could lead to unpredictable consequences, such as the collapse of the ecosystem balance [4]. The eutrophication of surface water and decline of fisheries stock around the planet are particularly acute examples [5].

These findings urge the development of food production solutions addressing this double challenge of feeding growing urban population while reducing its environmental impact.

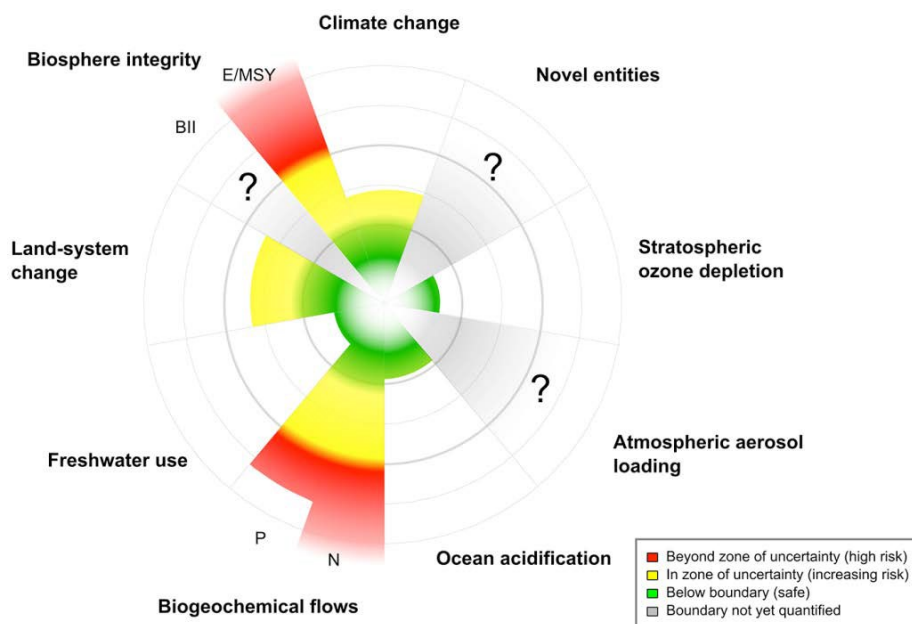


Fig 1.1. The current status of the control variables for seven of the nine planetary boundaries [4]. One can see that P and N biochemical flows are in red and so, one of the most urgent topics to address.

Urban agriculture (UA) which is generally defined as the practice of growing crops and grazing livestock in urban, suburban and peri-urban areas [6], has become a popular topic to overcome the accessibility of food in metropolitan areas by producing food in places where population density is highest, and at the same time reducing transportation costs. UA aims to use urban areas efficiently by directly connecting people to food systems [7]. UA is often proposed as an environmentally friendly agricultural production method [8] and is expected to develop more and more in the future, worldwide [9]. However, UA practices need to develop and adopt more eco-intensive food production methods i.e., methods producing high yields of food on small areas with low environmental impact.

Some of the most advanced eco-intensive agricultural practices that UA should adopt are recirculating aquaculture systems (RAS) and hydroponics (HP). RAS produce fish and other aquatic animals off ground on small areas [10]. HP consistently produces the highest yield of vegetables [11,12]. Both technology consume and discharge less water into the environment compared to conventional farming methods. Life cycle assessments (LCA) of such systems show that their main impact on the environment is due to their feeding input (i.e. fish feed for RAS and fertilizer for HP) and the energy consumption to operate these systems [13–15].

It is possible to innovate for reducing the environmental footprint of the feeding input and the energy consumption of such systems. Energy use linked to fossil energy source depletion and global warming is an issue that could be addressed among others by developing technology producing renewable energy [16]. But this thesis is not devoted to the renewable energy thematic.

A way to reduce the respective feeding input impacts of RAS and HP on the environment can be the coupling of both systems. This combination of RAS and HP is called aquaponics (AP) [17]. Indeed, the fish feed input in RAS, after being processed by fish and microorganisms, is then used as fertilizer in hydroponics. Nutrient contained in fish feed will be distributed to the fish and to the plants. The feed impact on the environment of fish feed is thus reduced because its use is improved. The fertilizer impact on the environment for hydroponics is removed or consistently diminished because it is not needed anymore (i.e. only RAS nutrient are used) or consistently reduced.

LCA of RAS showed that fish production is the most contributing factor to the eutrophication potential [13,14]. This comes down to the fact that fishes release up to two thirds of the nutrients they ingest as feed [18,19]. In conventional and recirculating aquaculture these nutrients are released in the environment, impacting P and N biochemical flows (Fig 1.1), and thus leading to eutrophication of surface water. In AP, however, the nutrient rich water discharged from RAS is used as fertilizer in hydroponics in order to strongly reduce its release into the environment. Aquaponics is then a very promising innovative technique for producing aquatic animals and plants (e.g. vegetables, fruits, flowers, etc.).

As N and P biochemical flows are highly perturbed and safe planetary boundaries have been breached, one of the big stakes for aquaponics is to prove its ability to drastically reduce the quantity of nutrients released from fish and plant production into the environment. Ensuring the efficient use of nutrients within aquaponic systems is then a primary concern. Therefore, this thesis focuses on the nutrients available in aquaponics and more precisely on the following mineral elements necessary to grow plants: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), iron (Fe), bore (B), copper (Cu), Zinc (Zn), manganese (Mn) and molybdenum (Mo).

2. Objectives of the thesis

The scientific scope of this thesis concerns the mineral elements available in aquaponics to grow plants. A number of more specific questions related to this topic were investigated. Regarding the input of mineral elements via fish feed, to what extent is aquaponics really closing the loop and how to improve the recycling of these elements? In such systems, are enough soluble mineral elements released by fish to assure healthy, consistent plant growth? If the amount of soluble mineral elements is increased what is the impact on plant productivity?

To address these questions different objectives were defined here below. Each chapter of the thesis focuses mainly on one of the objectives.

The first objective was to deepen the aquaponic concept. Therefore, the concept was properly defined and the state of the art was established. The challenges to make aquaponics a breakthrough technology sustainable and economically viable were exposed. Especially, a focus was made on the challenges concerning the mineral element available to grow plant in aquaponics. All this was addressed and published in a review which constitutes the chapter 3 of this thesis.

The second objective of the thesis was to determine the impact of aquaponics on the environment in relation with the performances of the system. Therefore, the performances of a one loop aquaponic system, in terms of yields of fish and plant, energy and water consumption, and nutrient mass balances were studied. The mineral nutritive elements were also characterised. The results have been published as a manuscript which is the chapter 4 of this thesis.

The third objective was to determine if aquaponics can assure consistent plant growth compared to conventional systems. Therefore, lettuce growth has been compared in controlled condition, between a one loop aquaponic solution, a hydroponic solution and a complemented aquaponic solution. The latest allowed studying also the growth when nutrient concentrations are increased in the aquaponic solution. For experimentation convenience, lettuce was taken as a model plant. The results have been published and are presented in the chapter 5 of this thesis.

The fourth and last objective of the thesis was to analyse the potential for the improvement of nutrient recycling by sludge digestion onsite in aquaponic systems. Therefore, the mineralisation performance of sludge has been explored in aerobic and anaerobic reactors to assess the potential for the reintroduce of plant-assimilable nutrient forms back into the aquaponic solution. Exploratory results are presented in chapter 6 under the form of a short submitted communication. Because up-flow anaerobic sludge blanket (UASB) has been identified in literature as the most promising biodigestion technique, its performances for aquaponic sludge digestion in terms of total solids (TS), chemical oxygen demand (COD), fat, and fibres reduction, plus the macro and microelements mineralisation ability have been evaluated in a set of UASB and expanded granular sludge blanket (EGSB) reactors. The results are presented in chapter 7 under the form of a manuscript.

3. Challenges of sustainable and commercial aquaponics.

This chapter has been published as a manuscript entitled “. Challenges of sustainable and commercial aquaponics.” By Delaide, B.; Goddek, S.; Mankasingh, U.; Ragnarsdottir, K. V.; Jijakli, H.; Thorarinsdottir, R, in *Sustainability (MDPI)*, 2015, 7, 4199–4224.

3.1. Introduction

Aquaponics is an integrated multi-trophic system that combines elements of recirculating aquaculture and hydroponics [20], wherein the water from the fish tanks that is enriched in nutrients is used for plant growth. It is a soil-free down-sized natural process that can be found in lakes, ponds and rivers. Using fish waste as fertilizer for crops is an ancient practice. The most well-known examples are the “stationary islands” set up in shallow lakes in central America (e.g., Aztec’s *Chinampas 1150–1350 BC*) [21], and the introduction of fish into paddy rice fields in South-East Asia about 1500 years ago [22]. In the late 70s and early 80s, researchers at the New Alchemy Institute North Carolina State University (USA) developed the basis of modern aquaponics [23]. The probably most known example was set up at the University of the Virgin Islands (UVI) in 1980 [20]. A survey, conducted by Love *et al.* [23], shows that aquaponics has been receiving growing interest since then [24], which underpins its increasing significance for society as an innovative response for food security.

Its role for food security would be particularly relevant because the global population now exceeds 7.2 billion and is growing rapidly. It is expected to reach 9.6 billion around 2050 with more than 75% living in urban areas [25]. Urban population growth will require an increasing demand for animal protein [26]. However, the future of conventional farming, including intensive animal protein production, in meeting this demand is challenged by rising but fluctuating energy and oil costs, climate change and pollution. Resource limitations including the decrease of arable surfaces, constrained freshwater supplies, soil degradation and soil nutrient depletion also add to these challenges [27,28]. This alerts researchers to the necessity to compensate existing sustainability deficits in agricultural food systems.

The interlinking of aquacultural and hydroponic procedures allows some of the shortcomings of the respective systems to be addressed, and this represents a promising sustainable food production method. Aquaponics can be considered a sustainable agricultural production system regarding the definition of Lehman *et al.* [29], who define sustainable agriculture as a process that does not deplete any non-renewable resources that are essential to agriculture in order to sustain the agricultural practices. Francis *et al.* [30] add that sustainable agricultural production can be achieved by resembling natural ecosystems and “designing systems that close nutrient cycles”, which is one of the main characteristics of aquaponics.

Mineral transfers from aquaculture to hydroponics support efficient nutrient recycling, while water recirculation reduces the water use [21]. High yield hydroponic systems require a considerable amount of macro- and micronutrients from industrial and mining origin, leading to high energy (*i.e.*, for production and transport) and finite resources use (e.g., phosphorus and oil) [11,31,32]. Also, in no-recirculating systems, intermittent disposal of the considerable amounts of nutrient rich water leads to high water consumption as well as surface and groundwater pollution [33]. The regular exchange of water performed in conventional aquacultural systems is not necessary in aquaponics. In

this respect, 1 kg of beef meat requires between 5000 and 20,000 L of water [34] and the same amount of fish bred in semi-intensive and extensive conventional aquaculture systems requires a range of 2500–375,000 L [35]. Recirculating aquaculture systems, on the other hand, have a high degree of water reuse (*i.e.*, 95%–99%) [36], with water usage down to below 100 L kg⁻¹ of fish produced [10]. In aquaponics, nitrate in excess is used for valuable plant production instead of being removed in gaseous form in denitrification units [37].

Although preliminary research has shown that developed aquaponic system components are not yet fully realized in view of either cost effectiveness or technical capabilities [38,39], the aquaponics concept is promising to contribute to both global and urban sustainable food production and should at the same time diminish pollution and need for resources. In order to meet the goal of establishing large-scale eco-efficient and economically viable aquaponic farming projects, this paper reviews the technical and socio-ecological developments that have been undertaken to date and demonstrates which aspects still need to be addressed. The purpose of this paper is to highlight current aquaponics challenges and give directions for further research. For each challenge, various approaches are described.

3.2. Principles of Aquaponics

Aquaponics combines hydroponics and recirculating aquaculture elements. Conventional hydroponics requires mineral fertilizers in order to supply the plants with necessary nutrients but the aquaponics systems use the available fish water that is rich in fish waste as nutrients for plant growth. Another advantage of this combination lies in the fact that excess of nutrients does not need to be removed through periodical exchange of enriched fish water with fresh water as practiced in aquaculture systems. The system results in a symbiosis between fish, microorganisms and plants, and encourages sustainable use of water and nutrients, including their recycling (Figure 3.1). Within this synergistic interaction, the respective ecological weaknesses of aquaculture and hydroponics are converted into strengths. This combination substantially minimizes the need for input of nutrients and output of waste, unlike when run as separate systems.

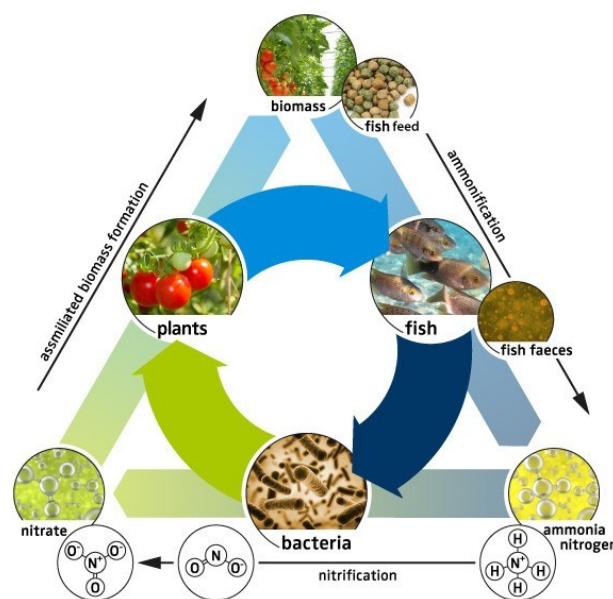


Figure 3.1. Symbiotic aquaponic cycle.

Plants need macronutrients (e.g., C, H, O, N, P, K, Ca, S and Mg) and micronutrients (e.g., Fe, Mn, B, Zn, Cu and Mo), which are essential for their growth. Hydroponic solutions contain well-defined proportions of these elements [12] and are added to the hydroponic solution in ionic form with the exception of C, H, and O, which are available from air and water. In aquaponics systems, plant nutrient input from the fish tanks contains dissolved nutrient rich fish waste (gill excretion, urine and faeces), comprising of both soluble and solid organic compounds that are solubilized to ionic form in the water and assimilated by the plants. To sustain adequate plant growth the concentrations of micro- and macronutrients need to be monitored. Periodically some nutrients may need to be added to adjust their concentration, for example iron is often deficient in fish waste [40,41].

Aquaponic systems need to be able to host different microorganism communities that are involved in fish waste processing and solubilisation. Ammonia (NH_3) from fish urine and gill excretion can build up to toxic levels if not removed from the system. This can be done by step-wise microbial conversion to nitrate. One of the most important microbial components is the nitrifying autotrophic bacteria consortium that is established as a biofilm on solid surfaces within the system and is principally composed of nitroso-bacteria (e.g., *Nitrosomonas* sp.) and nitro-bacteria (e.g., *Nitrospira* sp., *Nitrobacter* sp.). The ammonia within the system is converted into nitrite (NO_2^-) by nitroso-bacteria, before being transformed into nitrate (NO_3^-) by the nitro-bacteria [42]. The final product of this bacterial conversion, nitrate, is considerably less toxic for fish and due to its bioconversion, is the main nitrogen source for plant growth in aquaponics systems [43–45]. In most systems, a special biofiltration unit where intensive nitrification occurs is required.

The optimal ratio between fish and plants needs to be identified to get the right balance between fish nutrient production and plant uptake in each system. Rakocy [46] reports that this could be based on the feeding rate ratio, which is the amount of feed per day per square meter of plant varieties. On this basis, a value between 60 and 100 g day⁻¹ m⁻² has been recommended for leafy-greens growing on raft hydroponic systems [47]. Endut *et al.* [48] found an optimum ratio of 15–42 grams of fish feed day⁻¹ m⁻² of plant growing with one African catfish (*Clarias gariepinus*) for eight water spinach plants (*Ipomoea aquatica*). Hence, finding the right balance necessitates fundamental knowledge and experiences with regard to the following criteria: (1) types of fish and their food use rate; (2) composition of the fish food, for example, the quantity of pure proteins converted to Total Ammonia Nitrogen (TAN); (3) frequency of feeding; (4) hydroponic system type and design; (5) types and physiological stages of cultivated plants (leafy greens vs. fruity vegetables); (6) plant sowing density, and (7) chemical composition of the water influenced by the mineralisation rate of fish waste. Additionally, since fish, microorganisms and plants are in the same water loop, environmental parameters such as temperature, pH and mineral concentrations need to be set at a compromise point as close as possible to their respective optimal growth conditions.

3.3. System Description

As outlined above, the aquaponics system can be seen as the connection between a conventional recirculating aquaculture systems (RAS) and hydroponics components. In short, water recirculates in a loop as it flows from the fish tank to filtration units, before it is pumped into the hydroponic beds that are used as water reprocessing units. The filtration units are composed of mechanical filtration units for solid particles removal (e.g., drum filter or settling tank), and biofilters for nitrification processes (e.g., trickling or moving bed biofilter). Although system configurations and complexity can vary greatly, Figure 2.2 illustrates a typical layout.

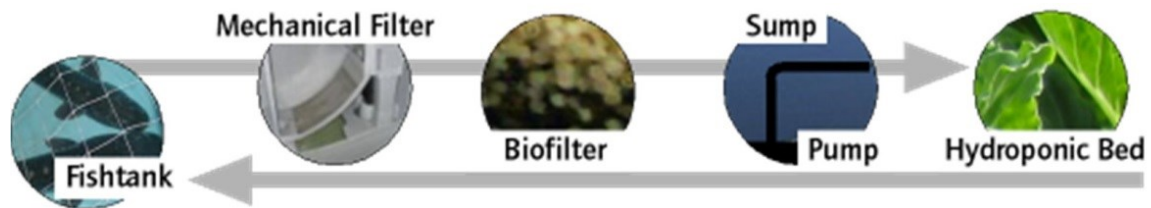


Figure 2.2. Basic aquaponic system layout.

Three types of hydroponic beds are commonly used: media-based grow bed, Deep Water Culture (DWC) bed, and Nutrient Film Technique (NFT) gutter shaped bed. The media-based grow bed is a hydroponic trough filled with inert substrate (e.g., expanded clay, perlite, pumice, gravel), serving as root support and microbial substrate. The water is commonly supplied in an ebb and flow pattern, ensuring sequential nutrition and aeration. The DWC system consists of large troughs with perforated floating rafts, where net plant pots are inserted. In the DWC system, these plant pots are generally filled with media, such as Rockwool, coco or pumice that support the roots, which are then continually submerged in the water tank. The Nutrient Film Technique (NFT) consists of narrow channels of perforated squared pipes where the roots are partially immersed in a thin layer of streaming water. A comparison of the advantages and disadvantages of these hydroponic beds *versus* soil culture is presented in Table 3.1. With respect to a holistic system approach, there are many ways to frame an aquaponic system in terms of hydrological and functional design. A few scientific papers provide working knowledge about different design and key parameters. Table 3.2 gives an overview of these.

Table 3.1. Advantages, disadvantages and nutrient uptake for different grow components in aquaponics with regard to different practical and productivity aspects.

	<i>Media-Based Growing Bed</i>	<i>DWC</i>	<i>NFT</i>	<i>Soil</i>
Advantages	<ul style="list-style-type: none"> - Biofiltration: media serves as substrate for nitrifying bacteria [49]; - Act as a solids filtering medium - Mineralization in grow bed; - Colonized by a broad microflora 	<ul style="list-style-type: none"> - Constant water flow; - Small sump tank needed; - Ease of maintenance and cleaning [50] 	<ul style="list-style-type: none"> - Constant water flow - Small sump tank needed; - Ease of maintenance and cleaning; - Require smaller volume of water; - Light hydroponic infrastructure, suits well for roof farming 	<ul style="list-style-type: none"> - Less infrastructure - Natural roots environment; - Colonized by broad microflora and fungi [51]; - Accepted as “organic way of production”
Disadvantages	<ul style="list-style-type: none"> - If flood and drain method: sizing and reliability plus large sump tank needed; - Heavy hydroponic infrastructure; - Maintenance and cleaning difficult; - Clogging leading to water channeling, inefficient biofiltration and inefficient nutrient delivery to plants [50] 	<ul style="list-style-type: none"> - Separate biofilter needs to be added [49]; - Require large volume of water; - Heavy hydroponic infrastructure; - Device for roots aeration mandatory [52] 	<ul style="list-style-type: none"> - Separate biofilter needed; - Lower yields (showed for lettuce by) [49]; - Expensive material; - the system is less stable as there is less water, 	<ul style="list-style-type: none"> - Small control on the soil nutrient solution; - Good soil not available everywhere; - More vulnerable for diseases; - Lower basil and okra yield than in aquaponics [45]
Nutrient uptake	<ul style="list-style-type: none"> - High 	<ul style="list-style-type: none"> - High 	<ul style="list-style-type: none"> - Lower because smaller root-water contact area 	<ul style="list-style-type: none"> - Lower

Table 3.2. Comparison of design and key parameters in well described aquaponic systems found in scientific articles.

	<i>System A</i>	<i>System B</i>	<i>System C</i>	<i>System D</i>
System Type	Nutrient Film Technique (NFT) configured in the conveyor production system.	Deep Water Culture (DWC)	Deep Water Culture (DWC)	Deep Water Culture (DWC)
Source	Adler <i>et al.</i> [53]	Roosta and Hamidpour [54]	Rakocy <i>et al.</i> [55,56]	Endut <i>et al.</i> [57]
Location	The Conservation Fund's Freshwater Institute, Shepherdstown, W. Va., USA	University of Rafsanjan, Iran	University of Virgin Islands, USA	University of Malaysia Terengganu
Based on	The system was theoretically valuated using data from studies conducted at the Conservation Fund's Freshwater Institute during 1994 and 1995 [42]	UVI-System	Own setup (UVI-System)	Own Setup
Volume RAS (m^3)	>38	0.848	43	3
Size Hydroculture (m^2)	498	Unknown (consisting of 8 plants)	220	2
Plant Density (pcs/m^2)	5.7 per meter of NFT trays	ND	8 (basil); 2–4 (okra)	ND
Fish Density (kg/m^3)	113.4	17.69 (Common Carp), 23.58 (Grass Carp), 17.69 (Silver Carp)	61.5–70.7	ND
Daily feed input/plant growing area ($g/day/m^2$)	ND	ND	81.4–99.6	15–42
Fish:Plant Ratio (kg)	ND	ND	ND	1:8
Plants Used	Basil (<i>Ocimum basilicum</i>); Lettuce (<i>Lactuca sativa</i> L. "Ostinata")	Tomato (<i>lycopersicon esculentum</i>)	Basil (<i>Ocimum basilicum</i>); Okra (<i>Abelmoschus esculentus</i>)	Spinach (<i>Spinacia oleracea</i>)

Fish Used	Rainbow Trout (<i>Oncorhynchus mykiss</i>)	Common Carp (<i>Cyprinus carpio</i>), Grass Carp (<i>Ctenopharyngodon idella</i>), Silver Carp (<i>Hypophthalmichthys molitrix</i>)	Nile Tilapia (<i>Oreochromis niloticus</i> L.)	African Catfish (<i>Clarias gariepinus</i>)
Hydroculture (Wet) Biomass (kg/m²)	ND	ND	2 (basil); 2.9 (okra)	1.16
Biofiltration	Fluidized Sand Filter + Carbon Dioxide Strippers	Net Filter	Net Filter	Rapid Sand Filters
Mechanical Filtration	Drum filter	Clarifier plus Net Plastic Filter	Clarifier plus Net Plastic Filter	Rapid Sand Filters
Water Parameters (pH; °C)	pH 7.2; Temp : ND	pH 7.0–7.7; Temp : 25.7 °C	pH 7.0–7.5; Temp : 28 °C	pH 5.6–7.3; Temp: 27.5–28.8 °C
Temporal length of experiment	ND	108 days	28 weeks (basil); 11.7 weeks (okra)	35 days
Cost of setup (\$ U.S.)	\$100,120 (hydroponic part) *	ND	ND	ND
Cost of annual running (\$ U.S.)	\$204,040 (lettuce); \$194,950 (basil)	ND	\$24,440 (tilapia+basil)	ND
Break-even price (\$ U.S.)	\$13.80 (per box of 24 lettuces); \$0.53 (per basil plant)	ND	\$3.23 (per kg of tilapia); \$1.66 (per kg of basil)	ND
Potential annual profit (\$ U.S.)	\$12,350–\$44,350 (for box of 24 lettuces sold at \$14–\$16); \$27,750–\$66,090 (for basil plant sold at \$0.60–\$0.70)	ND	\$116,000 (for tilapia sold at \$5.50/kg and basil sold at \$22.50/kg)	ND

With respect to Table 3.2, it is particularly noticeable that DWC systems are mainly used, and important design parameters such as fish to plant ratio or daily feed input are sometimes missing from the literature. It must be mentioned that some costs (*i.e.*, labour costs) are not taken into account, so the financial viability can only be partially estimated.

Apart from the UVI system, there is a lack of scientific literature when it comes to aquaponic experiments on large scale and during long time sequences. Moreover, many experimental setups published are small-scale replicates of the UVI design. Limited data on cost and potential profit of such systems are available [55,57–59]. As aquaponics is still in a maturing experimental phase, scientific research has focused more on technical aspects than economic viability. However, economic challenges need to be addressed. Experiments covering bigger production systems exist, but they are performed by private research centres or companies, whereby confidential findings are not always made accessible to third parties.

3.4. Technical Challenges

Aquaponics system design and application can be considered a highly multidisciplinary approach drawing from environmental, mechanical and civil engineering design concepts as well as aquatic and plant related biology, biochemistry, and biotechnology. System specific measurements and control technologies also require knowledge of subjects related to the field of computer science for automatic control systems. This high level of complexity necessarily demands in-depth knowledge and expertise of all involved fields. The biggest challenge in commercial aquaponics is its multidisciplinary nature, needing further expertise in economics, finance and marketing. Thus, a high degree of field-specific insight in terms of both practical and in-depth theoretical knowledge is required. This leads to an increasing level of complexity, which directly affects the efficiency factors of the running system. In the interest of highest efficiency and productivity, some numerical trade-offs are recommended and are outlined below. They include pH stabilization, nutrient balance, phosphorus, and pest management.

3.4.1. pH Stabilisation

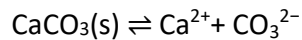
A crucial point in aquaponic systems is the pH stabilization, as it is critical to all living organisms within a cycling system that includes fish, plants and bacteria. The optimal pH for each living component is different. Most plants need a pH value between 6 and 6.5 in order to enhance the uptake of nutrients. The fish species Tilapia (*Oreochromis*) is known to be disease-resistant and tolerant to large fluctuation in pH value with a tolerance between pH 3.7 and 11, but achieves best growth performance between pH 7.0 and 9.0 [60]. The nitrifying bacteria have a higher optimum pH, which is above 7. Villaverde [61] observed that nitrification efficiency increased linearly by 13% per pH unit within a pH range between 5.0 and 9.0 with the highest activity of ammonium oxidizers at 8.2. Similar observations were made by Antoniou *et al.* [62], who report the overall nitrification pH of approximately 7.8. There are three major bacteria, for which optimal pH conditions are as follows: (1) *Nitrobacter*: 7.5 [63]; (2) *Nitrosomonas*: 7.0–7.5 [64], and (3) *Nitrospira*: 8.0–8.3 [65].

Based on these data, the highest possible pH value should be consistent with the prevention of ammonia accumulation in the system. Then, the ideal pH value for the system is between 6.8 and 7.0. Although root uptake of nitrate raises pH as bicarbonate ions are released in exchange [66], the acidity producing nitrification process has a higher impact on the overall system pH, leading to a constant and slight decrease in the pH-value. There are two approaches to counteract that trend:

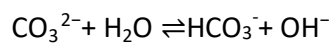
(1) Nutritional supplementation is the most applied method in use. By adding carbonate, bicarbonate or hydroxide to the system, the pH value can temporarily be adjusted in line with the requirements. Also, they increase the alkalinity parameter that prevents large fluctuations in pH and

thus keeps the system stable. The buffers should preferably be based on calcium, potassium, and magnesium compounds, since they compensate for a possible nutritional deficiency of those essential nutrients for plants [46]. Regarding the composition of the supplementation, it is important to seek a balance between those three elements.

(2) A proposed alternative approach is the implementation of the fluidized lime-bed reactor concept [67] into the field of aquaponics. This water neutralization concept consists of the controlled addition of dissolved limestone (CaCO_3) to the acid water that leads to a continuous pH-elevating effect due to carbonate solubilisation that releases hydroxide anions (OH^-).



Depending on pH, when CaCO_3 dissolves, some carbonate hydrolyses produce HCO_3^-



The degree to which the pH is raised is dependent on the adjustable flow rate. However, this concept requires preliminary empirical measurements with respect to the system's steady pH-drop in order to determine the size of the lime-bed reactor taking the specific flow-rate into consideration.

3.4.2. Nutrient Balance

As an innovative sustainable food production system, the challenge in aquaponics is to use the nutrient input efficiently, minimizing its discard and tending to a zero-discharge recirculating system [68,69]. Fish feed, the main nutrient input, can be divided into assimilated feed, uneaten feed, and soluble and solid fish excreta [19]. Soluble excreta are mainly ammonia and is the most available mineral until it is successively transformed into nitrite and nitrate by nitrifying bacteria [70,71]. Both uneaten feed and solid faeces need to be solubilized from organic material to ionic mineral forms that are easily assimilated by plants. Minerals have different solubilisation rates and do not accumulate equally [41,50], which influences their concentrations in the water. All involved microorganisms and chemical and physical mechanisms of solubilisation are not well understood [37,72]. Under current practices in RAS the solid wastes are only partially solubilized as they are mechanically filtered out on a daily basis [73]. These filtered wastes can be externally fully mineralized and reinserted into the hydroponic beds.

Given the objective of obtaining a low environmental footprint, a zero-discharge recirculating system concept should be achievable according to Neori *et al.* [69], but more research needs to be carried out on fish waste solubilisation with the objective to transform all added nutrients into plant biomass. There are two methods for mineralising organic material that could be implemented: (1) anoxic digestion in special mineralization or settling units using bioleaching abilities of heterotrophic bacteria (e.g., *Lactobacillus plantarum*) [74]; and/or (2) using earthworm species such as *Lumbricus rubellus* capable of converting organic wastes to water enriching compounds in wet composting or grow beds [75]. Vermiculture can facilitate a high degree of mineralization as worm casts contain micro- and macronutrients broken down from organic compounds [76,77]. Addition of external sources (e.g., food waste) of feed for the worms to provide the aquaponic system with additional organic fertilizers has also been suggested [78].

Feed composition directly affects the nutrient excretion by fish, consequently affecting the water chemistry [50,79]. One challenge is to find the right fish feed composition for aquaponics in order to attain a water composition that is as close as possible to hydroculture requirements. There is a need to establish the macro- and micronutrient proportion that fish can release in the water for a given feed in a given system; this depends on fish species, fish density, temperature, and type of plants (*i.e.*, fruity plants or leafy greens). This will allow prediction of the subsequent mineral addition needed to match optimal plant growth requirements. Inorganic mineral input adds extra cost and issues for sustainable resource management (e.g., global P peak production reality) [31,80]. Thus, fish feed composition should be adapted to minimize this mineral addition while ensuring required nutrition properties for fish yield and avoiding phytotoxic mineral accumulation (e.g., Na). The fish feed origin regarding its environmental footprint should also be taken into account. Low trophic fish species should be preferred and alternative production solutions should be promoted such as human food waste recycling [81], insects, worms, aquatic weed, and algae as a feed base [82,83]. Also, some fish–plant couples might be more appropriate than others in terms of overlap between nutrients profiles offered by excreta and nutrient profiles demanded by plants. Identifying these couples would assure an optimum use of the available nutrients.

A comparison of mineral concentrations in the published aquaponics literature (Table 3.3), with recommended recirculating hydroponics solutions leads to two main observations: (1) there is a lack of aquaponic data for some macro- and micro-elements, indicating the necessity of more research focus on them; (2) for the available data, the aquaponic concentrations are below the recommended hydroponic level. However, Rakocy and Lennard (pers. comm.) report that hydroponics and aquaponics nutrient solutions are not comparable for many reasons. The nature of the total dissolved solid (TDS) is not the same in these systems. In hydroponics, TDS consists mainly of mineral compounds, while in aquaponics it includes organic molecules wherein nutrients can be locked up and overlooked by measuring procedures such as electrical conductivity (EC) or aqueous sample filtration. Both aqueous sample filtration and the EC measurement methods only take nutrients that are available in ionic form into account. These suspended organic solids are assumed to promote growth because they might simulate natural growing conditions as found in soil, unlike the growing environment of hydroponics [84].

Table 3.3. Comparison of pH and nutrient concentrations in hydroponic and aquaponic solution for different plant species, all nutrients reported in mg L⁻¹.

Plant Species	System	pH	Ca	Mg	Na	K	TAN	NO ₃ -N	PO ₄ -P	SO ₄ -S	Cl	Fe	Mn	Cu	Zn	B	Mo	Source
Lettuce (<i>Lactuca sativa</i>)	Hydroponic	5–6.2	180	24		430	18	266	62	36		2.2	0.3	0.05	0.3	0.3	0.05	Sonneveld and Voogt, 2009 [11]
Lettuce (<i>Lactuca sativa</i>)	Hydroponic		200	50	50–90	210		190	50	66	65–253	5	0.5	0.15	0.15	0.3	0.05	Resh, 2012 [12]
Lettuce (<i>Lactuca sativa</i>)	Aquaponic	8				48		20	10									Al-Hafedh <i>et al.</i> , 2008 [85]
Lettuce (<i>Lactuca sativa</i>)	Aquaponic		180	44	17	106		137	9									Pantanella <i>et al.</i> , 2012 [86]
Basil (<i>Ocimum basilicum</i> ‘Genovese’)	Aquaponic	7.4	12	7		45	2.20	42	8			2.5	0.8	0.05	0.44	0.19	0.01	Rakocy <i>et al.</i> , 2004a [40]
Water spinach (<i>Ipomoea aquatica</i>)	Aquaponic	5.6–7.3						20	17									Endut <i>et al.</i> , 2010 [48]
Tomato (<i>Solanum lycopersicum</i>)	Hydroponic	5–6.2	110	24		254	18	151	39	48		0.8	0.6	0.05	0.3	0.2	0.05	Sonneveld and Voogt, 2009 [11]
Tomato (<i>Solanum lycopersicum</i>)	Aquaponic	7.7	34			27	0.33	35	8			0.2		0.04	0.37			Roosta and Hamidpour, 2011 [54]
Okra (<i>Abelmoschus esculentus</i>)	Aquaponic	7.1	24	6	14	64	1.58	26	15	6	12	1.3	0.06	0.03	0.34	0.09	0.01	Rakocy <i>et al.</i> , 2004b [87]

There is a lack of knowledge about the nature of organic molecules and the biochemical processes occurring for their assimilation by plants. Some can be taken up directly or need complex biodegradation to make them available. Another difference is the microflora inherent to aquaponics while sterilization occurs in hydroponics. This microflora can have significant beneficial effects on plant growth and organic molecules' assimilation. Hence, some aquaponics investigators report similar or even better yield than hydroponics for some crops, despite lower concentrations of mineral nutrients [86,88–90].

Voogt [91] identifies three aspects of the hydroponic nutrient solution composition that should be taken into account in aquaponics: (1) elemental uptake ratio compared to nutrient composition; (2) ease of uptake of specific elements; (3) the type of growing system that also require a specific nutrient composition. The composition of a nutrient solution must reflect the uptake ratios of individual elements by the crop, otherwise it will lead to either accumulation or depletion of certain elements. As the demand between crops differs, the basic compositions of nutrients solutions are crop specific [92]. The uptake of elements differs widely, the absorption of some can be more difficult and necessitates relatively higher ratios than the straightforward uptake ratio of the crop.

The optimal nutrient levels for leafy and fruity vegetables in aquaponics systems are not yet well established. Additional research should be carried out to assess the optimum value of mineral concentration per single crop or hybrid multi-crop systems regarding growth rate and crop yield. Optimal suspended organic solids' level should be identified with respect to its impact on vegetative growth. Also, a special emphasis should be placed on crop quality since productivity should not be the only argument for competitiveness. For output purposes, this should be compared to (1) hydroponic crop grown with mineral nutrient solution; (2) conventionally soil-based agricultural methods; and (3) organic soil-based agricultural methods. Within-system comparative studies address the productivity, as the macro- and micronutrient composition of the products will play a decisive role with respect to future orientation of healthy and efficient quality food production. A deeper understanding of the biochemical processes occurring in solid fish waste solubilisation is necessary with the aim to increase mineral levels in aquaponic water by implementing process and specific waste biofiltration units.

3.4.3. Phosphorous

Among the different minerals, phosphorus (P) deserves a specific attention. It is a macronutrient, which is assimilated by plants in its ionic orthophosphate form (H_2PO_4^- , HPO_4^{2-} , PO_4^{3-}). It is essential for both vegetative and flowering stages of plant growth [93]. In RAS, 30-65% of the phosphorus added to the system via fish feed is lost in the form of fish solid excretion that is filtered out by either settling tanks or mechanical filters [18,41]. Moreover, organic P solubilised as orthophosphate can precipitate with calcium (e.g., hydroxyapatite— $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$) making these elements less available in solution [41,72]. Consequently, aquaponic experiments report a range of 1-17 $\text{mg L}^{-1} \text{PO}_4\text{-P}$ [56,85,88,94]. However, recommended concentrations in standard hydroponics are generally between 40 and 60 $\text{mg L}^{-1} \text{PO}_4\text{-P}$ [11,12,95]. This discrepancy suggests that phosphate should be added to aquaponic systems, especially for fruity vegetables that do not yet show satisfying yields in aquaponics [96]. Phosphorus is a finite and scarce mining resource and subsequently, an expensive component of hydroponic solutions. Sufficient phosphorus production will certainly be a major concern in the near future [80]. Therefore, solutions to reuse the discharge of P-rich effluents must

be explored [97,98]. As up to 65% of P can be wasted in form of aquaculture effluent sludge, recovery solutions should be developed to achieve zero-discharge systems. For example, leachate rich in P could be obtained by sludge digestion with selected P-solubilising microorganisms [74] and then reinserted in the hydroponic part of the system. The ultimate objective is to develop a zero-discharge recirculating system with maximum nutrient recycling transformed into plant biomass and improved yield.

3.4.4. Pest and Disease Management

The challenges of pest and disease management is another aspect that needs further improvement [39]. Aquaponic systems are characterized by a broader range of microflora than conventional hydroponic systems, especially because the breeding of fish and biofiltration occurs in the same water loop. Conventional pesticides that are used in hydroponics cannot be used in aquaponics because of toxicity risk to the fish and to the desired biofilm (e.g., autotrophic nitrifying biofilm) [96]. The need to maintain the nitrification biofilm and other nutrient solubilizing microorganisms also prevents the use of antibiotics and fungicides for fish pathogen control and removal in the aquatic environment. Furthermore, antibiotics are not allowed for plant application so their use against fish pathogens must be avoided in aquaponic systems. These constraints demand innovative pest and disease management solutions for fish and plants that minimize impacts on fish and desired microorganisms. Plant and fish pests and pathogens can be divided into four different categories based on specific alternative treatment solutions. These are (1) plant pests—mostly insects that damage the leaves and roots (e.g., aphids, spider mites); (2) plant diseases—microorganisms (e.g., bacteria, fungi) and viruses that attack plants; (3) fish parasites (e.g., monogenea, cestoda); and (4) fish diseases caused by viruses and microorganisms.

Rearing and crop practices that decrease the occurrence of diseases could be applied such as preventive sanitary measures, low density of fish and/or plants, and/or control of environmental conditions, which decrease relative humidity around the plants. In addition to these practices, a few innovative methods of biocontrol already exist for plants cultivated under field or greenhouse conditions. These methods are based on the use of microorganisms with biocontrol activity [99,100], or extracts of such microorganisms or extracts of plants (including essential oils) that show high antimicrobial efficiency and short residence time [101,102]. It will be a challenge to select and adapt these methods to aquaponics systems, considering their compatibility with the other living organisms of the system. Furthermore, microbial diversity can be beneficial for plants. The presence of some mutualistic microorganisms in the plant biosphere can retard the development of pathogens [103,104] while promoting growth (e.g., plant growth-promoting rhizobacteria and plant growth-promoting fungi).

Since the presence of a broad range microflora belongs to aquaponic practices, the occurrence of pathogens and risk for human health should also be established, in order to assess the safety of aquaponics and to conduct appropriate quality control. These challenges can lead to the production of products that are quality and pesticide free certified (e.g., organic) and thereby achieve a higher prize in the market and leads to a healthier population [105].

3.4.5. Other Technical Challenges

The regulation of the nitrate level in aquaponics is another challenge. Leafy vegetables need 100–200 mg L⁻¹ of NO₃-N concentration, while fruity vegetables need lower level at species specific growth stages [12]. Intermittent intervals of high nitrate can be harmful for fish and nitrate concentration must stay under a certain threshold to avoid adverse physical effects to sensitive species (e.g., 100, 140, 250 mg L⁻¹ NO₃-N for *Oncorhynchus mykiss*, *Clarias gariepinus*, *Oreochromis niloticus*, respectively [106–108]). Therefore, it is of particular relevance to determine the best practical means fish:plant ratio before setup and/or implement a flow-controlled denitrification unit in the system in order to be able to adjust the desired nitrate level. Some denitrification tanks are already used in RAS [10], however, the technology is not yet fully developed. The approach involves creating anoxic conditions in a column by using the sludge as an organic carbon source for heterotrophic denitrifying microorganisms and recirculates the nitrate-rich water through it. If anoxic conditions are applied in sludge, heterotrophic microorganisms are able to use nitrate instead of oxygen as electron acceptor and reduce it successively to gaseous nitrogen (N₂) [109]. A critical step is to guarantee additional bio filtration before discharging the treated water back into the system to reduce the risk of toxic NO₂⁻ ions from the denitrification process entering the system.

Together with environmental conditions, the population density is the most important parameter for the fish well-being. In outdoor aquaponics facilities such as the UVI system, the common tilapia fish density without use of pure oxygen is around 30–40 kg m⁻³. A higher density up to 60 kg m⁻³ can be achieved in greenhouses [110]; this may be due to more algae and cyanobacteria blooms under longer daylight conditions, producing more oxygen from increased photosynthesis. These characteristics, however, cannot be generalized. In fact, different fish species require different optimal water quality; e.g., warm water species tilapia require a dissolved oxygen (DO) level of 4–6 mg L⁻¹, whereas the cold water species trout needs at least 6–8 mg L⁻¹ DO [111]. Dissolved oxygen is not the only factor that needs to be kept stable. Large fluctuations in temperature and pH might harm fish, plants, and nitrifying microorganisms [112,113]. Despite this fact, temperatures for warm water species such as tilapia and nitrifying bacteria can be 25–30 °C, whereas most plants rather prefer colder water temperatures (approx. 20–25 °C).

Thus far, aquaponics has been built on a trade-off between the needs of fish and plants, respectively. Development is now needed to achieve optimal conditions for both fish and plants with either: (1) emphasis on interdependent parameters of both system components (e.g., combining fish and plant species that preferably require similar environmental conditions within the same range of temperatures and pH that ensure bacterial nitrification); or (2) the physical separation in two recirculating loops, *i.e.*, an aquaculture and hydroponic loop, described as decoupled systems, where optimal condition for each system is applied with periodic water exchange between them. These are different types of solutions that may contribute to the breakthrough of commercial aquaponics.

3.5. Socio-Ecological Challenges

Aquaponics as such is also responding to diverse ecological and social challenges, which point to the importance to focus on efficient and sustainable forms of agricultural production. Socio-ecological challenges include mineral recycling, water scarcity, energy availability, overfishing, as well as urban farming and short supply chains. They are outlined below.

3.5.1. Mineral Recycling

In terms of sustainability, both phosphorus and potassium are major components of agricultural fertilizers, and like oil, they are non-renewable resources. Therefore, increasing use and depletion of these minerals without reuse or recapture has a negative impact on and is of significance to their future supply. This in turn would have dramatic consequences for global food security. Nutrient recycling policies, especially for phosphorus, are crucial in order to avoid global food shortages [31,32].

3.5.2. Water

An increasing number of countries are facing economic and physical water scarcity, leading to a growing incapability in feeding their people [114]. On average, global agriculture uses around 70% of the available freshwater resources. In arid climate zones such as the Middle East and North Africa, the agricultural water consumption can even be up to 90% [115]. Compared to conventional agriculture, aquaponics uses less than 10% of water, depending on the climatic conditions [116]. Aquaponics can reduce fresh water depletion associated with irrigation whilst guaranteeing safe encouraging sustainable farming and food production practices, which in turn reduces the freshwater consumption in countries facing water stress. System related water losses that occur in evaporation, plant transpiration and the water content of the agricultural products can be compensated for by capturing water from air humidity [117] or by reverse osmosis desalination plant in coastal areas [118,119].

3.5.3. Energy

The energy requirements of aquaponics are likely to be based on system configuration (design, species, scale, technologies) and geographic location (climate, available resources). For each location, different measures are needed in order to ensure that each system will have a suitable sustainable energy source all year round to provide stable conditions for fish and plants. This is crucial, as fluctuations in temperature might harm fish, plants, and nitrifying microorganisms [113]. This requirement constitutes a mandatory factor in regions with constantly and seasonally changing climatic conditions as well as in hot and arid climatic zones. Ensuring stable conditions may be achievable in equatorial areas without additional technology. Harnessing solar energy can be beneficial in order to either run climate control systems within greenhouses (e.g., via air conditioning operated by solar photovoltaic modules), or to heat up a low-energy greenhouse with passive solar heating [120]. The latter option is practicable for small sized non-commercial (passive solar) greenhouses, but may not be suitable for larger greenhouses because of the high thermal resistance and high energy losses, associated with medium and large greenhouses. These larger structures may require alternative solutions. In countries such as Iceland and Japan, near-surface geothermal energy can be used by means of heat pumps and direct geothermal heat for maintaining the indoor temperature at the desired level [121,122]. Countries with comparatively unfavourable geological conditions still might assess possible options in terms of using waste heat of combined heat and power (CHP) units to heat the greenhouse during cold days [123] or cool them down during hot days. Those CHP units can mostly be found in combination with agricultural biogas plants, whereby surplus heat is fairly cheap for further disposal. Alternatively, they might consider using fish and plant species that are more suitable for the respective climatic conditions in order to avoid the expensive heating or cooling down of the system's water.

3.5.4. Overfishing

Eighty percent of the world's oceans are fully- or over-exploited, depleted or in a state of collapse. One hundred million tons of fish are consumed worldwide each year, providing 2.5 billion people with at least 20% of their average per capita animal protein intake [124]. Fish is one of the most efficient animal protein producers, with a food conversion ratio (FCR) between 1 and 2 [125]. Since fish demand is increasing whilst the fishing grounds are overexploited [126], aquaculture is the fastest growing sector of world food production [124]. Adverse effects of this development include the high water consumption in case of conventional fish protein production [127], and release of up to 80% of N and 85% of P per kg of fish feed [18,37] into the environment. This causes the loss of valuable nutrients, resulting in eutrophication in rivers, lakes and coastal waters, and excessive productivity leading to vast dead zones in the oceans [128]. However, it has to be noted that high-protein fishmeal and fish oil are still key components of aquaculture feeds [129]. Between 2010 and 2012, 23% of captured fish was reduced to fishmeal and fish oil [129]. Decreasing the proportion of both fishmeal and fish oil in fish feed is thus a challenge that needs to be addressed.

3.5.5. Urban Farming and Short Supply Chains

Aquaponic systems can be set up almost everywhere and have the potential to (sub-)urbanise food production. This could bring important socio-environmental benefits. Aquaponic farming plants could be implemented in old industrial neglected buildings with the advantages of re-establishing a sustainable activity without increasing urbanization pressure on land. Roof gardens would be another possibility, allowing the saving of space in urban areas. If greenhouses are used on roofs, they can insulate buildings while producing food [130]. Another important aspect is minimizing the distance between the food producer and consumer. The longer the supply chain, the more transport, packaging, conservation and labour needed, leading to substantial decreases of resources and energy (e.g., up to 79% of the retail price in US conventional food distribution [131]). Shortening and simplifying the food supply chains can drastically diminish their environmental impacts, while providing cities with fresher products. This also allows the consumer to clearly identify his food origin [132,133]. Nevertheless, one should not underestimate the development of rural locations, where farmland is plentiful. As aquaponics can be considered a high-tech agricultural method, it is necessary to assure knowledge transfer in this field to maintain skilled labour forces.

3.6. Economic Challenges

The current literature cannot be used to critically assess and predict economic challenges; as presented in Table 3.2, only two economic sub-studies are available in the peer-reviewed literature [53,55]. At this early stage of scientific research, the main focus has been on technical aspects of aquaponics; financial figures held by private research entities are not shared with the public. Furthermore, it is difficult to compare the two systems to determine which is better as information may not be available for all system parameters and outputs. For example, light intensity (lm) was not reported by Rakocy *et al.* [55], yet this is one of the major factors affecting plant growth and thus the harvested biomass. Overall, system costs can be measured in the cost per square meter, which is influenced by the complexity of the system and this is closely related to climatic and geographic conditions such as seasonal daylight availability, temperature extremes, and fluctuation of warmth and cold. Also, dynamic costs such as maintenance costs (*i.e.*, price per kWh and labour) and sales

revenues in regional markets might differ, making it more difficult to make accurate economic evaluations. Even comparing the most expensive item within a system is difficult, as it differs per region and country (e.g., electricity prices, heat availability, *etc.*). Consequently, there is no general optimal system, as the system must be adjusted to environmental conditions. Another approach could be to calculate the cost savings by comparing the cost of RAS and hydroponics separately to the same system and integrated to an aquaponic system, under the same environmental and market conditions. Hence, Rupasinghe and Kennedy [134] calculated an improvement of the net present value of 4.6% in an integrated aquaponic system of lettuce and barramundi. Unfortunately, there are no other studies available for comparison.

Market prices, one of the major factors for profit, can greatly vary between countries for several (e.g., cultural, historical availability) reasons. However, the profit margins will definitely be higher if the product manufacturing costs are low and the food distribution supply chain is short. The transport, packaging and conservation of the food are time and energy consuming, which has an effect on the additional costs and freshness of the products. In order to meet these problems, more urban and peri-urban fresh food production plants need to be implemented to guarantee efficient short food supply chains [133].

Rakocy [38] showed with respect to the crop choice, leafy greens generally achieve a higher profitability than fruity vegetables. In an initial economic analysis, given the University of Virgin Islands (UVI) system design, they had a profit margin with basil exceeding almost by a factor 4 of that of lettuce. This finding should be viewed with a degree of caution because of different domestic market dependencies. Nonetheless, when addressing economic optimization, the three most important factors are: (1) sustainability considerations, which, in the case of aquaponics, are interrelated with economic profits, since the reuse of resources should cut costs for the producer and for the customer; (2) technical optimization of processes (e.g., nutrient availability in different growth stages, nutrient recycling, *etc.*), and; (3) system components (e.g., design of the hydrological regime, P recycling unit, pH stabilizing reactors, *etc.*).

Although Vermeulen and Kamstra [39] state that the actual perceived environmental benefits of nutrient reuse, energy efficiency and land use seem only marginally cost-effective, the aspects of possible differences in product quality and societal value are not necessarily reflected in business costs. Also, the use and cost of fertilizers in hydroponic production systems has an increasing importance, as fertilizer costs lie between 5% and 10% of the overall costs, and scarce fossil fuels are required in their manufacture [135]. The costing forecasts for fossil fuels could rather exacerbate the situation further and increase the demand of alternative fertilizing solutions such as using waste. Another resource that becomes increasingly scarce is fresh water. Reprocessing instead of discharging contaminated water will be a big challenge that needs to be met in the future. Taxes for wastewater discharge or strong limitations in discharge by local or national policies might become a factor as all point source discharges are regulated by water quality policies. Anticipating this trend will ensure economic and financial advantages with respect to conventional agriculture or hydroponic approaches.

3.7. Education as a Necessity

A broad range of knowledge is required to understand and implement the multidisciplinary concept of aquaponics. From the theoretical perspective, the multidisciplinary nature of the field and a lack of training in holistic thinking is a hurdle to fully comprehend the concept of aquaponics covering all interrelating issues. The bundling of field-specific in-depth knowledge is required in order to consolidate available scientific knowledge and evidence. At most universities, the two main disciplines, *i.e.*, hydroponics and aquaculture, are either not taught, or offered in different schools, which could complicate access and exchange of knowledge. In practice, aquaculture and hydroponic technologies are well-known. The problem lies in the fact that those disciplines need to be connected. This lack of information-sharing shows the necessity for developing an education network dealing with the improvement of the interconnection between (scientific) disciplines involved in this field. Aquaponic stakeholders, including researchers, entrepreneurs and technicians, need to have basic knowledge covering all disciplines that are involved in this field. Furthermore, experts within every connected field are required to address specific issues within theoretical, scientific, financial as well as practical frameworks.

3.8. Discussion

Challenges underlying sustainable socio-ecological, technical and economic factors pertaining to aquaponics are discussed in this paper to demonstrate the need and the means of extensively investing in more research and development and education in the aquaponics sector. Taking these factors into account is necessary because a pure financial perspective faces significant constraints, notably in terms of natural resource scarcity and their long-term economic consequences. The commercial development of socially, ecologically, and environmentally sustainable aquaponic systems confronts several technical challenges that need to be addressed further: (1) improved nutrient solubilisation and recovery for a better use of the nutrient input and reducing extra-mineral addition, *e.g.*, phosphorus recycling; (2) adapted pest management; (3) reduce water consumption to a high degree by limiting the need for water exchange; (4) use of alternative energy sources for hot/cold and arid areas (*e.g.*, CHP waste heat, geothermal heat, *etc.*); and (5) innovative pH stabilization methods by implementing fluidized lime-bed reactors that have successfully been used in natural waters [67].

All the factors mentioned above require additional attention, because some production parameters still need to be determined and optimized to prepare aquaponics for commercial use as some components and their interactions are not technically mature yet. This cannot be sufficiently achieved without a greater focus on combining existing knowledge of the different involved fields within a scientific and international framework. These aspects are important, as the commercially aligned technology should not be restricted by certain external conditions. Instead, the systems to be developed should be universally applicable, which implies resource–economic (*i.e.*, resource-saving) production systems that can be run in arid, hot, cold, and urban areas or any combination thereof.

Vermeulen and Kamstra [39] report only a marginal cost reduction for environmental benefits of nutrient reuse and energy efficiency when aquaponics is compared to RAS and hydroponics run separately. However, this study did not take socio-ecological factors into account, such as operating in a resource (*e.g.*, phosphorous, water) limited world. Energy cost and fertilizer prices are constantly

rising and governmental policies encourage reduction of emitted pollution (e.g., tax incentive schemes), so this cost margin benefit of aquaponics is expected to rise. Although the highest financial profit margin has been shown with leafy greens, it is still necessary to determine the purpose and the scale of the respective systems before building them; the needs on a microeconomic level in terms of food self-sufficiency or local food supply might differ from profit-oriented approaches and from country to country.

3.9. Conclusions

Given the fact that aquaponics follows nutrient and water reusing principles, it seems to be a promising solution for sustainable aquaculture and hydroponic practices. However, further research and developments are needed as demonstrated by the challenges described in this paper. These challenges need to be resolved with the aim to establish fully controlled and standardized aquaponic systems that will be easy to handle and economically viable. The competitiveness of the production method depends on technological developments, local markets, and climatic and geographic conditions that need to be assessed and cannot be generalized. Only addressing those factors thoroughly will eventually validate aquaponics as a sustainable food production alternative.

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Author Contributions

Simon Goddek and Boris Delaide both have equally added to the manuscript. They were assisted by the other co-authors in form and content.

Conflicts of Interest

The authors declare no conflict of interest.

4. Plant and fish production performance, nutrient mass balances, energy and water use of the PAFF Box, a small-scale aquaponic system.

This chapter has been published as a manuscript entitled “Plant and fish production performance, nutrient mass balances, energy and water use of the PAFF Box, a small-scale aquaponic system.” By Delaide, B.; Delhay, G.; Dermience, M.; Gott, J.; Soyeurt, H.; Jijakli, M. H. *Aquacultural Engineering*, 2017, 78, 130–139.

4.1 Introduction

The increased demand for food from a growing world population is intensifying the pressures on natural resources and ecosystems. Recent studies suggest that the planetary boundaries of biosphere integrity, nitrogen (N) and phosphorus (P) cycles have been or are soon to be overtaken. Overtaken planet boundaries can lead to unpredictable consequences, as deep changes of the ecosystem balance [4]. Solutions urge to be found. The potential for more efficient resource use through the tightening nutrient cycles and reduction of waste may explain the increasing interest in aquaponics [23] as an innovation for the rapid expanding sectors of recirculating aquaculture systems and hydroponic productions [11,129].

Aquaponics is an integrated farming concept that combines fish, hydroponic plant production, and nitrifying bacteria in a symbiotic environment. The most common form is the integration of hydroponic beds in the water circuit of a recirculating aquaculture system (RAS) [47,136]. This integration aims to convert the normally wasted nutrients excreted by fish into valuable plant biomass. This allows for lower water exchange and spillage which should significantly reduce the environmental impact of fish and hydroponic plant production.

Recirculating aquaculture systems are composed of fish tanks, a mechanical filter, a biofilter (i.e. for autotrophic conversion of ammonia into nitrate) and a sump. A pump recirculates the water constantly. Oxygen is supplied by air blower or air cones and water temperature is controlled. In this system configuration, nitrate tends to accumulate in water and is the first toxic factor for fish, necessitating water exchange. The idea to introduce hydroponic beds in the water loop is originally due to address this issue [50]. However, the impact on fish welfare of the water quality in such highly recirculated system has not yet been clearly established and would need special attention [137].

Healthy plant growth requires the presence of additional macro- and micronutrients (i.e., potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), Sulphur (S), iron (Fe), boron (B), copper (Cu), zinc (Zn), manganese (Mn), and molybdenum (Mo)) in specific proportion and concentration in the water [11,12]. To date, the mass balance dynamics and budget of these nutrients, with the exception of N, P, K [138], have not yet been fully studied in aquaponic systems while these data are fundamental for better system sizing, design and feed formulation [136].

Three types of beds are most frequently used in aquaponics: nutrient film technique (NFT); ebb-and-flow (EAF); and deep water culture (DWC or RAFT) beds. EAF beds composed of heavy substrate (e.g., clay balls, perlite, etc.) and siphon bells seem to be less practical for maintenance [50] and there is only a few reports on their production performance compared to DWC or NFT [139]. Aquaponics is a new research field and a theoretical lower environmental footprint compared to conventional farming methods is expected but there is a lack of data establishing it. Namely, the water and energy used but also the expectable plant yields are not yet well documented and need to be compared to

RAS and hydroponic systems. The ability of aquaponic systems to produce the same yield and quality as conventional one needs to be also reported.

Using a small-scale aquaponic system, the objectives of this study were threefold: 1) Comparing the plant yield between ebb and flow and DWC hydroponic beds in order to select the most productive one. 2) Describing the plant and fish production capacity, as well as water and energy consumption over one season's production. 3) Analysing all macro- and micronutrient mass balances. In the attempt to establish the environmental advantages of aquaponics in terms of plant and fish production, nutrient fluxes and energy and water uses, these data have been confronted to conventional systems and other small-scale aquaponic systems.

4.2. Material and methods

4.2.1 Experimental setup and operation

The PAFF Box aquaponic system was situated at Gembloux Agro-Bio Tech - University of Liège, in Gembloux, Belgium (latitude 50° 33'N, longitude 4° 41'E, altitude 157 m). The system was harboured in a two floor structure composed of a steel container measuring 2.21 m high, 5.72 m long, 2.17 m wide, insulated with 8.2 cm polyurethane foam, and topped with an aluminium and polycarbonate greenhouse (Euro-Maxi, Euroserre, Genk, Belgium) measuring 2.40 m x 6 m x 2.40 m, the whole occupying a space of 71.21m³ with a total water volume of 2.673m³.

The aquaculture part of the system was in the container and the plant production, consisting of bunk plant grow beds, was located in the greenhouse. The design and specifications are presented in Fig. 4.1. In 2014 half of the grow beds were used as EAF beds. These beds were filled with expanded clay balls (AH 7/16, Argex, Burcht, Belgium) and siphon bells were used to create a flood and drain period. The EAF and DWC beds were irrigated with the same water (i.e. same water quality). Then, in 2015 all beds were converted in DWC.

The mechanical systems and their energy demand are reported in Table 4.1. Mechanical components requiring electrical supply included: water pump, air blower, three submersible heaters, wall mounted greenhouse fan, fluorescent lighting, LED light, and an energy recovery ventilator (ERV). The greenhouse fan was thermostat-controlled to extract air when its temperature exceeded 25°C. Four 0.7 m x 0.9 m windows in the greenhouse roof were automatically opened by thermosensitive pistons (Ventomax, J.Orbesen teknik ApS, Asnaes, Denmark). In summer sunny days, shade cloths were installed on the roof and south side of the greenhouse to mitigate excessive temperatures. The air in the container was kept warm using an ERV. Target water temperature was set at 25°C with thermostat submersible heaters. The solid excretions of the fish and uneaten feed were retained in sieve and microbeads filters (Fig. 4.1) and removed daily from the system.

Prior to the studies, the system was operated for two months with fish and water as a recirculating aquaculture system, allowing the biofilter to mature and the nutrient levels to increase. The study started as soon as the full fish load and the first seedlings were introduced.

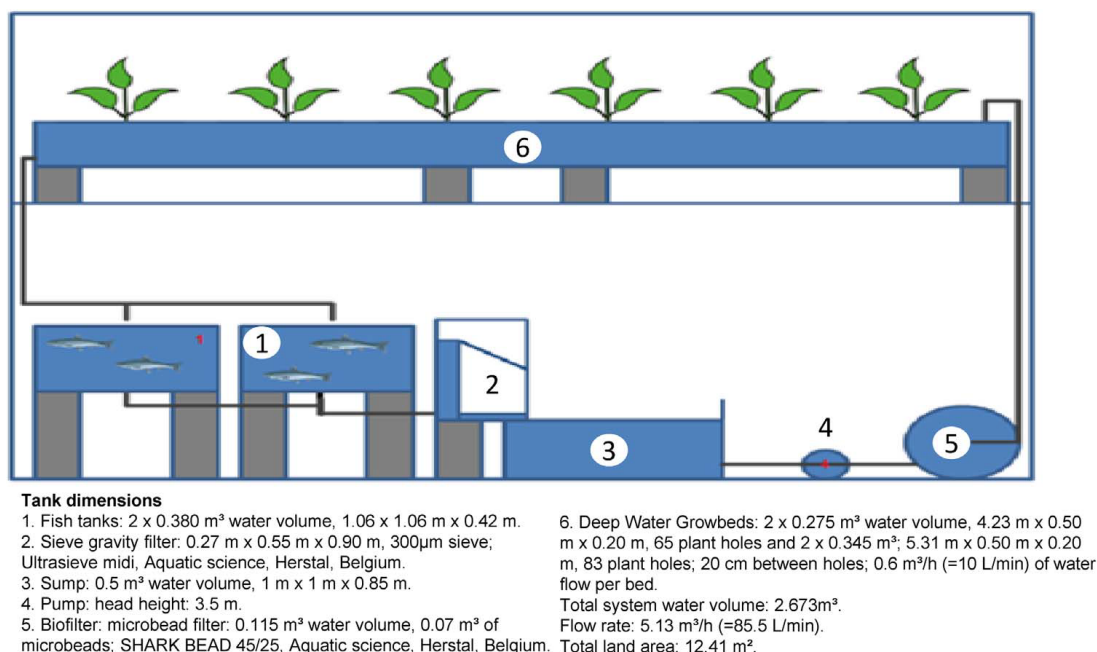


Fig. 4.1. Aquaponic plant and fish farming box (PAFF Box) system layout and dimensions. This system is a one loop aquaponic system.

Fish stocking

The system was stocked with 200 Nile tilapia (*Oreochromis niloticus*) obtained from the Centre de Formation et de Recherches en Aquaculture located at Tihange, Belgium. They had an average weight of $73.9 \text{ g} \pm 20.8$ for a total of 14,784 g. They were reared in tanks at a starting density of 23 kg/m³. Fish were fed 200 g of feed twice per day (i.e. 42 g per m² of DWC beds). This feeding rate was kept constant throughout the experiment in order to be able to easily draw conclusion on the nutrient mass balance dynamics in water. It was equivalent to 2.7% of the body weight at the beginning of the experiment and 0.9% at the end. The feed (3.2mm Omegabaars Grower, AQUA4C, Kruishoutem, Belgium) was 100% veggie based and had a content of 40% proteins, 12% lipids and 3.7 % crude fibre.

4.2.2 Sampling and analytical methods

Water quality

The water quality was closely recorded in order to follow its evolution with a daily feed input of 42 g per square meter of DWC beds. A temperature of 25 °C and a pH of 7 were targeted as trade-off value for plant, fish and nitrifying bacteria [42].

The temperature (T°), dissolved oxygen (DO), electro-conductivity (EC) and pH were measured daily. Total ammoniacal nitrogen (TAN), nitrite (NO₂⁻-N) and nitrate (NO₃⁻-N) were measured three times per week. Alkalinity, phosphate (H₂PO₄⁻, HPO₄²⁻ or PO₄³⁻), sulphate (HSO₄⁻, SO₄²⁻), Mg, Ca, K, Fe, Cu, Mn, Zn, B, Mo and Na were measured twice per week. Measures and samples were taken in the sump (T°, EC, nutrients) and fish tank (DO) in the morning before fish feeding and filter cleaning.

EC and T° was measured with a conductivity tester (AD31 Waterproof, ADWA, Szeged, Hungary), DO with DO meter (HI 9146, HANNA instruments, Woonsocket, RI, USA) and pH with a pH-meter (Inolab pH level 1, WTW, Weilheim, Germany). The concentration of nitrogen compounds, alkalinity, PO₄³⁻ and SO₄²⁻ were determined immediately after sampling with a multiparameter spectrophotometer (HI 83200, HANNA instruments, Woonsocket, RI, USA) using the following Hanna instruments' reagents: HI 93700 (TAN), HI 93707 (NO₂⁻-N), HI 93728 (NO₃⁻-N), HI 93713 (PO₄³⁻-P), HI 93751 (SO₄²⁻-

S), HI 93755 (Alkalinity). K ion (K^+), Mg ion (Mg^{2+}), Ca ion (Ca^{2+}), Fe ions (Fe^{3+} , Fe^{2+}), Cu ions (Cu^{2+} , Cu^+), Mn ion (Mn^{2+}), Zn ion (Zn^{2+}), B oxides (BO_3^{2-} , $B_4O_7^{2-}$), Mo oxide (MoO_4^{2-}) and Na ion (Na^+) concentrations were determined with a microwave plasma atomic emission spectrometer (MPAES 4100, Agilent Technologies, Santa Clara, CA, USA). Samples were collected at the inlet of the DWC beds, 0.45 μ m filtered and conserved in freezer (-20°C) prior to analysis.

Plant and fish production

The production of head lettuce (*Lactuca sativa* var. 'Grosse Blonde Paresseuse', Faulx-Les-Tombes, Semailles, Belgium) and basil (*Ocimum basilicum* var. 'Grand Vert', Semailles, Faulx-Les-Tombes, Belgium) were firstly evaluated and compared in DWC and in EAF beds from 18th March 2014 to 23th July 2014. Two cycles of lettuce and basil were evaluated. Then lettuce and basil were grown only in DWC from the 20th April 2015 to 29th September 2015. Cultures were staggered to maximize the number of cycles of lettuce and basil and to maintain an average constant plant biomass (i.e. nutrient uptake) during the experiment. Six crops of lettuce and four crops of basil were analysed. Sowing was done directly on the Rockwool support (RFX-1, 35x35x40, Terra Terra, Genval, Belgium), watered with tap water only, and housed in a climate and light controlled greenhouse. Seedlings were transferred into the aquaponic system 15 days after sowing. Harvesting was done after 28 and 35 days for lettuce and basil respectively, with replacement seedlings synchronized and planted the same day. The air humidity and temperature in the greenhouse was recorded every 10 min with a datalogger (MOINEAU Instruments, Chef-Boutonne, France). Once harvested, shoots and roots fresh biomass weight was immediately determined. The plant production performance was evaluated in terms of fresh plant biomass obtained after each crop cycle.

Fish mass increase was recorded twice a month by weighing 10% of the fish. The total fish biomass was obtained by weighing all the fish at the beginning and at the end of the experiment. The fish production performance was evaluated in term of feed conversion ratio (FCR), calculated by dividing the total feed administered during the experiment by the total fish biomass gain. The growth rate (GR) in grams per day was also calculated by dividing the total fish biomass gain by the number of days of the experiment.

Water use

Water consumption was measured with a flow meter throughout the experiment. Sources of water loss were evaporation, evapotranspiration, spillage, leakage and water exchange. To refill, tap water was directly added into the sump. The discharge of solution in this experiment was done for two main reasons: the cleaning of filters and the control of pH.

Energy use

A portable energy meter (E305EM5, Perel, Gavere, Belgium) was used to determine the daily energy consumption of each electrical components of the system. Total electricity used during the experiment was recorded by an electric meter integrated in the system's electric panel.

Table 4.1. PAFF Box mechanical systems characteristics and their energy demand.

Device and characteristics	Power (W)	Daily use (h)	Daily consumption (Wh)	Daily consumption proportion (%)
Submersible heaters (Eco heater, Superfish, Netherlands)	900	24	21600	57
Energy recovery ventilator (Double flux 90 pavillon'Air, Autogyre, France)	134	24	3216	9
Pump (Projet SE 20/8 tri, Aquatic science, Belgium)	280	24	6720	18
Air blower (HP40 Hiblow, Absolute Air & Gas, United Kingdom)	30	24	720	2
Greenhouse fan(AW 200E4 sileo, systemair, Sweden)	44	12	528	1
LED light (Flexible led strip 5050 60LED/m, Colasse, Belgium)	308.52	15	4627.8	12
Fluorescent light (TL-D super 80, Philips, Belgium)	35	12	420	1

4.2.3 Nutrient mass balances

The nutrient mass balances were established by quantifying the nutrient input, output and accumulation in the system following this equation:

$$\frac{dM}{dt} = M_{feed} + M_{tap\ water} + M_{chemical} - M_{fish} - M_{plant} - M_{sludge} - M_{solution} - M_{lost}$$

Where the nutrient inputs during the all experiment came from: fish feed (M_{feed}), tap water ($M_{tap\ water}$) and iron sulfate ($FeSO_4$) ($M_{chemical}$). The nutrient output comprised the ones trapped in: fish (M_{fish}), plant (i.e. lettuce and basil) (M_{plant}), sludge (M_{sludge}), some accumulated in solution ($M_{solution}$) and the rest was considered as lost (M_{lost}).

The nutrient masses were obtained by multiplying the mass of each component (e.g. fish, sludge...) by its nutrient content concentration following the equation:

$$N_x = Y_x M_y$$

Where N_x is the mass of the nutrient x , Y_x the nutrient concentration in the component y and M_y the total mass of the component y accumulated during the experiment. The values obtained were plotted as histogram in Microsoft Excel software (Microsoft Office 2010).

A target pH around 7 for the aquaponic water was maintained by tap water supply. Its high hardness and alkalinity allowed keeping an acceptable pH so that no addition of chemical base was needed throughout the experiment. $FeSO_4$ (0.5 g Fe/L) was spread on the leaves once per crop cycle.

The quantity in mass (for solids) or volume (for liquids) of each input and output was measured. Except that the mass of sludge produced was based on literature data. Neto and Ostrensky (2013) reported that 23% of the feed mass input was excreted as solids (i.e. sludge production) by tilapia, neglecting feeding loss by assuming that all the feed input was eaten. P, S, Mg, Ca, K, Fe, Cu, Mn, Zn, B, Mo and Na content was determined for feed, tilapia, sludge, lettuce and basil with an ICP-OES (5100 VDV ICP-OES, Agilent Technologies, Santa Clara, CA, USA), after the dry biomass was pulverized and acid mineralized with 1:1 nitric (65%) and perchloric acid (70%). The Total Kjeldahl Nitrogen (TKN) was analysed with a distillation unit (B-324, Buchi, Flawil, Switzerland).

The nutrient content in tap and aquaponic solution was determined with the same methods used for water quality analysis. Tilapia dried flesh was obtained at the Institute for Natural Resource Sciences, ZHAW (Wädenswil, Switzerland).

4.2.4 Statistical analysis

A one-way analysis of variance (ANOVA) was performed to test the significance of difference on the shoots fresh weight between both studied beds (i.e. EAF and DWC). The model included the bed type as fixed effect. Prior to this analysis, the homogeneity of variance was tested using Levene test and the normality of data was tested using Shapiro-Wilk test. All of these calculations were conducted using procedures PROC GLM and PROC UNIVARIATE in SAS software (SAS 9.4).

4.3. Results and discussion

4.3.1 Grow bed selection

During 2014, two production cycles of lettuce and basil were studied. The basil and lettuce shoots fresh weights were not normally distributed and variances were heterogeneous. Therefore, all data were log-transformed allowing a normal distribution and homogeneity of variance between EAF and DWC (Levene test p-value > 0.05 for lettuce and basil). ANOVA results showed a highly significant effect of the bed type on the log-transformed shoot fresh weight of lettuce and basil (p-value < 0.001). For both cycles, DWC beds had always a higher shoot mass production, lettuce had a 10-fold higher mass while basil had a 3 to 5-fold higher mass (Fig. 4.2). The lettuce and basil did not grow efficiently in clay balls contrary to Lennard and Leonard (2006) who compared their growth in NFT, DWC and EAF. This may be explained in our case by diminished nutrient uptake in EAF compared to DWC caused by a reduced water flow around the roots and hence reduced nutrient availability [140]. Also, clay that is negatively charged may adsorb some nutrients [141], making them less available for young roots. Considering the poor growth obtained in EAF beds, DWC beds were selected as only grow bed type in the PAFF Box. All EAF beds were replaced by DWC beds for the 2015 growing season.

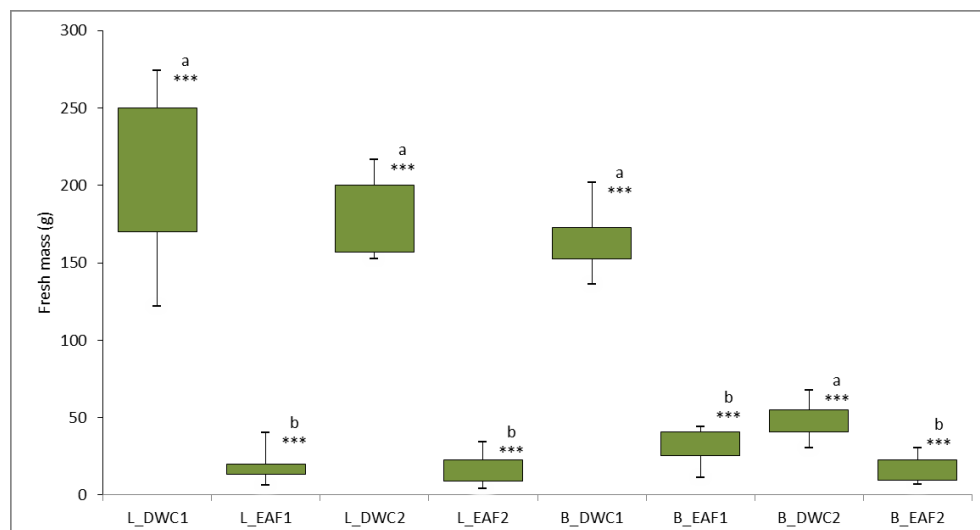


Fig. 4.2. Box-Plot and descriptive statistical data of lettuce (L) and basil (B) shoots harvested in deep water culture (DWC) and ebb and flow (EAF) beds during season 2014 for cycle 1 and 2. Bed types (DWC vs EAF) were compared per crop (L, B) for each cycle (1, 2). Box-Plot tagged with different letters (a, b) are significantly different at the 0.05 level. *** Equal significance level of $P < 0.001$. Low and up bar outside the box are the minimum and maximum of the shoot weight harvested.

4.3.2 Water quality

The water quality was recorded from 20th April to 1st July 2015 and from 15th August to 29th September 2015. On the all experiment, the average water temperature was $25.6 \pm 2.5^{\circ}\text{C}$ with a range of 22 to 31°C . This was suitable for tilapia but was often warmer than the optimum temperature needs of lettuce and basil [12]. Temperatures followed seasonal trends, with lower values at the beginning and at the end of the experiment. Although water heaters were used and the container was insulated and ERV equipped, this was not enough to maintain constant temperatures at night, most likely due to heat loss from the greenhouse. Indeed, air temperatures in the greenhouse were below 25°C at the beginning and end of the experiment with a minimum near 10°C during the night (Table 4.2).

Table 4.2. Relative humidity (RH) and air temperature (T) in PAFF Box greenhouse.

Month	T ($^{\circ}\text{C}$)				RH (%)				
	Average	SD ^a	Min	Max	Average	SD	Min	Max	(n) ^b
April	21.4	10.5	11.7	35.4	56.0	24.7	26.4	82.8	1584
May	24.4	8.3	15.5	34.2	47.9	20.3	25.8	72.3	1583
June	27.1	8.4	18.3	36.9	46.5	19.6	25.6	70.3	4320
August	26.5	8.8	19.3	37.4	55.3	19.2	33.6	75.3	2492
September	21.0	8.0	15.2	32.2	62.8	19.0	38.5	79.7	4176

^a Standard deviation. ^b Number of observations.

The pH and alkalinity decreased through the experiment from 8.71 to 5.5 for pH and 145 to 0 mg CaCO_3/L for alkalinity (Fig. 4.3A). High pH and alkalinity at the beginning is due to earlier PAFF Box filling with tap water that had an alkalinity of 305 ± 35.5 mg CaCO_3/L . During the experiment the feed input partly excreted in ammonia by fish and its conversion in nitrite and nitrate by nitrifying bacteria leaded to release H^+ [142]. This phenomenon acidified the water and is then supposed to be the main factor removing the alkalinity. The presence of plants in the water loop did not affect this well-known acidification tendency in RAS water. The sudden increase in alkalinity and pH during mid-August (day of experiment 116) is due to a replacement of about 1000 L of solution with tap water in order to restore acceptable value of alkalinity and pH for the second period of experiment. Regarding the constant decrease in pH and alkalinity, constantly adding base and carbonate source in the water, cannot be avoided.

The EC rose from 721 to 1238 $\mu\text{S}/\text{cm}$ during the first part of the experiment (April to July 2015, day 1 to 68), stayed around 1210 $\mu\text{S}/\text{cm}$ during the second part (August to mid-September, day 116 to 162)) and slightly decreased at the end of the experiment (Fig. 4.3B). Accordingly to the EC trend, the macro and micronutrients increased in concentration during the first 3 months of experiment but this stopped in the second part and concentrations even went slightly down at the end of the experiment. Especially, nitrate and calcium accumulated faster than other nutrients and impacted the EC (Fig. 4.3B). The dynamics of accumulation for each nutrient are reported in part 4.3.6. This EC rise indicates that nutrients accumulated in solution during the first 3 months due to feed input and fish excretions. During that period plant nutrient uptake and water exchange could not compensate the accumulation (i.e. daily water exchange of 2-3.6%, Fig. 4.4). However, another situation appeared

in August and September because of better plant growth but mainly because the water exchange rate was increased (i.e. 5-5.2%).

This shows thus the planting area could not balance the nutrient input itself necessitating a high exchange of water to control nutrient accumulation. Hence, the appropriate daily water exchange between 3.6 and 5.2 % prevented excessive accumulation, without removing too much nutrient content.

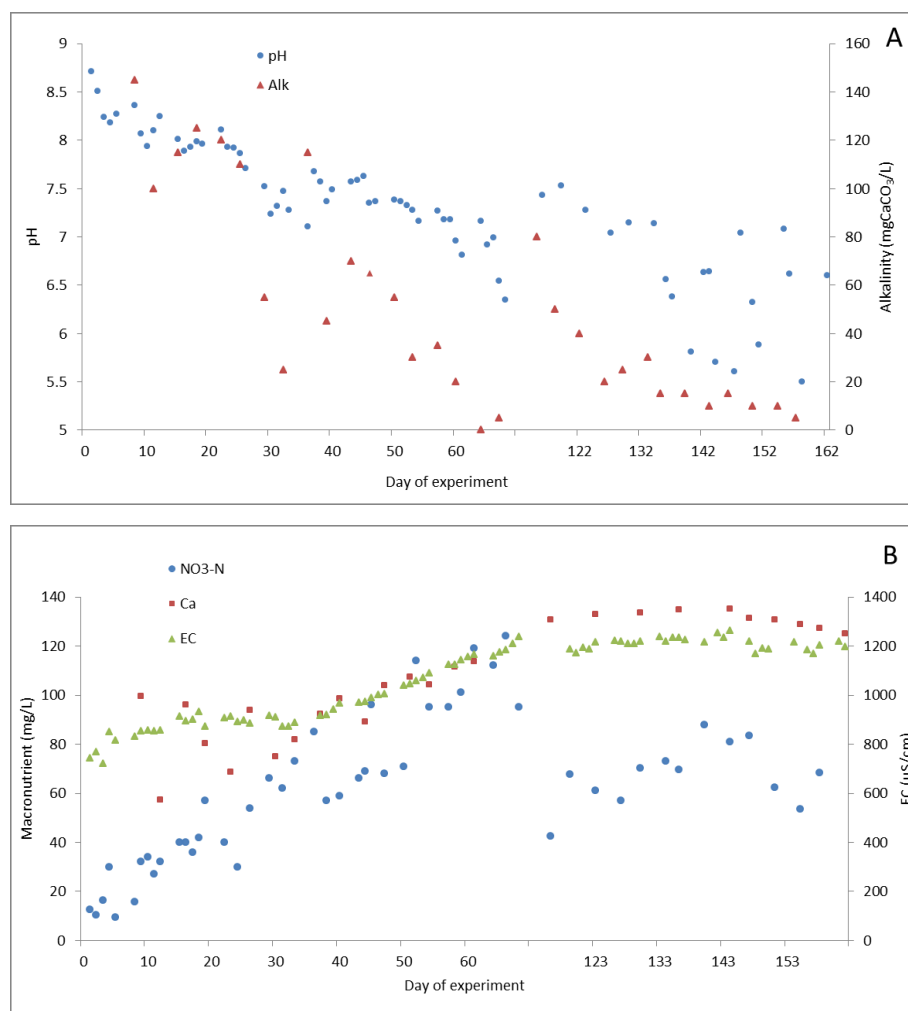


Fig. 4.3. Water quality: (A) pH and Alkalinity; (B) Electro-conductivity (EC), nitrate (NO₃⁻-N) and calcium (Ca²⁺) concentrations. The measurements were paused from day 69 to 115 for maintenance reason.

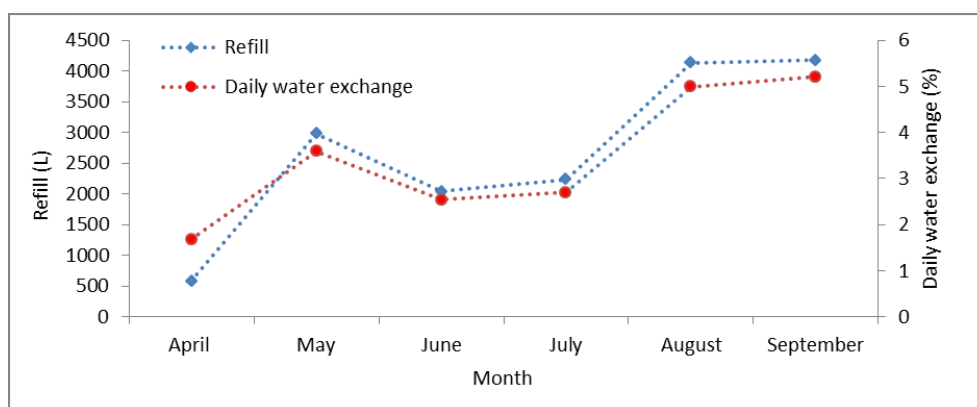


Fig. 4.4. Refill water use and daily water exchange.

4.3.3 Plant production

Every two weeks lettuce and basil were harvested. During July to mid-August (day of experiment 69 to 115) the production was paused for maintenance reason. The average and standard deviation (SD in brackets) shoots mass for the all season was 175.08 (\pm 71.51) and 125.41 (\pm 85.97) g for head lettuce and basil, respectively. In comparison in conventional hydroponics, Barbosa et al. (2015) reported that the yearly average of head lettuce shoots harvested after a complete 30 day cycle was 144.6 g. This is lower than the lettuce growth achieved in the PAFF Box during appropriate weather conditions (May to beginning of September). Good growth was obtained for plants grown in August and harvested beginning of September (Fig. 4.5) corresponding to yields of 2.4 kg/m² and 1.3 kg/m² for lettuce and basil, respectively. However, in hydroponic lettuce production monthly yields of 3.4 kg/m² are achievable year round [143]. This may be explained by higher plant density used in conventional setup.

The high harvests of September may be explained by better water quality and more appropriate environmental conditions. For that period (day 116 to 162) the pH oscillated between 6 and 7 while it was higher than 7 during May and beginning of June (day 1 to 68, Fig. 4.3A). The EC was at its highest value also at that time meaning higher nutrients concentration in solution. The air temperature in greenhouse was more stable with higher minimal temperature (i.e. close to 20°C) and so warmer night time (Table 4.2). Lower yields obtained at the end of September can be linked with lower temperature in the greenhouse and shorter daylight period. In brief, on the overall experiment a sustained growth was achieved and no nutrient deficiency or disease was visually observed.

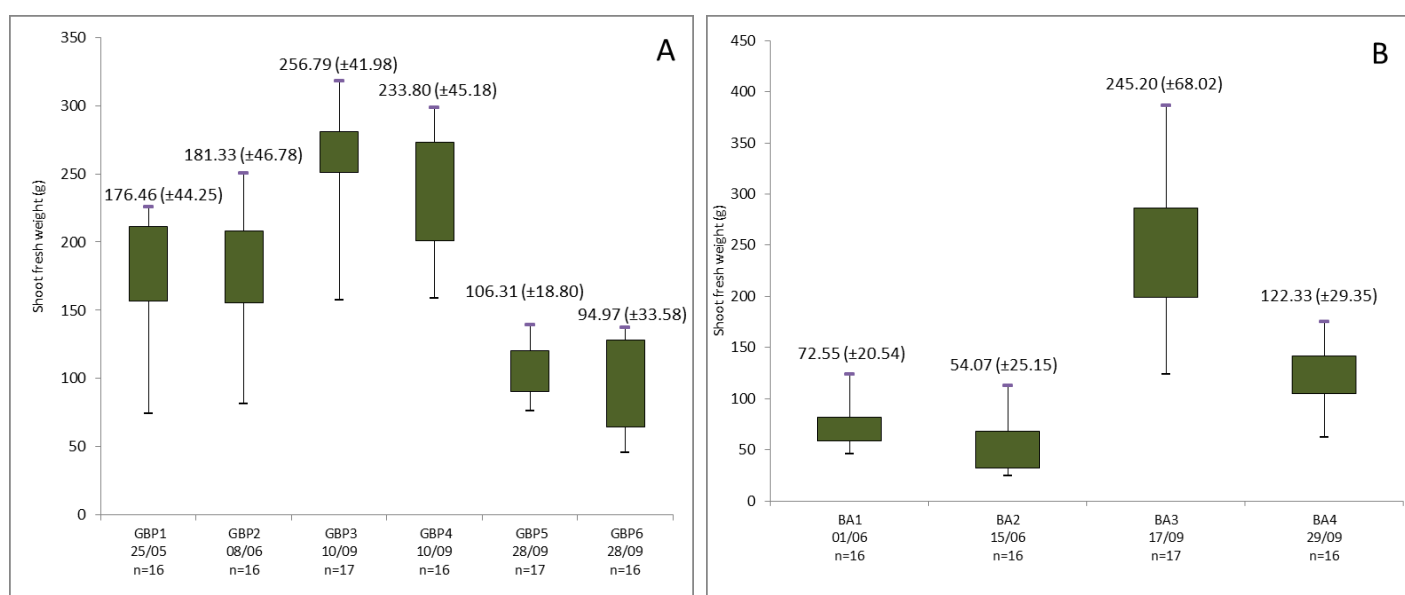


Fig. 4.5. Shoot fresh weight Box-Plot distribution for each crop cycle of (A) lettuce var. 'Grosse blonde paresseuse' (GBP 1 to 6), (B) Basil var. 'Grand vert' (BA 1 to 4). The harvest date (day/month) is mentioned under each crop name plus the number of heads (n). Above each Box-Plot is the average (\pm standard deviation). Min and max (low and up bar outside the box) are the minimum and maximum of the shoot weight harvested.

4.3.4 Fish production

Tilapia biomass gained a total of 30 kg during the 164 days of experiment with an average individual final weight of 236 g. No fish were harvested during the study but the mortality rate was 5% which can be considered as normal for tilapia [60,111].

The water quality was maintained in the right range (Fig. 4.3), the DO, TAN and NO₂-N in the fish tanks were on average 4.4 (\pm 0.8), 0.71 (\pm 0.52), and 0.30 (\pm 0.44) mg/L, respectively.

The FCR was 1.56 with a GR of 1 g/d. These results are better than 1.7 - 1.8 previously reported for tilapia reared in aquaponics [45] but lower than performance expected in productive recirculating aquaculture, where FCR of 1.25 and GR higher than 2 g/d are achieved [60,111]. In our experiment, the fish were fed at a constant rate independently of their body mass increase, in order to keep a constant nutrient input in the system and this might explain the lower GR obtained. The water temperature kept around 25°C for plant roots should also have contributed to slow tilapia growth as their growth rate is correlated to water temperature and 28°C is their optimal rearing temperature [111].

4.3.5 Water and energy used

At the start of the experiment, 2673 L of water were necessary to fill the system, and thereafter there was a regular need of refill water to compensate the loss due to evaporation, evapotranspiration, spillage, and water exchange (Fig. 4.4). A total amount of 16155 L of refill water has been used during the experiment, giving an average daily water need of 97 L. This represents a daily water exchange of 3.6% which is in the 0.5-10% range observed in aquaponics [45,144], lower than (5-20%) achieved in RAS [111]. Regarding the daily feed input, the water exchange rate was 243 L/kg feed added/d which is in the 100-1000L/kg feed/d range of conventional recirculating aquaculture systems [10]. These results confirm aquaponics as a water-efficient method for plant and fish production.

Electricity was the sole energy form used. During the 71 days of the measured period for energy consumption, the total consumption was 2641 kWh with an average daily consumption of 37 kWh. The water heating was the most consuming part with 57% of the daily consumption followed by the water pump (18%) (Table 4.1). The LED lighting took also an important part (i.e. 12%) but was only used to compensate for the shade of upper DWC beds cast on the lower ones.

In the studied aquaponic system, the water and energy consumption counted for simultaneous vegetable and fish production. Some water and energy needs overlap for plants and fish in a combined aquaponic system. Hence, if the same yields of fish and vegetables are achieved as in conventional systems, theoretically, the water and energy consumption will automatically be less than the sum of separated production systems. It is then necessary to link the resources consumption to fish and vegetables production together. In our system, the production of 1 kg of vegetable required, in average, 488 L of water and 169 kWh but simultaneously 0.878 kg of tilapia was also produced. The water and energy needs can be split (i.e., 50/50) then it can be considered that 1 kg of vegetable consumed 244 L and 84.5 kWh and proportionally 1 kg increase of tilapia consumed 278 L and 96.2 kWh.

As comparison, Love et al. (2015) reported 104 L of water, and 56 kWh used for 1 kg of crops and 292 L and 159 kWh to produce a 1 kg increase in tilapia. Our results are close but interestingly their crops consumed twice less water and energy, probably because they produced year round more vegetables than in the PAFF Box. Timmons and Ebeling (2013) predicted the use of 16.23 kWh to produce 1 kg of fish in a large scale RAS of 500 tons of fish produced per year but with a daily water

exchange of 5-20% of the total volume. Ayer and Tyedmers (2009) reported 84.83 kWh/ kg of Salmon production in RAS while d'Orbcastel et al. (2009) reported 10.68 kWh/kg for Rainbow trout in RAS. This confirms that larger scale production in RAS allows economy of scale for energy but not for water consumption. On the hydroponic production side, the survey of Barbosa et al. (2015) in Arizona climate conditions reported 20 L of water, and 25 kWh used for 1 kg of lettuce produced as yearly average in a warmed and lighted greenhouses.

Our system had consumption in the same range as other small scale system [144]. Compared to RAS alone, the system was very efficient in water but not in energy use. The reduction in water use is supposedly due to the nitrate uptake by plants enabling to reduce the water exchange rate. Compared to hydroponic alone, our system was less efficient. The water and energy consumption in hydroponics are lower because most of the energy is consumed only in winter for heating air and lighting the greenhouse while in our system energy was required permanently to warm up the water for fish. Also, in hydroponics, water exchange is only necessary to compensate the evaporation, plants evapotranspiration and annual cleaning [143].

To mitigate the high energy demand of our system, a considerable reduction in electricity consumption could be achieved by using fish species thriving in lower temperatures thus better adapted to the Belgian climate. Another solution could be the use of other technologies (e.g., solar panels) to warm up the water. A better sizing of the pump and flows will also reduce the energy demand. Pumping water in the greenhouse at the top of the container demands more pumping energy than a system build with fish and plant on the same floor. The LED lighting electricity demand could also be avoided. Therefore, the bed design should be revisited, for example by increasing the distance between or using vertical grow beds to minimize shading. So, the design of the PAFF Box system itself increases the use of energy for water pumping. The size of the system is also important as in larger system economies of scale are possible.

Table 4.3. Macro- micronutrient inputs and their ratio to N in PAFF Box compared to Resh (2012) hydroponic solution formulation.

	N	PO ₄ ⁻ P	K	Ca	Mg	SO ₄ ⁻ S	Fe	B	Cu	Zn	Mn	Mo
Total input in PAFF Box (kg)	1.17	0.40	0.47	2.47	0.49	0.90	0.01 27	0.00 06	0.00 10	0.00 48	0.00 24	-
Calculated ratio to N in Resh's (2012) solution	1	0.3	1.1	1.1	0.2	0.6	0.02 63	0.00 26	0.00 05	0.00 05	0.00 26	0.00 03
Calculated ratio to N in PAFF Box	1	0.3	0.4	2.1	0.4	0.8	0.01 08	0.00 05	0.00 09	0.00 41	0.00 20	-

4.3.6 Macronutrient mass balances

The record of the nutrient mass balances enabled identifying where the nutrients were trapped in the system and which proportion was recycled or lost. The fish nutrient input was constant during the period of study which allowed drawing a picture of their mass balances.

The total nutrient inputs during the experiment can be compared to the inputs used in conventional hydroponic solution in order to identify unbalance regarding the plant needs (i.e., for lettuce in this case). A practical way to do it is converting the inputs into ratio to N (Table 4.3). The comparison of ratios to N between Resh (2012) and PAFF Box shows that K input in PAFF Box were halved compared to Resh (2012) while Ca, and Mg were roughly doubled. P and S were quite equivalent. Almost all the N, P and K was introduced in the system from the feed, while more than 75% of Ca, Mg and S was introduced by tap water (Fig. 4.6A) because their concentrations were consistent with it (Table 4.2 in

supplementary material). Tap water contained low concentrations of NO_3^- -N and PO_4^{3-} -P (i.e. <3.5 mg/L) but also only 0.61 mg/L of K^+ . Nutrients present in tap water have the advantage to be directly available for plants unlike the ones in feed. Surprisingly, 75% of the Na was also introduced by tap water. Tap water can be a convenient way to input some nutrients provided its quality and nutrient content is appropriate. That was the case in this study even if some Na was present.

After being introduced from the feed or tap water, nutrients were allocated in different parts of the system as into tilapia body, lettuce and basil, solution and sludge. Some were also lost due to water spillage and/or trapped somewhere else in the system (Fig. 4.6). The allocation of the nutrient contained in the solution evolved during the experiment. The nutrient concentrations in solution used to build Fig. 4.6 are the concentrations measured at the end of the experiment. Nutrients accumulated in solution during the experiment. The accumulation rates are nutrient specific and depend mainly on their input quantity and under which form (i.e. soluble or insoluble) they are released by fish [41]. The dynamics of accumulation for each nutrient is of primary importance to identify the suitability of using fish water to produce plants.

Regarding the macronutrients dynamics in solution, at the end of the experiment NO_3^- -N, Ca^{2+} , SO_4^{2-} -S, Mg^{2+} , K^+ and PO_4^{3-} -P increased by 58, 31, 16, 12, 7 and 2 mg/L respectively (Table 4.4). NO_3^- -N and Ca^{2+} accumulated faster than other macronutrients. It can be assumed that if water exchange was reduced they could reach 160-200 mg/L, which is the concentration range found in conventional hydroponic solutions [145]. If nitrate and other macro nutrients concentrations increase, tilapia health has to be considered and appropriate solution would be needed not to overtake toxic threshold. Notably, tilapia tolerates quite high concentration of nitrate compared to other fish [146]. Mg^{2+} and SO_4^{2-} -S were in a suitable range that was facilitated by their substantial concentration in tap water. In turn, K^+ and PO_4^{3-} -P accumulated the least and remained very low in solution (i.e. < 10 ppm). They were thus far under the concentration recommended in hydroponic solution of 210-430 and 50-62 mg/L for K^+ and PO_4^{3-} -P respectively [11,12]. The high loss (> 80%) of K^+ and PO_4^{3-} -P is a special concern and is unfortunately not identifiable with the data available from this study. Most of the P is normally supposed to end up in sludge [18,19]. K^+ and PO_4^{3-} -P were low concentrated in solution and so had a higher proportion trapped in basil and lettuce compared to other macronutrients (Fig. 4.6B).

4.3.7 Micronutrient mass balances

The comparison between Resh (2012) and PAFF Box in terms of ratios to N shows that Fe input were halved compared to Resh (2012) while Cu was roughly doubled (Table 4.3). B input was 5 times lower in PAFF Box compared to Resh (2012) while Zn was 8 times higher. Mn was quite equivalent. Mo input was not detected. Almost all Fe, Zn and Mn was introduced in the system from the feed, while 50% of B and 30% of Cu were introduced by tap water (Fig. 4.6A).

Regarding the micronutrient dynamics in solution, at the end of the experiment B, Fe, Cu, Zn and Mn increased by 26, 6, 5, 4 and 2 $\mu\text{g/L}$ respectively (Table 4.4). All micronutrients at the end of the experiment, except Mo, were present in solution in a range from 5 to 50 $\mu\text{g/L}$. Though, they are used in a range of 100-500 $\mu\text{g/L}$ in hydroponics [11,12]. B accumulated most sufficiently in the water compared to the other micronutrients. Even then, it reached a concentration of 52 $\mu\text{g/L}$, which is still 6 to 10 times lower than the concentrations found in hydroponic solutions. Fe was present in a range of 5-10 $\mu\text{g/L}$ which is 500-1000 times lower to the concentration used in hydroponics (i.e. 2.2-5 mgFe/L). To prevent iron deficiency, FeSO_4 was sprayed on the plant leaves and no yellowing was noticed. Mn accumulated poorly and reached a concentration of only 6 $\mu\text{g/L}$. Regarding Na, which is

a constant concern in aquaponics as it is released in solution by feed and fish. It is poorly absorbed by vegetables and then tends to accumulate until toxic level for hydroponic production [147,148]. Indeed, in this experiment its concentration increased by 20 mg/L, from 20 to 40 mg/L. It was slightly trapped in vegetables (Fig. 4.6B) and only water exchange rate could control its accumulation in solution.

Mo was hardly detectable in solution, in tap water and feed. It was not quantifiable because it was too close to detection limits, and thus, to avoid deficiency its complementation is presumably required.

Key nutrients as K, P, Fe, Cu, Zn, Mn and Mo are the ones that accumulated the less and were very low in solution compared to standard solutions used in hydroponics. However, only FeSO_4 was once per crop sprayed on the leaves and sustained lettuce and basil growth was achieved and no visible deficiency symptoms appeared. Several reasons may explain this sustained growth while key nutrients were low concentrated. In DWC, nutrients can be absorbed at a constant rate regardless of the nutrient concentrations as soon as they are higher than a limit threshold [149]. To our knowledge these thresholds are not well yet established in aquaponic condition and may have not been overtaken during the experiment. Moreover, bacteria promoting nutrient uptake may have occurred in the aquaponic solution and reduced these thresholds. Also some organic nutritive material may have been present but not measured.

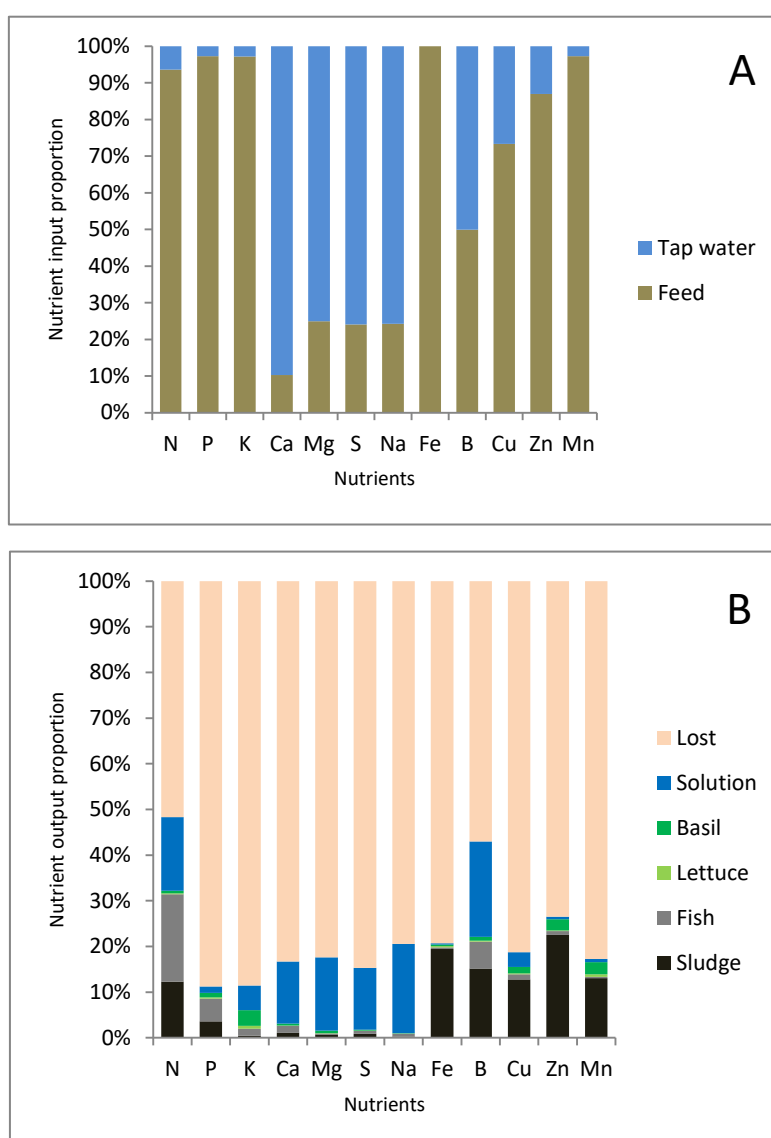


Fig. 4.6. Nutrient Budgets: (A) nutrient input as feed and tap water, (B) nutrient output, nutrients were allocated in different parts of the system as into tilapia, lettuce, basil, solution (i.e. PAFF Box water) and sludge. Nutrients lost are the one not trapped in the above cited parts. Lost is due to water spillage, untracked allocation somewhere else in the system and measurement preciseness.

4.3.8 Nutrient loss

An important proportion of N and P and micronutrients ended up in sludge and then were discharged out of the system (Fig. 4.6B). P, Fe and the other micronutrients were in low concentration in solution because they are released mainly as insoluble forms by fish and end up in sludge. In order to improve their recycling, a solution should be to solubilize and reinject them in solution. This could be achieved by sludge digestion in a bioreactor [150] and injection of nutrient rich supernatant back in the system.

As K, P, Fe, Mn and Mo seem to be not released by fish in sufficient soluble quantity and as K, Fe and Mo were not inputted in sufficient proportion (Table 4.3), complementing the aquaponic solution with all these nutrients seems necessary to reduce the potential risk of deficiency for plants in such system.

Interestingly, 50 to 88 % of the nutrients were lost (Fig. 4.6B). Presumably, most of the lost is due to water spillage but some nutrients could have been trapped somewhere else in the system like on tank walls, bottom of DWC beds, filter media, biofilm, etc.

The quantity of the water lost by evaporation and spillage was not measured during the experiment, but if we assume that with a daily water exchange of 3.6 %, evaporation counted for 1.5% and 2.1% for spillage, this gives an approximation of 9400 L discharged in total. Taking the average solution nutrient content, this would count for a loss of 49, 8, 18, 40, 45 and 38% of N, P, K, Ca, Mg and S, respectively. Close to 50% of the macronutrient could have been lost by water spillage.

These findings underline the necessity to minimize the water discharge for improving nutrient recycling in a one loop aquaponic system such the one studied here. In aquaponic system in production, water exchange could be reduced until about 1% [144]. Ideally, water loss would be due only by evaporation. However, in these conditions the fish welfare should be evaluated in order to make sure suitable rearing conditions are maintained. Fish species thriving in water with relatively high EC and nitrate concentration should be preferred for aquaponics.

Table 4.4. Macro- micronutrients average concentration in PAFF Box solution.

Nutrient	Average	SD ^a	Min	Max	(n) ^b
mg/L					
TAN	0.7	0.5	0.1	2.3	50
NO3-N	61.3	29.3	9.4	124.0	50
P	3.3	1.8	0.9	7.6	28
K	9.2	8.9	1.6	30.3	26
S	36.6	11.4	16.7	63.3	26
Ca	107.1	23.0	57.3	135.0	26
Mg	23.5	6.6	10.5	31.8	26
Na	31.8	7.8	14.3	46.6	28
µg/L					
B	39.3	10.1	16.6	52.3	26
Cu	10.9	3.6	2.8	17.9	28
Fe	7.5	2.0	3.2	12.0	16
Mn	4.4	3.5	1.9	13.8	19
Zn	8.7	9.2	0.0	39.9	26

^a Standard deviation. ^b Number of observations.

4.4. Conclusion

The data obtained during this study confirm aquaponics as a consistent alternative to produce fish and vegetable. Lettuce and basil growth obtained in DWC beds with only fish feed and tap water as input was sustained and no nutrient deficiency or diseases were observed. In terms of water consumption, the PAFF Box system was relatively efficient when compared to RAS. However, means to reduce energy consumption should be explored as alternative solutions for warming the water and fine-tuned pumping setup.

With a feed daily input of 42g per m² of DWC beds, the nutrients tended to accumulate in water correlated to a pH decrease. Key nutrients as K, P, Fe, Cu, Zn, Mn and Mo, remained low with a presumable risk of deficiency for plants. Complementing the aquaponic solution with these nutrients as well as additional base to counterbalance acidity seems unavoidable in such system. For the nutrients that accumulated quickly, only water exchange showed its ability to control their accumulation. However, our data shows that even a low water exchange rate (i.e. 3.6 %) implicates a high nutrient loss. In the willingness to decrease aquaponics environmental footprint, the optimal feed to plant area ratio should be further studied in parallel with solutions to reduce water spillage. Nutrient recovering from sludge should also be considered. Other designs could be explored such as decoupled aquaponics systems.

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Supplementary Material

Table 1. Balance sheet of energy and water used to produce tilapia, lettuce and basil in PAFF Box.

	May	June	August	September	Monthly average	1 kg of vegetables
Energy (kWh)	1173	1135	1173	1135	1154	169
Water (L)	2980.5	2039.8	4136.2	4177.2	3333.4	488.1
Tilapia (kg)	6.200	5.940	6.138	5.700	5.995	0.878
Lettuce and basil (kg)	2.980	6.781	0.000	17.559	6.830	-

Table 2. Quantity of matter (input and output) and its nutrient content in PAFF Box.

	Total amount (kg dry matter)	N	P	K	Ca	Mg	S	Na	Fe	B	Cu	Zn	Mn
		mg/g							µg/g				
Feed	46.90	23.4	8.33	9.69	5.41	2.60	4.60	2.84	270.8	6.4	16.4	88.7	49.1
Sludge	10.79	13.3	1.34	0.20	2.42	0.32	0.73	0.12	229.2	8.5	12.4	99.7	28.7
Tilapia ^a	8.43	26.6	2.37	0.85	4.48	0.13	0.66	0.41	5.2	4.2	1.4	4.9	0.8
Lettuce	0.12	17.3	9.15	22.23	12.67	3.25	4.22	1.96	243.5	10.5	14.5	63.1	105.9
Basil	0.46	14.9	8.63	34.94	24.75	5.75	3.38	0.80	109.5	11.1	32.7	251.3	135.8
	Volume (L)	mg/L							µg/L				
Tap water	21501.7	3.47	0.51	0.61	102.97	17.09	31.71	19.36	0	14	13	29	3
Solution	2673.4	70.7	2.05	9.48	125.13	29.33	45.43	40.30	12.0	47.0	12.7	9.7	6.0

^aObtained at the Institute for Natural Resource Sciences, ZHAW (personal communication)

5. Lettuce (*Lactuca sativa* var. capitata cv. Sucrine) growth performance in complemented aquaponic solution outperforms hydroponics.

This chapter has been published as a manuscript entitled “Lettuce (*Lactuca sativa* L. cv. Sucrine) growth performance in complemented solution encourages the development of decoupled aquaponics.” Delaide, B.; Goddek, S.; Gott, J.; Soyeurt, H.; Jijakli, H. M. In *Water (MDPI)* 2016, 1–11.

5.1 Introduction

Aquaponics is an integrated closed-loop multi-trophic food production system that combines elements of a recirculating aquaculture system (RAS) and hydroponics [44,48,136]. Aquaponic systems where the nutrient flows and concentrations within the different components (e.g., aquaculture and hydroponic parts) are independent of one another are called decoupled aquaponic systems (DAPS) [150], or double recirculation aquaponic systems (DRAPS) [151]. Aquaponic systems designed with independent loops offer greater control over the hydroponic component, where water can be complemented with mineral salts for increased nutrient concentrations, and pH adjusted to fall within an optimal range. A number of studies have attempted to show optimal nutrient solutions for growing lettuce in hydrocultural environments [11,12]. Table 5.1 provides the results obtained by Resh [12]. Several factors determine the nutrient uptake performance of plants, including the availability of all essential nutrients, their presence in appropriate ratios, and favourable external conditions, for instance, pH, temperature, O₂, and CO₂. According to Liebig’s ‘law of the minimum’ nutrient availability constitutes a critical factor; the nutrient least available determines the maximum growth rate. Several researchers [145,152,153] reported an enhanced NO₃⁻ uptake when the nutrient solution’s N source contained between 5% and 25% NH₄⁺. At a pH of 6.8, both NO₃⁻ and NH₄⁺ are equally absorbed, whereas NO₃⁻ is preferred in acidic and NH₄⁺ in alkaline environments [145]. The influence of pH on nutrient uptake is also observed for other macro- and micronutrients. Indeed, a pH from 6.0 to 8.0 is optimal for the uptake of macronutrients such as phosphorus (H₂PO₄⁻, HPO₄²⁻ or PO₄³⁻), potassium (K⁺), sulphur (SO₄²⁻), Calcium (Ca²⁺), and Magnesium (Mg²⁺). Considering that micronutrients such as Iron (Fe³⁺, Fe²⁺), manganese (Mn²⁺), boron (BO₃²⁻, B₄O₇²⁻), copper (Cu²⁺, Cu⁺), and zinc (Zn²⁺) are preferentially absorbed at pH values below 6.0 [154,155]; the trade-off pH in hydroponics is approximately 5.5–6.0 [12].

Table 5.1. Optimal nutrient solutions for lettuce growth using nutrient flow technique (NFT) and in the University of the Virgin Islands (UVI) system.

System	pH	EC mS/cm	NO ₃ ⁻ -N	NH ₄ ⁺ -N	PO ₄ ³⁻ -P	K ⁺	Ca ²⁺	Mg ²⁺	SO ₄ ²⁻ -S	Fe ²⁺
			mg/L							
Hydroponics (NFT) [12]	5.5–5.8	1.5–2.0	165	25	50	210	200	40	113	5
Aquaponics (UVI) [40]	7.0–7.6	0.7–0.8	42.2	2.2	8.2	44.9	11.9	6.5	15	2.5

In the domain of efficient agriculture the root: shoot ratio of plants has become an important issue. Root hairs will be limited or almost absent if the plants are exposed to NO₃⁻-N concentrations of at least 100 mg/L or to high P content. However, a phosphorus deficiency in the plant’s tissues can be observed if their Al³⁺ or Ca²⁺ concentrations are too high at the root surface. Sonneveld and Voogt

[11] showed that a Ca:P ratio of approximately 3:1 was the most efficient target value. Jones [145] also showed that the optimal Ca: Mg ratio was 3:1. Furthermore, uptake imbalance mostly occurs when K^+ concentrations are too high in the system in proportion to Ca^{2+} and Mg^{2+} . In such cases, K^+ is more readily absorbed than Ca^{2+} and Mg^{2+} .

Although lower nutrient levels are observed in one-loop aquaponic systems compared to hydroponic cultivation methods, a number of researchers have reported a similar lettuce yield [86,156]. In most recent studies the growth of lettuce has been measured only in aquaponic (AP) and hydroponic (HP) systems. However, the growth performance of aquaponic and hydroponic lettuce exposed to similarly high nutrient concentrations has not been comprehensively investigated. It remains unclear to what degree the aquaculture effluent generates an impact (negative, neutral, or positive) on plant growth performance.

The leaf nutrient content can give information on plant health (e.g., nutrient deficiency detection); however, this has not yet been investigated in aquaponics. The strict regulations within the EU concerning the maximum levels of contaminants in food [157] further the need for leaf composition analysis.

Consequently, the objective of this study was to compare shoot and root yields and leaf nutrient content of lettuce grown in conventional hydroponic solutions to those grown in complemented and normal aquaponic solutions.

5.2 Materials and Methods

Two identical trials (trial 1 and 2) were conducted between May and September 2015 in the climate-controlled experimental greenhouse of the Integrated and Urban Plant Pathology Laboratory of the University of Liège (Gembloux, Belgium, latitude 50°33' N, longitude 4°41' E, altitude 157 m). Trial 1 started on 21 May 2015 and trial 2 on 20 August 2015. The air temperature and relative humidity in the greenhouse were recorded every 30 min with a USB datalogger (MOINEAU Instruments, Chef-Boutonne, France) in order to control the similar climate conditions between trial 1 and 2. Light availability was dependent on the natural fluctuations of solar irradiance. The total accumulated solar radiant exposures measured from a local meteorological station (IRM-KMI Ernage, Gembloux, Belgium) were 316.21 and 180.94 MJ/m² for trial 1 and 2, respectively. The experimental setup consisted of three identical DWC systems (i.e., AeroFlo 28, GHE, Fleurance, France) that were exposed to the specific nutrient solutions. Each AeroFlo system comprised a sump that was connected to four DWC channels containing seven holes each. The total planting area was 1 m² per system with a water volume of 100 L that was constantly recirculated by a submersible pump.

For both trials 15-day-old lettuce seedlings (*Lactuca Sativa* 'Sucrine', Semailles, Faulx-Les-Tombes, Belgium) were placed into the AeroFlo and harvested after 36 days.

The AeroFlo systems were filled with a fresh 100 L solution on a weekly basis to maintain stable nutrient conditions for better reproducibility and comparison among treatments. In order to validate such stability, during trial 2 the water nutrient content of the one-week-old solution was sampled for analysis before spillage, and another sample of the fresh solution was taken directly after the refill.

5.2.1 Nutrient Solution Formulation and Control

To match the nutrient concentration targets high-purity mineral salts were added. The HP solution (i.e., the control) and the CAP solution were formulated to have their nutrient concentrations equal to conventional NFT lettuce nutrient solutions based on Resh [12]. The HP control solution was formulated with 100% rainwater and the added high-purity mineral salts. The CAP solution consisted of 100% RAS water complemented with high-purity mineral salts to reach the same nutrient concentrations as in the HP control solution. The RAS water was taken directly from the sump of a running tilapia RAS fed with a 40% protein, 12% lipid, and 3.7% crude fibre feed (Omegabaars, Lambers-Seghers, Baasrode, Belgium). The water did not receive any treatment prior to being used in the AeroFlo system. The AP solution was designed to reproduce the macro- and micronutrient concentrations found in the single loop aquaponic system of the University of Virgin Islands (UVI) published in Rakocy et al. [40]. It was formulated with RAS water. The concentrations of several nutrients in RAS water were higher than the concentration targets. RAS water was, therefore, diluted 1:10 in rainwater, and high-purity mineral salts added to match the nutrient concentration targets. For all treatments, the pH was adjusted by adding HCl and Na₂CO₃. PH, electrical conductivity (EC) and nutrient concentration targets of the three solutions are presented in Table 5.1.

The RAS water macronutrient content was analysed with a multiparameter spectrophotometer (HI 83200, HANNA instruments, Woonsocket, RI, USA) with the following reagents: HI 93700 (TAN), HI 93728 (NO₃⁻), HI 93717 (PO₄³⁻), HI 93751 (SO₄²⁻), HI 93750 (K⁺), HI 93752 (Ca²⁺), and HI 93752 (Mg²⁺). The macronutrient analysis allowed the calculation of salt quantities necessary to add to the AP and CAP solution formulations. Salt additions were calculated with the hydroponic-specific HydroBuddy free software (<http://scienceinhydroponics.com/category/hydrobuddy>) to match the target concentration values. Sulphate was used as a degree of freedom. For the first experimental week only half the quantities of salts were added in order to limit the EC and allow the seedlings to adapt to the nutrient solution and avoid osmotic shocks. The mineral salts used for the macronutrients were MgSO₄·7H₂O, NH₄NO₃, K₂HPO₄, Ca(NO₃)₂·4H₂O, KNO₃, K₂SO₄, and HNO₃ (65%), and for the micronutrients were Fe-EDTA, MnSO₄·4H₂O, CuSO₄·5H₂O, ZnSO₄·7H₂O, (NH₄)₆Mo₇O₂₄·4H₂O, and H₃BO₃.

The water EC, dissolved oxygen (DO), temperature, and pH were controlled regularly. EC was recorded with a conductivity tester (AD31 Waterproof, ADWA, Szeged, Hungary). The DO and temperature were measured with a DO meter (HI 98193, HANNA instruments, Woonsocket, RI, USA), and pH with a pH-meter (Inolab pH level 1, WTW, Weilheim, Germany).

To assess water quality, the concentrations of P, K, Ca, Mg, S, Fe, Cu, Zn, B, Mo, Mn, and Na in AeroFlo solutions were measured during trial 2 with an ICP-OES (5100 VDV, Agilent Technologies, Santa Clara, CA, USA). Total ammonia nitrogen (TAN) was measured with a spectrophotometer (HI 83200, HANNA instruments, Woonsocket, RI, USA) using the reagent HI 93700 based on the Nessler method. NO₃⁻-N was measured with a Nanocolor standard test (Ref 918 65, Macherey-Nagel, Düren, Germany) using the 2,6-dimethylphenol method. Samples of 150 mL of solution were taken directly from the sump of each AeroFlo just before and just after weekly renewal of the solution. Samples were 0.45-µm-filtered (Acrodisc, Pall corporation, Portsmouth, UK) and frozen immediately after collection. They were analysed for TAN within 24 h and for nitrate within 30 days. All measurements were performed in triplicate.

To detect potential differences in water composition among the used systems, the measured micro- and macronutrient concentrations and the key physiological macronutrient ratios (i.e., TAN:NO₃-N, Ca:P, Ca:K, Ca:Mg) were analysed using a repeated model because of week-dependent measurements. The model included the treatment as the fixed effect, the week as the repeated effect, and their corresponding interaction realized as shoot and root yields. All calculations used PROC GLM in SAS software (SAS 9.4., Cary, NC, USA), and a Duncan multiple-comparison was used to assess the significance of treatment differences. These differences are reported in this paper as least square (LS) means.

5.2.2 Lettuce Growth and Leaf Nutrient Content

During the lettuce harvests of trials 1 and 2, the weight of both shoots and roots were recorded and then analysed by a one-way analysis of variance (ANOVA). The fixed variation factor was the treatment (i.e., AP, CAP, and HP).

The lettuce leaf nutrient content (P, K, Ca, Mg, S, Fe, Cu, Zn, B, Mo, Mn, and Na) was measured during trial 2 with an ICP-OES (5100 VDV, Agilent Technologies, Santa Clara, CA, USA). Prior to the ICP analysis, six lettuce plants per treatment were randomly chosen and were dried in an oven at 105 °C for 48 h, pulverized together, and acid-mineralized with 1:1 nitric (65%) and perchloric acid (70%). Nutrient content was analysed by a one-way analysis of variance (ANOVA) using the treatment as the fixed effect. A Duncan multiple-comparison was used to assess the significance of treatment differences estimated using least square (LS) means. All calculations used PROC GLM in SAS software (SAS 9.4.).

5.3 Results

5.3.1 Shoot and Root Fresh Weight

In both trials, the average fresh weight of the harvested shoots from the CAP treatment was significantly higher ($p < 0.05$) than those observed for the AP and HP treatments, while no difference could be found between the latter two ($p > 0.05$) (Table 5.2). For both trials, the shoot weight of the CAP treatment showed a 39% higher growth rate compared to the HP treatment. In both trials, no difference of root fresh weights could be found between the CAP and AP treatments, while the one observed for the HP treatment was significantly lower. However, the shoot:root ratio observed for CAP and HP were not different, while it was significantly lower for AP.

Table 5.2. LS means of shoot and root fresh weight and shoot:root ratio of harvested lettuce.

Treatment ¹	(N) ²	Shoot Fresh Weight (g/plant) ³	Root Fresh Weight (g/plant)	Log ₁₀ (shoot:root)
Trial 1				
CAP	26	136.28 ^a	4.86 ^a	1.47 ^a
HP	26	98.17 ^b	3.58 ^b	1.47 ^a
AP	25	80.55 ^b	5.80 ^a	1.14 ^b
Significance		*** ⁴	*	***
Trial 2				
CAP	24	55.05 ^a	1.71 ^a	1.52 ^a
HP	20	39.64 ^b	1.08 ^b	1.53 ^a
AP	25	35.72 ^b	1.52 ^a	1.39 ^b
Significance		**	**	**

Notes: ¹ CAP: complemented aquaponic solution, HP: hydroponic solution, AP: aquaponic solution; ² (N): number of observations; ³ within columns, LS means followed by different letters (a, b) are significantly different at the 0.05 level; ⁴ *, **, *** Equal significance level of $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

A two-fold difference in the harvested biomass between trial 1 and 2 was observed for all treatments. This finding may be explained mainly by different external environmental conditions that affected lettuce plant growth. The only substantially identified change was the total accumulation of solar radiant exposure, which was 316.21 and 180.94 MJ/m² for trial 1 and 2, respectively. This uncontrolled parameter was nearly halved for trial 2 because of shorter daily light periods and cloudier days.

5.3.2 Nutrient Solutions

Within each trial, the environmental conditions affecting growth, such as water temperature, water DO, light intensity, air temperature, pH, and relative humidity, were similar with the exception of the pH value that was slightly different in the AP system (Table 5.3).

Table 5.3. Growth environmental conditions for trial 1 and 2.

		pH ¹			EC (μS/cm)			DO (mg/L)			Water T (°C)			Air T (°C)	Air RH (%)
		CAP	HP	AP	CAP	HP	AP	CAP	HP	AP	CAP	HP	AP	GH	GH
Trial 1	Mean	5.59	5.73	7.32	2606	2453	823	- ⁴	-	-	20.01	21.07	19.60	22.84	58.21
	SD ²	0.69	0.45	0.50	297	206	163	-	-	-	1.46	1.28	1.43	3.78	14.69
	(N) ³	21	14	18	7	7	9	-	-	-	10	10	10	4461	4461
	Min	4.30	4.76	6.50	2236	2189	630	-	-	-	17.50	18.60	17.20	15.60	27.20
	Max	7.55	6.56	8.20	2945	2710	1014	-	-	-	22.30	22.50	21.40	35.60	86.90
Trial 2	Mean	5.87	5.77	7.50	2493	2418	642	7.51	7.14	7.36	20.68	20.96	22.28	22.15	71.29
	SD	0.43	0.34	0.25	116	140	48	0.34	0.53	0.32	1.39	1.26	0.96	2.58	10.26
	(N)	19	20	17	9	9	16	10	10	10	15	16	15	1162	1162
	Min	5.24	5.32	7.10	2318	2237	567	6.92	6.12	6.91	19.10	19.50	20.90	18.50	37.90
	Max	6.84	6.80	7.94	2656	2672	749	7.91	7.82	7.92	24.70	24.80	25.00	33.20	88.30

Notes: ¹ CAP: complemented aquaponic solution, HP: hydroponic solution, AP: aquaponic solution, GH: greenhouse; ² SD: standard deviation; ³ (N): number of observations; ⁴ Missing data.

Water composition during trial 2 was assessed through the average of weekly LS means for each measured macro and micronutrient in order to improve the clarity of results (Table 5.4). The averages of weekly LS means for all concentrations measured were close to the desired macronutrient target value for each treatment (Table 5.1). Depending on the nutritive mineral, AP treatment had four-to ten-fold lower macronutrient concentrations compared to the other treatments, whereas the micronutrient concentrations were similar in all treatments. Hence, the average EC was three to four times lower in the AP treatment compared to CAP and HP (Table 5.3).

The solution nutrient concentrations and macronutrient ratios for both CAP and HP treatments were compared for each sampling time (i.e., just before and just after weekly renewal of the solution) and were significantly different (data not shown). However, for trial 2 the differences recorded were on average 22, 2, 2, 29, 23, 31, and 0 mg/L for NO₃⁻-N, TAN, PO₄³⁻-P, SO₄²⁻-S, K⁺, Ca²⁺, and Mg²⁺, respectively. Only SO₄²⁻-S concentrations had a consistent difference in CAP compared to HP (i.e., approximately 30% lower in CAP) because sulphate was used as a degree of freedom for the adjustment of mineral concentrations, which is a common practice in hydroponic solution formulation [12].

The evolution of physiological ratios between macronutrient concentrations (Figure 5.1) calculated for each sampling time showed considerable smaller differences between CAP and HP than with AP treatment. For each treatment, the ratio tended to slightly increase between the fresh and the old solution. This was due to water evaporation, which was not balanced with the plant nutrient uptake. The exception was the TAN:NO₃-N ratio that was systematically lower before solution exchange. Notably, these crucial ratios stayed closed to the targets throughout the experiment.

In this study, the Na⁺ concentrations were 6-9 times higher in both AP and CAP treatments compared to the HP treatment, with a maximum of 93.5 mg/L in the CAP system in trial 2. Substantial Na⁺

concentrations were present because some Na^+ was present in the RAS water but mostly because, in CAP and AP solutions, Na_2CO_3 was used to control the pH, which tended to drop during aquaponic solution formulation and throughout the experiment.

Table 5.4. Average of the LS mean of macro- and micronutrients concentration in CAP, HP, and AP treatments for trial 2 (mg/L).

Element	Treatment ^{1o}	(N) ²	Average	SD ³	Min	Max
NO_3^- -N	CAP	6	215.54	28.13	164.00	245.80
	HP	6	193.29	12.35	181.23	211.55
	AP	8	50.31	1.80	46.57	52.39
TAN	CAP	4	25.79	3.09	22.83	29.87
	HP	6	23.95	2.51	20.53	26.67
	AP	8	1.82	1.35	0.25	3.32
PO_4^{3-} -P	CAP	6	52.66	2.42	50.03	56.27
	HP	5	50.93	4.47	44.20	55.57
	AP	6	7.83	0.52	7.06	8.49
SO_4^{2-} -S	CAP	6	66.72	6.97	57.33	77.60
	HP	5	95.36	4.72	87.77	99.97
	AP	8	10.99	1.17	9.24	12.30
K^+	CAP	6	219.31	39.46	169.13	260.60
	HP	5	242.27	36.69	212.67	295.90
	AP	8	59.51	7.89	48.87	73.03
Ca^{2+}	CAP	6	175.09	14.87	154.43	192.63
	HP	4	205.68	12.58	192.30	217.27
	AP	8	14.72	2.03	12.73	19.07
Mg^{2+}	CAP	6	43.02	4.44	36.70	49.40
	HP	5	43.11	3.15	39.13	45.83
	AP	8	7.36	0.64	6.76	8.56
Fe^{3+}	CAP	6	4.40	0.20	4.19	4.69
	HP	5	3.83	0.29	3.39	4.11
	AP	8	3.47	1.05	1.58	4.33
B^{3+}	CAP	6	0.59	0.03	0.54	0.63
	HP	5	0.51	0.08	0.37	0.59
	AP	8	0.47	0.13	0.24	0.60
Cu^+	CAP	6	0.12	0.01	0.11	0.13
	HP	5	0.09	0.01	0.07	0.11
	AP	8	0.09	0.03	0.05	0.12
Mn^{2+}	CAP	6	0.66	0.06	0.58	0.73
	HP	5	0.64	0.10	0.48	0.75
	AP	4	0.50	0.12	0.32	0.60
Mo^+	CAP	6	0.33	0.02	0.29	0.35
	HP	5	0.32	0.04	0.25	0.36
	AP	8	0.32	0.10	0.14	0.41
Zn^{2+}	CAP	6	0.16	0.03	0.11	0.19
	HP	5	0.15	0.01	0.13	0.16
	AP	8	0.14	0.03	0.11	0.19
Na^+	CAP	6	71.67	18.24	40.20	93.53
	HP	5	7.95	4.52	4.22	13.77
	AP	8	49.73	20.98	5.01	74.37

Notes: ¹ CAP: complemented aquaponic solution, HP: hydroponic solution, AP: aquaponic solution; ² (N): number of observations; ³ SD: standard deviation

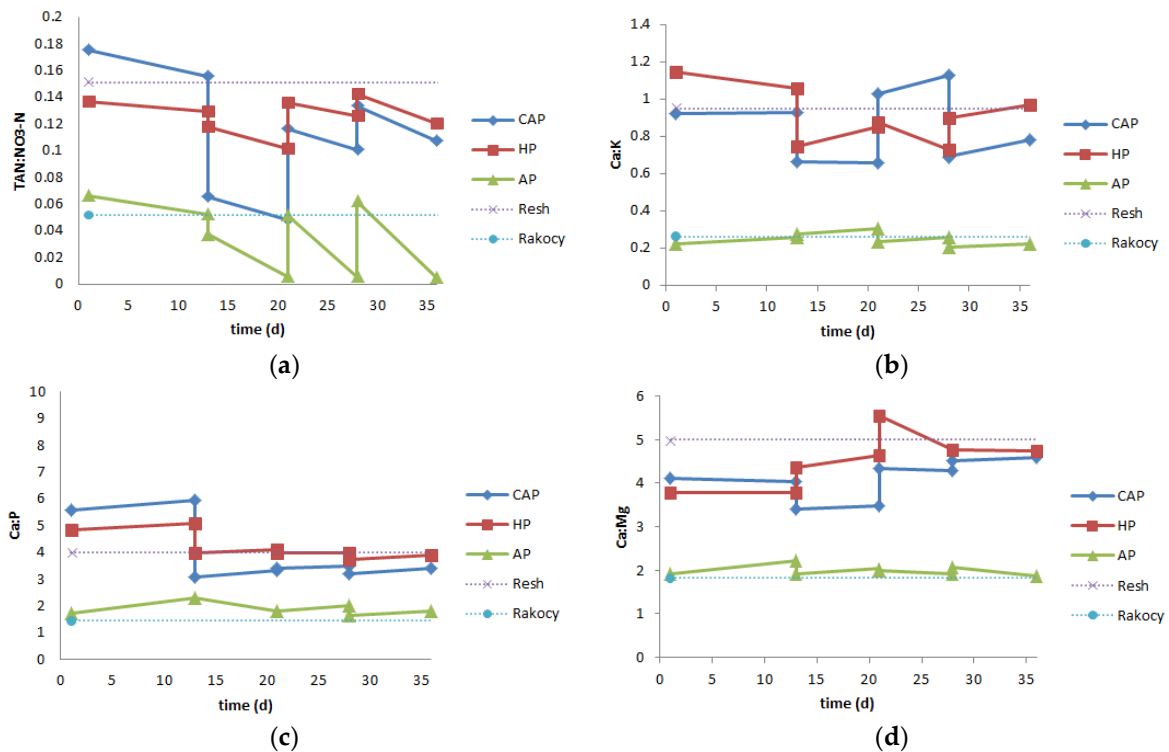


Figure 5.1. LS mean of macronutrient concentration ratio evolution in complemented aquaponic (CAP), hydroponic (HP), and aquaponic (AP) treatments during trial 2. Resh's and Rakocy's ratios are given for comparison. (a) TAN to NO₃⁻-N ratio; (b) Ca²⁺ to K⁺ ratio; (c) Ca²⁺ to P ratio; (d) Ca²⁺ to Mg²⁺ ratio.

5.3.3 Lettuce Leaf Nutrient Content

Leaf nutrient content showed a significant difference ($p < 0.05$) among each treatment for each nutrient, except for K between AP and HP, and B between CAP and HP (Table 5.5). The CAP lettuce leaves had a significantly ($p < 0.05$) higher macronutrient content for all nutrients. AP had the lowest content for each nutrient. With respect to the micronutrients, the contrasts were greater; Fe and Zn content were significantly higher ($p < 0.05$) in HP, while AP had the highest content of Mn and Mo.

The Na content showed the highest observed values in the AP treatment, closely followed by CAP. The Na content was almost 10 times higher in the AP than in the HP treatment.

Table 5.5. LS mean of lettuce leaf nutrient content in trial 2.

Treatment ¹	(N) ²	P ³	K	Ca	Mg	S	Na	Fe	B	Cu	Zn	Mn	Mo
AP	3	5.47 ^a (0.02)	24.6 ^a (0.0)	6.36 ^a (0.01)	2.28 ^a (0.00)	1.97 ^a (0.01)	3.70 ^a (0.00)	739 ^a (5)	8.1 ^a (0.1)	12.6 ^a (0.1)	37.0 ^a (0.3)	1343 ^a (3)	26.5 ^a (0.1)
CAP	3	9.25 ^b (0.01)	29.8 ^b (0.1)	11.3 ^b (0.0)	3.36 ^b (0.01)	2.75 ^b (0.01)	2.80 ^b (0.01)	935 ^b (4)	19.4 ^b (0.1)	20.2 ^b (0.2)	69.1 ^b (0.8)	208 ^b (2)	19.8 ^b (0.3)
HP	3	8.56 ^c (0.02)	24.7 ^a (0.1)	10.8 ^c (0.0)	3.00 ^c (0.01)	2.56 ^c (0.01)	0.40 ^c (0.00)	1511 ^c (4)	19.3 ^b (0.1)	15.3 ^c (0.1)	102 ^c (0)	202 ^c (1)	19.0 ^c (0.1)
Significance		*** ⁴	***	***	***	***	***	***	***	***	***	***	***

Notes: ¹AP: aquaponic solution, CAP: complemented aquaponic solution, HP: hydroponic solution; ² (N): number of observations; ³ within columns, LS means followed by different letters (a, b, c) are significantly different at the 0.05 level. Na and macroelements are reported in mg/gDM and microelements in µg/gDM. Standard deviations are between brackets; ⁴ *, **, *** Equal significance level of $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

5.4 Discussion

While the experiment was conducted to keep the pH, the macro- and micronutrient concentrations, and the macronutrient ratios of HP and CAP treatment in a very close range in order to have the water origin as the only difference (i.e., rain and RAS), a significant difference between most values of macro- and micronutrient concentrations was observed. Due to technical limitations, it is very difficult to obtain concentrations significantly similar in both solutions. However, lettuce growth differences between CAP and HP treatments must not be attributed to the concentration differences recorded and, especially, the small macronutrient ratio variations. Indeed, previous reports have shown that growth was not affected by the fluctuation of a given concentration of a specific nutrient in conditions where lettuce roots are directly exposed to the flowing nutrient solution (e.g., NFT and DWC). Unlike in soil conditions, where there are both diffusion gradients and nutrient depletion, a given constant concentration can be maintained at the root surface. Consequently, nutrients can be absorbed at a constant rate regardless of the nutrient solution's concentrations [149]. However, the concentrations must be maintained above a minimum threshold. Santos et al. [158] showed that by increasing the $\text{PO}_4^{3-}\text{-P}$ concentration, whilst keeping other nutrients constant, lettuce growth and final weight remained constant as long as the $\text{PO}_4^{3-}\text{-P}$ concentration exceeded 20 mg/L. Similar observations have been made previously in other plants for $\text{NO}_3^-\text{-N}$ with a minimum concentration threshold of 1 mg/L [159–161]. Letey et al. [162] reported no significant differences on average shoot and root fresh weight of Romaine lettuce cultivated in DWC for 26 days with different $\text{NO}_3^-\text{-N}$ concentrations (i.e., from 5 to 105 mg/L).

In both trials a similar shoot mass between AP and HP treatment was recorded. In line with previous studies [86,156] these results confirm AP systems as an alternative to conventional hydroponic systems, producing similar yields. Importantly, this study shows that considerable lower nutrient concentrations and different macronutrient ratios in AP solution did not alter yields. When the RAS water was complemented (i.e., CAP treatment) to reach nutrient concentrations and macronutrient

ratios close to the HP control solution, to our surprise, 39% higher shoot mass was obtained in both trials. These results indicate that a 39% yield increase can be achieved if lettuces are grown in RAS water where mineral salts are added and pH kept around 5.5. Such production implicates a specific design that could be achieved with DAPS [150,163].

Trial 2 had lower yields in all treatments. This reduced growth was due to lower light intensity and is a well-known phenomenon. Burns et al. [164] confirmed these results by reporting that lettuce yield in fresh weight was halved in their 28-day trial when reducing the light intensity by 50%, which was close to the light intensity reduction measured for trial 2. Sucrine is a lettuce that is close to the Bibb butterhead type [165]. The biomass of the Sucrine lettuce obtained in HP treatment in trial 1 was 98.2 g per shoot, which is in the range of Bibb lettuce produced in hydroponics with Resh's solution [166].

The shoot:root ratio in AP treatment was significantly lower than in CAP, but CAP and AP treatment had similar root mass. Hence, the lettuce produced less shoot mass in the AP solution. This could have been due to a higher pH and/or to unfavourable nutrient ratios that hindered lettuce nutrient uptake and then limited shoot growth. Interestingly, the shoot:root ratio was similar for both HP and CAP treatments. The increase in shoot mass for CAP seems thus to be related to an increase in root mass. It can be suspected that this increase in root mass has been influenced by others factors that were present in solution rather than the observed small differences in the nutrient concentrations.

The lettuce leaf nutrient content supports these assumptions. The low nutrient content in the leaves of the AP treatment indicates less favourable nutrient solution for nutrient uptake. Leaves in the CAP treatment had higher nutrient content. This could be correlated to the water's EC. However, it is not certain that the small difference in average ECs of 75 $\mu\text{S}/\text{cm}$ between CAP (2493 $\mu\text{S}/\text{cm}$) and HP (2418 $\mu\text{S}/\text{cm}$) can explain this; other factors present in the RAS water might have boosted the nutrient uptake and the shoot and root mass.

The superiority of shoot weight and nutrient uptake in CAP treatment, and especially the superiority of root weight in both AP and CAP treatments compared to the HP treatment (Table 5.2), indicate that RAS water must contain factors that stimulate root growth. Presumably, these factors also stimulate the nutrient uptake. Two factors having a plant growth-promoting effect can be assumed to be present in RAS water: (1) dissolved organic matter (DOM), and (2) plant growth-promoting rhizobacteria and/or fungi (PGPR and/or PGPF). Several humic-like and protein-like DOM components have been identified that tend to accumulate in RAS water [167]. Humic acids, such as fulvic acid, and also certain phenolics can increase shoot and root growth as well as root ATPase activity [168–171]. Haghiaghi [172] showed that humic acid added to a hydroponic solution was also able to improve the nitrogen metabolism and photosynthetic activity of lettuce, which leads to an improved yield. Similar to DOM, PGPR were also identified to be able to promote plant growth and improve root development. PGPR can release phytohormones or induce hormonal changes within plants that stimulate plant cell elongation and division [173]. Mangmang et al. [174] inoculated *Azospirillum brasilense* to lettuce grown on perlite/vermiculite substrate irrigated with fish effluent. The author recorded an increase in endogenous levels of indole-3-acetic acid (IAA), peroxidase activity, total leaf chlorophyll, and protein content in lettuce. IAA is known to regulate biochemical signals controlling plant growth and development. A special focus on DOM and PGPR occurring in water is, thus, required to better understand their impact and potential for improving plant

production in aquaponics. Interestingly, while Na^+ concentrations were considerably higher in the AP and the CAP treatments, this did not seem to have a negative effect on lettuce growth. Moreover, the Na content in the leaves of these treatments highlights the ability of lettuce to absorb some Na^+ and subsequently remove it from aquaponic water. These conclusions are important because substantial Na^+ concentrations in aquaponic waters occur and are unavoidable due to Na release by the fish [147]. Na tolerance and assimilation in lettuce should be more specifically studied in aquaponics in order to define the Na^+ toxic threshold.

5.5 Conclusions

The purpose of the current study was to determine differences in growth rates when exposing lettuce plants to normal (i.e., AP), CAP, and HP solutions. The findings of this study indicated that there was a significantly higher growth rate in the CAP treatment. These findings highlight the potential usefulness of aquaponic systems because it was previously considered that the decisive competitive advantage of HP systems was the enhanced growth potential. This research has demonstrated that aquaponic systems could surpass the growth rates found in conventional HP systems. Notably, with respect to the increasing scarcity of phosphorus [175], it is remarkable that, in AP solution, significantly lower nutrient concentrations gave equivalent yields to HP solution.

From these results, we can conclude that the application of RAS water stimulates both root and shoot growth. It is difficult to ascertain which mechanism led to the increase in this particular case but microorganisms and DOM are suspected to play an important role. A special emphasis should be placed on the DOM species present, their effect on plant growth, and their optimal concentrations. Additionally, microbiota available in both water and the rhizosphere should be identified; it can be assumed that they host efficient growth-promoting rhizobacteria and/or fungi.

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Author Contributions: Boris Delaïde, Simon Goddek and M. Haïssam Jijakli conceived and designed the experiments; Boris Delaïde and James Gott performed the experiments; Boris Delaïde and H  l  ne Soyeurt analyzed the data; M. Haïssam Jijakli contributed for reagents, materials and analysis tools; Boris Delaïde and Simon Goddek, M. Haïssam Jijakli wrote the paper. James Gott corrected the English.

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6. Exploring the potential of nutrient recycling of aquaponic sludge by aerobic and anaerobic digestion.

This chapter has been submitted as a research note in Biotechnologie, Agronomie, Société et Environnement (BASE, Gembloux) in September 2017. By Boris Delaide, Simon Goddek , Karel J. Keesman and M. Haissam Jijakli.

6.1. Introduction

Aquaponics is a major area of interest within the field of sustainable food production. Decoupled multi-loop aquaponics combines the multi-trophic food production systems of both recirculating aquaculture systems (RAS) and hydroponics. This concept of aquaponics leads to a sustainable production system as it re-utilizes RAS wastewater to fertilize the plants [21,44,156,176]. Since most of the nutrients that enter aquaponic systems via fish feed accumulate in the fish sludge [18,19,177], there is a high potential to recycle these nutrients [74,178]. Reintroducing nutrients into the aquaponic water via natural mineralisation of fish sludge, while reducing the sludgy water spillage seems to be a promising way to improve the aquaponic system production performance. Hence, sludge mineralisation could be a contributing factor to close the loop to a higher degree to save water and thus lowering the environmental impact [136].

To validate the interest of aquaponic sludge treatment onsite, a deeper evaluation is required on the mineralisation performance of all macro and micronutrients that are beneficial to plants. To date, there has been little conclusive evidence on mineralisation performance of fish sludge under aerobic and anaerobic conditions.

The objective of this research note was to compare aerobic and anaerobic digestion performance with respect to COD oxidation, TSS reduction in order to evaluate the sludge degradation in reactors, and its mineralisation into dissolved macro- and micronutrients. This short note aimed to produce exploratory results in order to evaluate the interest of developing such technique for nutrient recovery in aquaponic systems.

6.2. Materials and methods

6.2.1 Description of the experiment

Aquaponic sludge digestion performance in term of COD oxidation, TSS reduction and nutrient mineralisation were analysed in an aerobic reactor (AER) and an anaerobic reactor (ANR) (Figure 6.1). The temperature inside both reactors was constantly held at 28°C using an aquarium heater. To work in a semi-continuous mode, reactors were manually batch-fed three times per week with fresh sludge derived from a tilapia (*Oreochromis niloticus*) aquaponic system situated at Zürich University of Applied Sciences (ZHAW). A hydraulic retention time (HRT) of 15 days was applied to both reactors, since the same volume of water (i.e. supernatant) was discharged from the outlets of each reactor. The reactors were operated for 42 days. No solids were discharged during the experiment. To check the operational stability the temperature, pH, EC, ORP, and DO were measured at each batch-fed time with a portable multi-parameter meter (HQ40d, HACH Lange, Loveland, CO, USA).

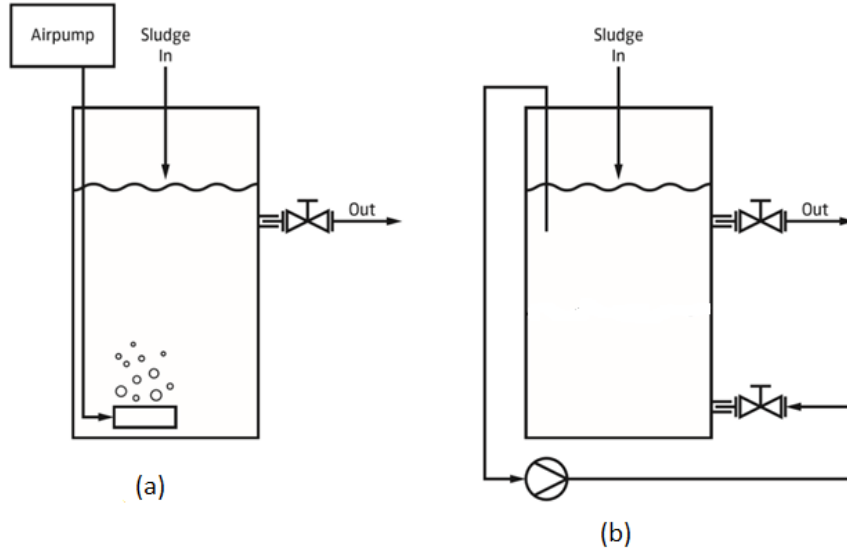


Fig. 6.1 - (a) aerobic digester, constantly aerated and mixed; (b) anaerobic digester, in order to assure a slow mixing of the sludge, a constant up flow velocity of 0.9 m/h was applied by a small pump recirculating constantly the top water of the reactor into the bottom inlet. Both reactors were 30 cm in diameter and 70 cm high with an operating volume of 45 L.

6.2.2 Determination of COD oxidation, TSS reduction and nutrient mineralisation

To determine the digestion performance, a mass balance approach was followed at the end of the experiment. The corresponding equation is as follows:

$$\frac{dM}{dt} = \frac{F_{in}}{V} M_{in} - \frac{F_{out}}{V} M - r \quad (1)$$

Where M is the mass (as TSS or COD or specific nutrient mass inside the reactor), M_{in} is the mass in the effluent, F is the flow rate (in $L T^{-1}$), V the volume (in L^3), and r the reaction term (in $M T^{-1}$).

To calculate the reactors' TSS reduction performance (η_{TSS}) (i.e. the capacity to degrade the solid matter into soluble particles, ions and gas) the equation (1) was integrated from t_0 to t_i , giving:

$$\Delta TSS = TSS_{in} - TSS_{out} - R_{TSS} \quad (2)$$

where ΔTSS is the TSS inside the reactor at the end of the experiment (t_i) minus the TSS inside the reactor at the beginning of the experiment (t_0), $T_{TSS out}$ is the total TSS outflow, $T_{TSS in}$ is the total TSS inflow and R_{TSS} the total reaction term (in M).

With reactors' TSS reduction performance formulated as:

$$\eta_{TSS} = \frac{R_{TSS}}{T_{TSS in}} \quad (3)$$

and by combining equation (2) in (3), the following equation was used:

$$\eta_{TSS} = 1 - \frac{\Delta TSS + T_{TSS out}}{T_{TSS in}} \quad (4)$$

Similarly the reactors COD oxidation performance (η_{COD}) (i.e. the capacity to remove the COD from the sludge input), follows from:

$$\eta_{COD} = 1 - \frac{\Delta COD + T_{COD out}}{T_{COD in}} \quad (5)$$

Where ΔCOD is the COD inside the reactor at the end of the experiment minus the one at the beginning of the experiment, $T_{COD out}$ is the total COD outflow, and $T_{COD in}$ is the total COD inflow.

Considering the nutrient mineralisation performance of the reactor η_N , (i.e. conversion of macro- and micronutrients contained in sludge into soluble ions), the following formula was used:

$$\eta_N = 1 - \frac{\Delta N + T_{N out}}{T_{N in}} \quad (6)$$

where ΔN is the mass of the undissolved nutrient inside the reactor at the end of the experiment minus the one at the beginning of the experiment, $T_{N out}$ is the total undissolved mass nutrient in the outflow and $T_{N in}$ is the total undissolved mass nutrient in the inflow. Thus, similarly to the COD and TSS performances, the smaller the accumulation and undissolved nutrient content in the outflow, the higher the mineralisation performance.

TSS, COD, and nutrient masses were determined from fresh sludge as well as input and reactor effluent samples at each time the reactors were fed with fresh sludge. The reactor contents were sampled at beginning and at the end of the experiment. TSS and COD were determined in triplicate following the APHA protocol [179]. For determination of nutrient content in sludge (i.e. undissolved nutrients), the samples of fresh sludge and sludge inside the reactor (beginning and end of experiment) have been decanted in cylinder for 24h at 0°C and the supernatant has been removed. Then sludge has been dried at 70°C for 96h, pulverized and acid mineralized with 1:1 nitric (65%) and perchloric acid (70%) prior to analysis. The samples' composition in terms of sodium (Na), macronutrient as P, potassium (K), calcium (Ca), magnesium (Mg), sulphur (S) and micronutrient as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), and molybdenum (Mo) were determined by inductively coupled plasma optical emission spectrometer (5100 VDV ICP-OES, Agilent Technologies, Santa Clara, CA, USA). This device gave a measure with a coefficient of variation of 0.51 %. The total Kjeldahl nitrogen (TKN) was analysed with a distillation unit (B-324, Buchi, Flawil, Switzerland). All the analysis were carried out in triplicate.

6.3. Results and Discussion

6.3.1 TSS reduction and COD oxidation

The TSS reduction performance after 42 days for ANR and AER was 49.0 and 60.8 %, respectively (Table 6.1). This shows an 11.8% performance difference between ANR and AER. With respect to COD, the oxidation performance was 56.9 % and 68.5 for ANR and AER showing an 11.6 % performance difference between AER and ANR.

Regarding literature, aerobic digestion seemed to be more performant for COD oxidation and TSS reduction on short period [180–183]. However, experiments realised on short period give only an indication on the easily degradable sludge compounds. The recalcitrant particles identified by van Rijn et al. [184] as being the carbohydrates (e.g. cellulose, lignin) take a long time to be degraded. Therefore, the highest performance of sludge reduction reported in literature are found in AN

digestion in long term experiments with a long sludge retention time (SRT) in up-flow anaerobic sludge blanket reactor (UASB) [185,186]. Under these conditions, the recalcitrant carbohydrates that were contained in the sludge might eventually have been converted into volatile fatty acids (VFAs), carbon dioxide (CO₂), methane (CH₄), and thus left the reactor. Under AE conditions the microorganism growth is much higher than under AN conditions and a considerable higher part of the sludge is converted into new biomass that accumulates in the reactor instead of leaving it as degraded organic matter as in AN conditions [187]. UASB technology consequently seems to be the most interesting option to treat aquaponic sludge on-site. UASBs also have the advantage that they consume less power to run (no aeration needed, lower operational cost) and the CH₄ produced can be a source of thermal and electric energy for the system [187].

6.3.2 Sludge mineralisation

The AER showed better mineralisation performance for most of the nutrients except for N and K. Indeed P, Ca, Mg and B were in a range of 54.2 to 63.0 % for AER while 2.5 to 35.8% for ANR. Cu, Zn and Mn were in a range of 13.2 to 24.6 % for AER while 5.7 to 21.9 % for ANR (Table 6.1). Unfortunately, due to missing data we were not able to assess the mineralisation performance of S, Fe and Mo. Since assessing mineralisation performance is quite innovative, there are not many studies in literature to confront our results. Jung and Lovitt [74] studied nutrient leaching from trout sludge during AN digestion in broth boosted by a lactobacillus inoculum and they observed results for P, Mg, K, and Ca are in the same range as in this study (i.e., 7 – 66 %).

6.4. Conclusion

The obtained results in this study show that sludge digestion in AER and ANR was able to remove at least 50% of the TSS and COD of the sludge input. Also the sludge mineralisation in both treatments was consistent with a 10 - 60% range for all macro- and micronutrients. This makes AE and AN digestion a promising way of treating aquaponic sludge on-site in order to reduce aquaponic sludge discharge and save water. Our results showed slightly better mineralisation performance under AE conditions. However, regarding performances reported in literature with long SRT in UASB, this technology should be deeper explored for aquaponic sludge treatments with a special focus on its mineralisation performance.

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Table 6.1. TSS reduction, COD oxidation and nutrient mineralisation performance of aerobic and anaerobic digestion of aquaponic tilapia sludge. Reactors were operated under conditions detailed in materials and methods.

Reactor	Temperature (°C)	DO (mg/L)	pH	EC (µS/cm)	TSS remov al (%)	COD remov al (%)	N1	P	K	Ca	Mg	S	Fe	B	Cu	Zn	Mn	Mo	Na
Aerobic	28.0 ± 0.6	5.10 ± 1.74	6.54 ± 0.61	1328 ± 465	60.81	68.48	58.7 5	54.2 5	40.4 6	62.9 5	57.4 9	36.2 2	25.1 0	62.9 8	21.7 9	24.6 0	13.1 8	62.9 8	55.9 8
Anaero bic	28.1 ± 0.7	0.11 ± 0.03	6.65 ± 0.27	1867 ± 740	49.02	56.89	61.5 2	28.4 0	42.2 7	8.41	35.7 7	-	-	2.50	10.7 4	21.9 1	5.74	-	32.0 9

¹ Numbers indicate the % of element input that have been mineralised. Presented for all macro- and microelements. - missing data.

7. Nutrient Mineralisation and Organic Matter Reduction Performance of RAS-based Sludge in Sequential UASB-EGSB Reactors

This chapter will be submitted as a manuscript entitled “Nutrient mineralisation and organic matter reduction performance of RAS-based sludge in sequential UASB-EGSB reactors.” By Boris P. L. Delaide, Simon Goddek, Rolf Morgenstern, Sven Wuertz, M. Haissam Jijakli, Amit Gross, Ep H. Eding, Ingo Bläser, Paul Keizer, Alyssa Joyce, Oliver Körner, Johan Verreth and Karel J. Keesman.

7.1 Introduction

In recirculating aquaculture systems (RAS), solid sludge is produced that must be removed from the system; one potential solution is to introduce this sludge into aquaponics systems using bioreactors where it can be broken down into bioavailable nutrients and used to fertilize plants [178,188]. In decoupled aquaponic systems (DAPS), bioreactors for sludge treatment must be designed to both reduce waste production and optimise nutrient re-utilisation [150]. A large percentage of nutrients from fish feed inputs end up as uneaten feed or faeces but are released in insoluble form, thus are not easily assimilated by plants [150,19,18]. In particular, phosphorus, calcium, magnesium, and most of the micronutrients (i.e. Fe, Zn, Cu, Mn and Mo) are not bioavailable and must be mineralized prior to delivery in hydroponics systems [41,177]. The challenge therefore with respect to digester design is to ensure that nutrients in suspended solids are effectively mineralised (i.e. recycled).

The use of upflow anaerobic sludge blanket reactors (UASB) in domestic wastewater treatment [187,189] and in aquaculture-derived fish sludge treatment [185] has been shown to result in a reduction of up to 90% of total suspended solids (TSS). Moreover, expanded granular sludge bed (EGSB) reactors have the potential to further treat UASB effluents [190]. The advantages of a combined UASB-EGSB system are that a UASB reactor mainly removes the TSS, while a EGSB can remove any remaining organic matter such as volatile fatty acids (VFAs) [190–192]. The UASB and EGSB are the most commonly used anaerobic reactors for sludge digestion not only due to their high TSS and chemical oxygen demand (COD) removal rates, but also because of their low operating costs and their ability to extract methane for energy recovery (i.e. heat or electricity generation) [189,193,186]. The very high rates of sludge decomposition possible in UASB-EGSB systems make them ideally suited for treating RAS-based sludge in DAPS systems.

The fish species cultivated in the RAS system, the microbial composition of the fish gut, and the composition of fish feeds being fed all have a strong influence on the mineralisation efficiency of RAS-based sludge. For instance, faeces from fish fed plant-based diets, compared to fishmeal-based diets, contain more soluble and insoluble non-starch polysaccharides (NSPs). NSPs remain largely undigested and directly affect the composition of the sludge [194]. The amount of NSPs in sludge will impact sludge degradation as well as the potential for biogas production [195]. In this study, it is assumed that NSPs will also impact the remineralisation efficiency. Therefore, when determining the mineralisation efficiency and biogas potential of the anaerobic digestion reactors, it is important to characterise the composition of treated waste (substrate) based on its components, and in particular lignocellulosic compounds such as lignin, cellulose and hemicellulose [195]. VFAs, especially C2-C6 VFAs, are also important indicators of the performance of a digester; VFAs are produced during anaerobic fermentation but a marked increase in their concentration indicates a perturbation of the digestion process [196–198].

In most studies on aquaculture sludge digestion in UASBs, the main focus has been on methane (CH_4) production as well as sludge reduction (i.e. solids and COD) [185,186,37,199] rather than the macro and microelements mineralisation capacity. For suitable use in aquaponic systems, it is important to evaluate whether the treatment could mobilise the sludge-trapped macro and microelements to be reintroduced into the aquaponic system. Only a few recent studies have addressed the mineralisation issue in aquaponics and results have shown only small differences when treating fish sludge in simple anaerobic (AN) and aerobic (AE) reactors [178,188]. The question remains whether AN or AE digestion methods are preferable for such purpose. Hence, in this paper we study the macro and microelements mineralisation efficiency in UASB-EGSB reactors treating freshwater RAS-sludge and in simple AN and AE reactors as control. As the nutrient mineralisation is assumed to be dependent of the reactors' performance for reduction of total solids (TS), chemical oxygen demand (COD), volatile fatty acids (VFA), and lignocellulosic compounds (i.e. hemicellulose, cellulose and lignin) these compounds have been measured, as well.

7.2. Materials and Methods

7.2.1. Experimental Setup

Lab-scale UASB and EGSB reactors were set up in series (Fig. 1). The Aquaculture and Fisheries Group at Wageningen University (WUR), Wageningen, the Netherlands housed two sets of these reactors (UASB I + EGSB I and UASB II + EGSB II) while the Integrated Urban and Plant Pathology Laboratory of the Université de Liège (ULg), Gembloux, Belgium operated a third set of reactors.

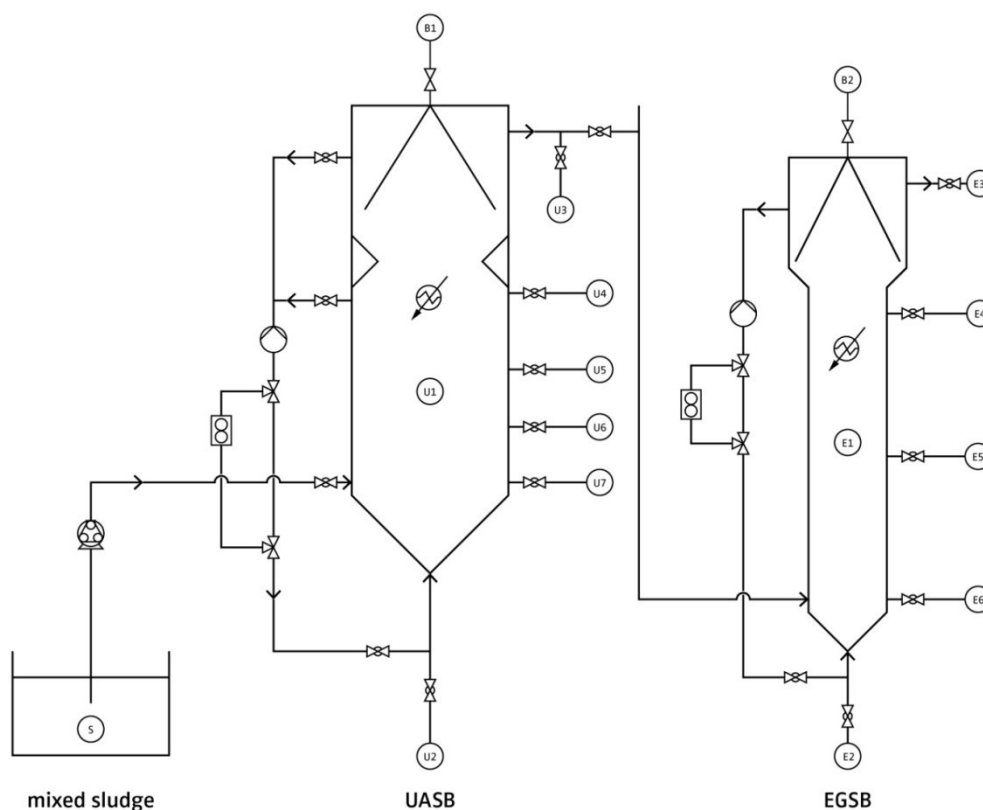


Fig. 7.1. Schematic drawing of experimental setup with UASB reactor (left) and EGSB reactor (right). The circles indicate the reactors' sampling points for fresh sludge (S), biogas (B), UASB sludge/supernatant (U), EGSB sludge/supernatant (E).

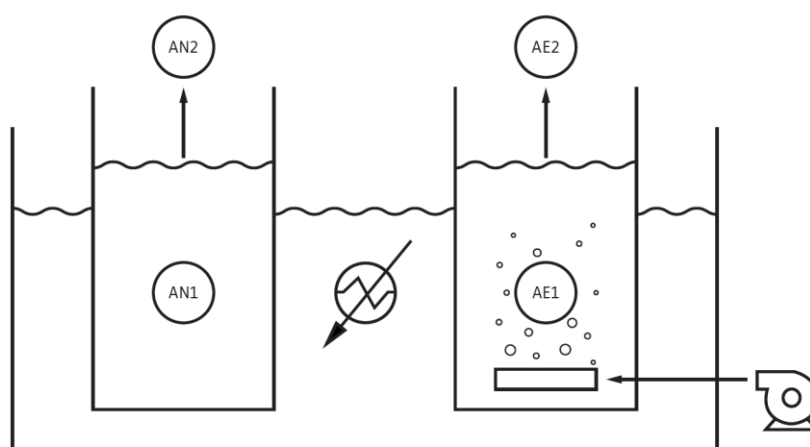


Fig. 7.2. Reference systems. Anaerobic and aerobic controls standing in a water bath heated at 28°C. The aerobic was constantly aerated with an air blower.

One aerobic (AE) and one anaerobic (AN) batch reactor served as controls at each facility (Figure 2). All UASB-EGSB reactors (Aquaponik Manufaktur GmbH, Germany) were of rectangular glass and custom-made. The reactors at ULg were fed with RAS sludge from tilapia (*Oreochromis niloticus*) fed with plant ingredient-based feed. The feed (Omegabaars Grower, AQUA4C, Kruishoutem, Belgium) contained 40% raw protein, 12% raw fat, and 3.7% crude fibre. The reactors operated in WUR were fed with sludge collected from a RAS rearing African catfish (*Clarias gariepinus*). The plant-based feed (C-3 Carpe F, Skretting, France) contained 33% raw protein, 8% raw fat, 3.8% crude fibre and 8% crude ash. After a start-up phase of 2 weeks, the experiment ran for 21 consecutive days and was then replicated under the same conditions. The study was executed from September until December 2016.

7.2.1.1 Two-stage anaerobic treatment

The UASB reactor had an effective volume of 25.5 L, and the EGSB of 11.5 L, respectively. Due to the considerably long hydraulic retention time (HRT) of both UASB and EGSB, a recirculation pump (universal 300, EHEIM, Germany) was required to maintain a sludge blanket in the UASB with an upflow velocity of 1-3.3 m/h, and an expanded granular sludge bed in the EGSB with an upflow velocity of 15-18 m/h. The flows were controlled by two flow-meters (k25, Singflo, China). The temperature inside reactors was maintained at 28°C by a heating controller (TRD 112, Schego, Germany) and a submerged heater (537, Schego, Germany).

7.2.1.2. Anaerobic and Aerobic Batch Control

Buckets served as anaerobic and aerobic batch reactors (Fig. 2) and had an operational volume of 5 L each. Both buckets were temperature controlled in a water bath heated at 28°C with an electric heater. In the AN reactor, the sludge was left to deposit on the bottom of the bucket, while in the AE

reactor the sludge was constantly aerated (relative dissolved oxygen of +50%) using aquarium air blowers.

7.2.2. Start-up phase

As Chernicharo and van Lier [193] previously reported that seed sludge could reduce the total start-up period to 2-3 weeks, 20% seed sludge of the total volume for UASB (i.e. 4.6 L) and EGSB (i.e. 2 L) was inoculated to the respective reactors. For comparison, both control batch reactors received the exact same inoculation as the two anaerobic reactors (i.e. 0.5 L each). The seed sludge was composed of granular sludge and sawdust and was directly coming from a biogas plant (HydroBusiness B.V., Boxtel, The Netherlands). The occurrence of granules was verified by microscopy.

All reactors were filled with water from the same RAS from which the sludge was coming. During the 2 weeks of start-up phase reactors were conducted in special condition to promote the establishment of the anaerobic microbiota and the formation of granules. Psychrophilic conditions were maintained with a water temperature at 30°C. The upflow velocity was slightly increased in UASB and EGSB to speed up blanket mixing. Reactors were fed with fresh RAS sludge 3 times a week and the equivalent volume of reactor supernatant water was removed.

7.2.3. Operation and sampling

An HRT of 10 days was applied for the UASB, and the control reactors. Consequently, three times a week 5.4 L and 1.2 L of fresh RAS sludge with targeted TS of 0.5-3% were manually added to the UASB and control reactors, respectively. To obtain the required volume and TS, the collected fresh sludge was diluted with RAS water if necessary, stirred, and added to the respective reactors. The equivalent supernatant volume (equivalent to the outflow) was removed from the respective reactors. 4.75L of UASB supernatant (i.e. its effluent) was used to feed the EGSB resulting in 5.75 days HRT. The equivalent supernatant volume was removed from the EGSB.

Temperature, EC, DO, and pH in all reactors were measured in the middle of the EGSB and control reactors, and in the sludge blanket of the UASB reactor. The same parameters were recorded in fresh sludge and supernatant every time sludge was added to the reactors. The frequencies of the measurements and the devices used are summarized in Table 1.

During the experimental period the content of the reactors (i.e. sludge) and their effluents (i.e. supernatant) were sampled in order to determine the total solid (TS), COD, dissolved nutrients, undissolved nutrients (i.e. nutrients trapped in sludge), VFAs, fat, and lignocellulosic (lignin, cellulose and hemicellulose) content.

Before the start and at the end of the experiment repetitions both UASB and EGSB were perfectly mixed and 20% of their content was removed and sampled to determine their initial and final composition. The aerobic and anaerobic control groups were treated the same. The respective volume was compensated with distilled water at the start of each repetition giving an initial state equal to 80% of the sample taken at the start.

Simultaneous to each feeding of fresh sludge to the reactors, 500 mL of samples were taken from the fresh mixed sludge, 200 mL from the aerobic and anaerobic control supernatant, and 650 mL from the UASB supernatant. The whole EGSB supernatant was sampled to obtain enough dry matter (DM)

for analysis. Before sampling the supernatant of UASB and EGSB, the pumps were switched off for 15 min so the solids could settle. For the aerobic control, the air pump was switched off. For each repetition and reactor, supernatants were sampled and merged. The corresponding analysis of the merged samples gave us the average supernatant compositions.

Table 7.1. Operation and Control Measurements.

<i>Measurement Parameters</i>	<i>WUR</i>	<i>ULg</i>
pH , EC, temperature	Hach HQ40d ¹	
DO meter	Hach HQ40d ¹	HI 9146 ²
Measurement frequency supernatant outflow	Thrice / week	
Measurement frequency inside reactor	Thrice / week	

¹ Hach Lange, Loveland, CO, USA; ²HANNA instruments, Woonsocket, RI, USA.

7.2.4. Analytical methods

TS and COD were determined in triplicate following APHA protocols [179]. For determination of dissolved nutrients, samples were 0.2 µm filtered and acidified to a pH of 2 with hydrochloric acid (25%) and stored at -20°C for later analysis. Sample (duplicate) content in macroelements as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S) and microelements as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and bore (B) were determined by inductively coupled plasma optical emission spectrometer (5100 VDV ICP-OES, Agilent Technologies, Santa Clara, CA, USA). The total ammonia nitrogen (TAN), nitrate (NO₃-N) and nitrite (NO₂-N) for the samples from the ULg reactors were determined by spectrophotometry using commercial reagent. TAN and NO₂-N were determined with reagent HI 93700-01 and HI 93707-01 (HANNA instruments, Woonsocket, RI, USA), respectively. NO₃-N was measured with a Nanocolor standard test (Ref 918 65, Macherey-Nagel, Düren, Germany). All analysis were carried in triplicate. TAN, NO₂-N, and NO₃-N for the samples of the WUR reactors were determined using an autoanalyzer (SAN Plus, Skalar, Breda, The Netherlands) and Skalar protocol number 155-006 for TAN, Skalar protocol number 467-033 for NO₂-N and Skalar protocol number 461-318 for NO_x-N [179]. NO₃-N was calculated as NO_x-N - NO₂-N.

For determination of nutrient content in sludge (i.e. undissolved elements), the samples were dried at 70°C for 96h, pulverized and acid mineralized with 0.8M H₂SO₄ prior to analysis. Then, the sample content in P, K, Ca, Mg, Fe, Zn, Cu, Mn, and B were analysed (in duplicate) as described before. Proximate composition of sludge samples was determined as dry matter (DM; ISO 6496, 1983), crude protein (ISO 5983, 1997, crude protein = Kjeldahl-N x 6.25), and crude fat (ISO 6492, 1999) using a bomb calorimeter (IKA model C7000; IKA-Werke GmbH & Co. KG, Staufen, Germany).

The determination of VFA, i.e., acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid and valeric acid was achieved by gas chromatography separation (Thermo, Trace GC Ultra with a GS column (Grace EC-1000 length 30 m, ID 0.53 mm, 0.2µm) and detected by flame ionization detector, Thermo, Interscience, Australia), following the method described in Ottenstein et al. [200].

7.2.5. Mass balances equation

In order to determine the organic reduction performance (i.e. TS, COD, fat, hemicellulose and cellulose) overall reactor mass balances were formulated from start to end of the experiment. The performance was calculated by using the equations described in Delaide et al. [188].

Derived from the mass balances equation in Delaide et al. [188], the nutrient mineralisation performances (or the nutrient recovery efficiency) were determined using the following equation:

$$NR = 100\% \times \left(\frac{DN_{out} - DN_{in}}{TN_{in} - DN_{in}} \right)$$

Where NR is the nutrient recovery at the end of the experiment in percent, DN_{out} is the total mass of dissolved nutrient in the outflow, DN_{in} the total mass of dissolved nutrient in the inflow, and TN_{in} the total mass of dissolved plus undissolved nutrients in the inflow.

7.2.6. Statistical analysis of data

The data of organic reduction and mineralisation performances were analysed using R.

7.3. Results

7.3.1 Sludge input characteristics

Sludge characteristics of ULg and WU are presented in Table 2. Average mineral elements in both liquids and solids are displayed. From the data, we can see that the solid part of ULg sludge had two times the nitrogen content of WU sludge. ULg solid sludge contained very low K. While the EC was higher in WU, the ULg liquid sludge contained concentrations of P, K, Ca and Mg more than twice as high as in WU. Microelements were mainly contained in the solids for both sludge provenances. WUR sludge had a higher hemicellulose and cellulose content of 5.3 and 1.7-fold, respectively. ULg sludge contained twice as much lignin and four times more fat.

Table 7.2. Fresh sludge description used to feed reactors in Wageningen (WUR) and Université of Liège (ULg) during experiment repetitions.

		WUR ^a		ULg ^b	
	Unit	Liquid part	Solid part	Liquid part	Solid part
<i>pH</i>		6.86 ± 0.19		6.48 ± 0.13	
<i>EC</i>	$\mu S/cm$	2626.94 ± 858.67		1607.00 ± 154.86	
TS	<i>g/L</i>	10.77 ± 0.00		8.6 ± 0.42	
COD		14.21 ± 0.83		9.89 ± 2.86	
Fat	<i>mg/gDM</i>		19.1 ± 0.9		81.0 ± 0.6
Hemicellulose			292.1 ± 23.9		54.6 ± 4.9
Cellulose			205.2 ± 12.0		123.1 ± 10.7
Lignin			39.4 ± 0.0		80.2 ± 2.1
TKN ^c	<i>mg/L</i>	53.80 ± 23.72	177.07 ± 45.38	33.06 ± 28.84	362.63 ± 28.28
P		17.13 ± 5.66	149.78 ± 24.71	43.85 ± 3.89	133.19 ± 16.32
K		16.58 ± 4.47	27.01 ± 1.31	36.65 ± 6.29	8.26 ± 2.67
Ca		26.62 ± 6.36	273.96 ± 67.14	173.35 ± 2.05	239.09 ± 46.70
Mg		7.41 ± 3.72	20.33 ± 4.06	39.35 ± 5.30	21.97 ± 6.16
S		7.42 ± 2.91	-	243.30 ± 293.03	-
Fe		0.03 ± 0.00	9.9 ± 1.43	0.10 ± 0.05	18.69 ± 1.60
B		0.03 ± 0.02	0.85 ± 0.21	0.064 ± 0.01	0.53 ± 0.08
Cu		0.01 ± 0.00	0.43 ± 0.02	0.01 ± 0.00	0.69 ± 0.04
Zn		0.10 ± 0.03	7.10 ± 0.05	0.02 ± 0.00	4.91 ± 0.65
Mn		0.09 ± 0.00	2.32 ± 0.28	0.28 ± 0.02	1.37 ± 0.27

^a Fresh catfish sludge from the Aquaculture and Fisheries Group at Wageningen University (WUR). ^b Fresh tilapia sludge from the Integrated Urban and Plant Pathology Laboratory of the Université de Liège (ULg).

7.3.2 pH

Measured pH during the experiment repetitions (i.e. repetition 1 from day 1 to 21 and repetition 2 from day 21 to 42) is presented in Figure 3. Aerobic reactors in WU and ULg had the highest pH that oscillated between 7.5 and 8.5. UASB and anaerobic reactors had all a pH that oscillated between 6.5 and 7. The WUR UASB II reactor had the lowest pH observed, beginning at 6.5 and declining slowly during repetition 1. At the beginning of repetition 2, adjustments were made using sodium bicarbonate to counter the decline in pH. Finally, the decision was made to run the WUR UASB II reactor on low pH as it could not be adjusted (i.e. kept on dropping; see Figure 3). Thus, during repetition 2, the pH was between 5.5 and 6.

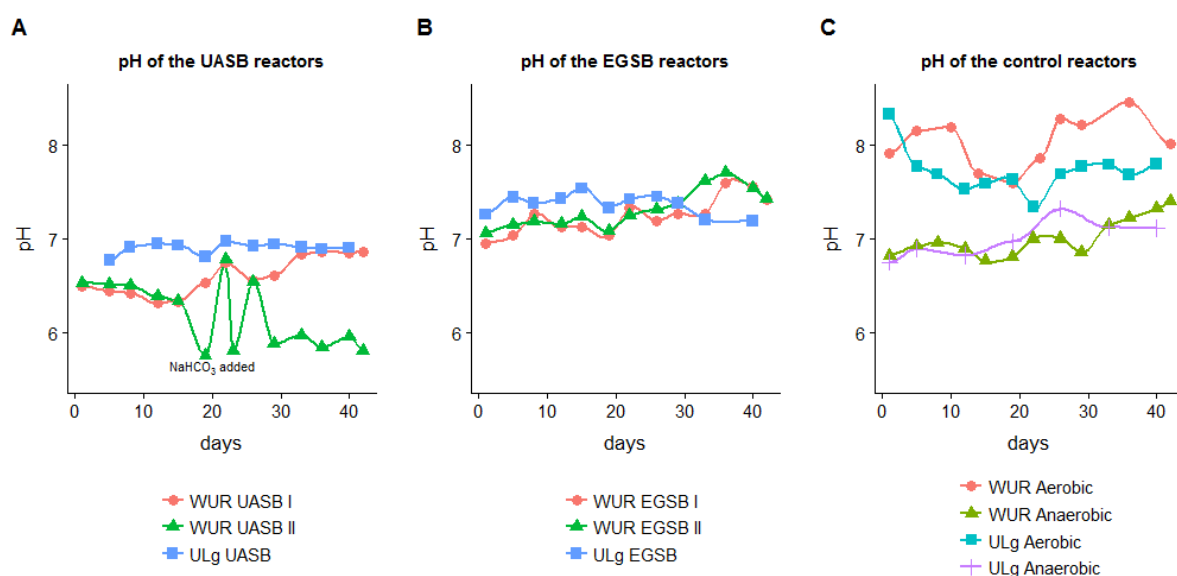


Fig 7.3. pH inside aerobic, anaerobic and UASB reactors in WUR and ULg.

7.3.3 VFA

The VFAs inside the reactors were recorded at 3 days intervals. Figure 4 presents the concentration of total VFA measured in WUR (A, C and D) and ULg (B) reactors. It is apparent from this figure that only UASBs had an increase in VFA during the experiment. In particular, the acidic UASB (WUR UASB II) increased from 0.6 to 36 mmol/L. Within the VFA measured in UASB, the most concentrated were acetic and propionic acids. The other reactors maintained a low level of VFA during the experiment and ended with a concentration lower than 2 mmol/L.

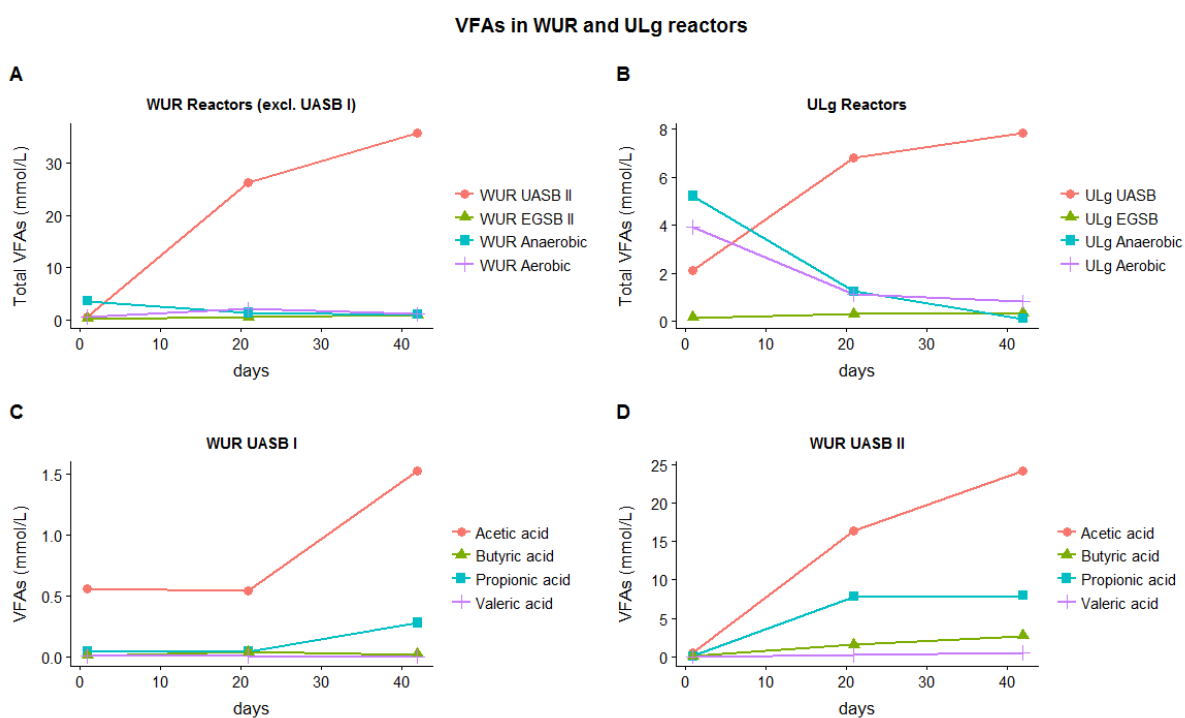


Fig 7.4. Total VFA inside UASB (and connected EGSB compared to WUR (A; excluding WUR UASB I) and ULg (B) combined aerobic and anaerobic control reactors. Concentrations of the different VFA inside the WUR UASB I (C) and WUR UASB II (D).

7.3.4 Reactors organic sludge reduction and mineralisation performance

The results of the TS, COD, hemicellulose and cellulose reduction performances of reactors are displayed in Figure 5A; mineralisation performances of macroelements are displayed in Figure 5B. The data obtained from UASBs at WUR and ULg at pH 6.5-7 from both locations, and the data from AN and AE reactors were pooled. The data of WUR UASB II that turned acidic (i.e. pH between 6.5 and 5.5) was not combined with the other UASB data and was analysed separately. A full performance analysis of the EGSBs could not be achieved due to inaccurate measuring equipment, thus only their effluents have been analysed.

TS

No statistical difference of TS reduction was found between the UASBs conducted at pH 6.5-7 and the control reactors. The UASB reactors had the highest performance with a reduction close to 50%. There was a significant difference between the WUR UASB II (pH 5.5-6.5) and the UASB running on the higher pH. The control reactors had the lowest performance, with a negative reduction.

COD

The UASBs running at a pH between 6.5-7, as well as the AE reactors, had the highest COD oxidation performance. The WUR UASB II had a lower performance with only 11 % oxidation.

Fibers

Compared to other UASB and control reactors, WUR UASB II clearly had the lowest performance for hemicellulose and cellulose reduction.

Fat

There was a significant difference in fat reduction between the ULg-digesting reactors and the WUR-digesting reactors. Indeed, the reduction was higher in the ULg reactors with values in a range of 62 to 97 % while the performance was in a range of 0 to 49 % for the WUR reactors (results not presented).

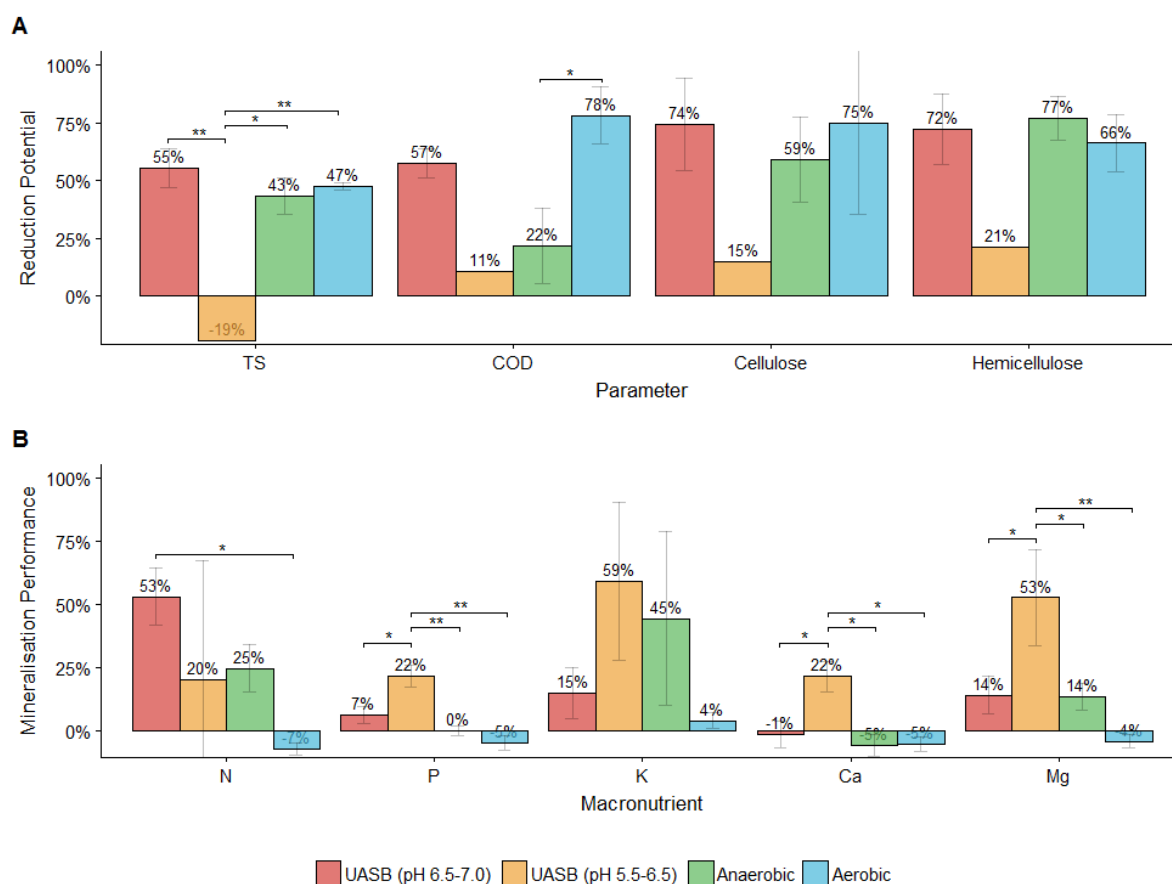


Fig 5. Organic sludge reduction performances (A) and mineralisation performances (B) for the following reactors: UASB with pH between 6.5 and 7 and between 5.5 and 6.5, anaerobic (AN) and aerobic (AE). Data from reactors of same type and pH were pooled together.

Mineralisation performance

Figure 5B shows that the significant (highest) performance of WUR UASB II (pH 5.5-6.5) for mineralising P, K, Ca and Mg. Mineralisation performance was on average in a range of 20 to 59 % while in a range of 0 to 15 % for the other UASBs (pH 6.5-7). The opposite was true for N, which had the best mineralisation rate in the UASB with pH 6.5-7. AE controls always had the lowest mineralisation performance. Least mineralised were the macroelements Ca and P. In control reactors, Ca and P even accumulated in sludge. Interestingly, no mineralisation but accumulation of N occurred in AE controls.

Mineralisation of microelements was very low and the results are therefore not shown. No mineralisation was observed for Mn and Zn. Instead, they actually accumulated in all reactors. Cu and Fe mineralised less than 1 % in all reactors. The best mineralisation performance was 1.74 % in UASB for B.

7.3.5 Effluents

The reactors' effluents were analysed and compared to hydroponic standards in order to evaluate their suitability for plant growth. Fig 6A displays the macroelements concentration in UASBs effluents with the concentrations used in lettuce hydroponic (HP) solution [12] for comparison. ULg effluents

had the highest concentrations for most of the macroelements. Almost no $\text{NO}_3\text{-N}$ was measured in UASB effluents while their TAN concentrations were 1.9 to 9.2 times higher than the HP one. P concentrations in most UASB effluents were close to the HP one. K concentrations in all effluents were 4.9 to 15 times lower than the HP one.

Figure 6B shows the microelements concentration in UASB with the concentrations used in lettuce hydroponic (HP) solution [12]. , All the microelements studied were far below the recommended HP concentrations, except for Mn. They all had concentrations higher than 0.01 mg/L, except for Cu.

Figure 6C displays TS and COD in UASB, EGSB, AN and AE effluents. The values for each reactor type were pooled together. From the data in this figure, it is apparent that EGSB effluents were always lower in TS and COD concentrations. EGSB were able to remove the TS and COD of UASB effluents (i.e. EGSBs influents) by 25 and 50 % on average, respectively. In control reactors effluents TS and COD comprised a range of 1 to 2 and 0.5 to 0.9 g/L, respectively.

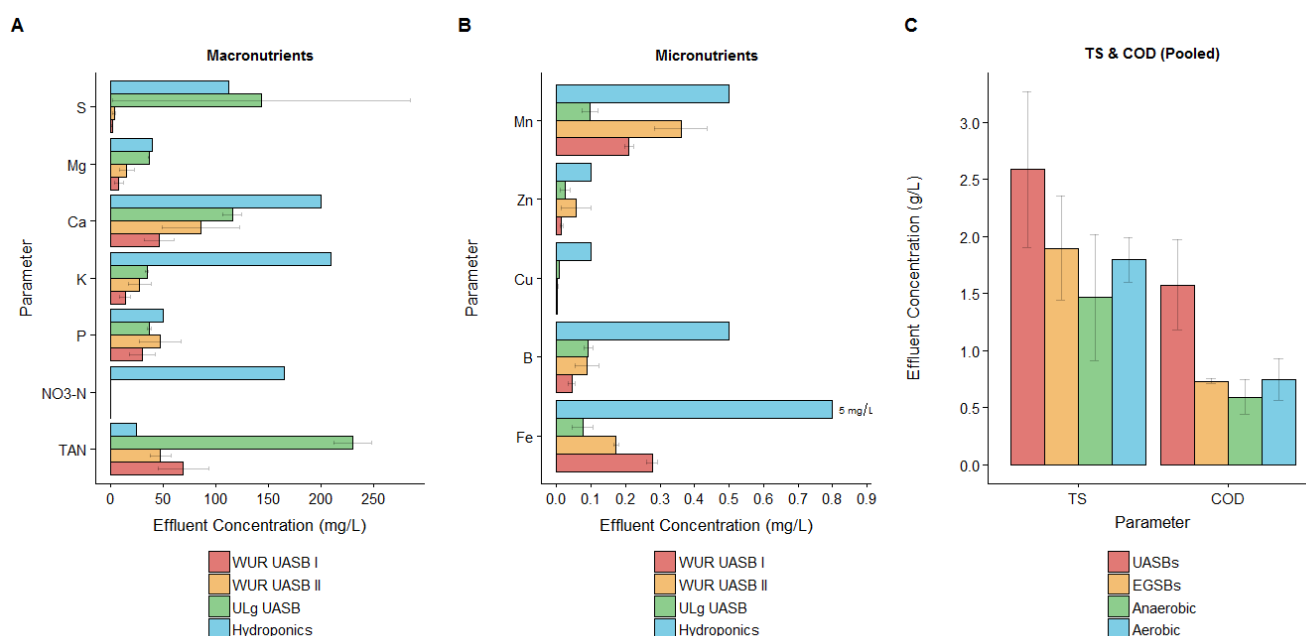


Fig 6. A. TS and COD in UASBs , EGSBs , Anaerobic and Aerobic effluents. In this case, the values for each reactor type were pooled together. B. Average macroelement concentrations in UASB reactor effluents for WUR and ULg compared to concentrations used in hydroponic solution for lettuce (HP) [12]. C. Average microelements concentrations.

7.4. Discussion

During the present study pH and VFA were recorded in reactors. The results showed clearly that the pH was quite stable in all reactors except for the WUR UASB II. This reactor had a drop in pH and turned acidic, as a result of VFA production, as consistent with prior experiments [196,197].

Regarding the reduction performances of the other reactors, aerobic digestion profiles obtained in this study are consistent with our previous results [188]. Performances of the anaerobic reactors were also in accordance with the results available in the literature [184,201]. The COD, cellulose and hemicellulose reduction obtained in the UASB reactors with high pH had higher levels than results reported by Meriac et al [202] who obtained 44 % COD reduction and around 50 % cellulose and

hemicellulose reduction. However, the COD and TS reduction performances observed in our UASB reactors were lower than the results reported by Mirzoyan and Gross [185] who achieved COD and TSS reduction up to 99 and 92 %, respectively. A possible explanation for this is that our experiment continued for only 21 days. As no sludge was discharged during this period, the sludge retention time (SRT) was 21 days while Mirzoyan and Gross [185] had a SRT of 335 days. Recalcitrant compounds, such as aromatic hydrocarbon compounds, asphaltenes and resins take a long time to be degraded [184,203–205], thus 21 days was not enough time to achieve more than 48 and 57% of COD and TS degradation in the UASB reactors respectively. On the basis of degradation rate measurements, van Rijn et al. [184] predicted that with a constant daily input it would take 400 days to reach the asymptotic maximum of sludge accumulation in an anaerobic reactor. This would correspond to a state where almost total TS reduction is achieved. Estimates provided in Van Rijn et al. are in accordance with the findings of Mirzoyan and Gross [185]. Hence, our results are promising and consistent with the results from several prior studies, thus supporting the potential feasibility of treating sludge anaerobically, notably with UASB, in aquaponic systems without discharge.

Perhaps the most interesting finding is the high mineralisation performances of P, K, Ca and Mg observed in the WUR UASB II. This is likely related to the pH drop under 6.5 observed in this reactor. Indeed, previous studies have demonstrated that a decrease in pH promoted macro and microelements mineralisation in fish sludge [74,206]. Interestingly, our results are consistent with those of Conroy and Couturier [206] who observed an increase in P, K, Ca and Mg mineralisation during anaerobic digestion of smolt sludge when the pH dropped from 7.8 to 5.5. They showed that the effect of pH on the phosphorus and calcium mineralisation is well described by an equilibrium model based on the solubility of calcium orthophosphates. As pH drops under 6.5 these minerals start to dissolve in water [207]. It can be assumed that the increase in K and Mg mineralisation is also due to the same phenomenon, as pure calcium orthophosphates are never found in biological systems and a portion of the calcium ions in the crystal lattice are normally replaced by smaller cations such as magnesium and potassium [208]. However, other equilibrium models should be established to describe more accurately the mineralisation of these elements. Regarding microelements, very low mineralisation (i.e. < 1.7 %) was observed in all reactors, even in the acidic UASB. However, Jung and Lovitt [74] obtained very high mineralisation of both macro and microelements and other heavy metals from trout sludge by lowering the pH until 4. For example, they achieved up to 92 % Fe mineralisation in 7 days by inoculating sludge with glucose and lactic acid bacteria, and observed that best heavy metal mineralisation rates could be achieved with organic acids, presumably due to their chelating capacity when complexed with the metals [74]. Jung and Lovitt also reported that under high acidic conditions, sludge reduction stopped, as observed in WUR UASB II. In accordance with their prior findings, our results also showed that the anaerobic digestion process slowed when the pH dropped under 6.5. This is confirmed by literature reporting that a pH value below 6.0 inhibits methane-producing microorganisms [187,209].

Under acidic conditions, our results showed lower nitrogen mineralisation performance than in the UASBs with a pH between 6.5 and 7. These reactors achieved also the best sludge reduction, which appears to indicate that N mineralisation is correlated to sludge reduction. A possible explanation is that nitrogen is released mainly in the form of ammonium during the breakdown of proteins that occur only when the anaerobic sludge digester is working correctly. With regard to nitrogen mass balances in the aerobic reactors, our data show that nitrogen was lost during the experiment, suggesting that microbial processes led to denitrification and/or N₂O emission [210]. P, Ca and Mg

accumulated inside the aerobic reactors, potentially due to the high pH (i.e. 7.5-8.5) that induces precipitation of these elements in the form of calcium orthophosphate and possibly other minerals [207], or due to microbial uptake [211].

Our results suggest that the best mineralisation of N is achieved in UASB when sludge reduction is high while the other macro and microelements would be efficiently mineralised only in acidic condition. Unfortunately, when acidic conditions occur, sludge reduction stops, methanogenesis fail and so the production of methane ends abruptly. This indicates that efficient mineralisation of all macro and microelements while producing methane is not possible in a single UASB. This antagonism between nutrient mineralisation and sludge reduction performance demonstrates that such processes should be carried out in separate reactors. Indeed, sludge digestion could be achieved in two stages. The first stage could be the sludge reduction promoting methanogenesis, followed by a second acidic stage to mineralise the nutrients contained in the outputs from the first reactor.

Concentrations of mineral elements in effluents were consistent with the analysis of the mineralisation performance of the reactors. Logically, higher concentrations in all ULg effluents occurred because higher concentrations of dissolved elements were found in fresh sludge. Compared to the concentration found in hydroponic solutions [12], the concentration in S, Mg, Ca and P were close to hydroponic target concentrations. Because of the very low mineralisation rates, however, microelement concentrations were low and far below the hydroponic recommendations. The high concentrations of TAN and the absence of nitrate in all anaerobic effluents is concerning. The especially high concentration of TAN measured in the effluent of UASB from ULg is consistent with the high N content measured in ULg fresh sludge. There is also evidence that fish sludge did not contain enough K to reach the concentrations required in hydroponic solution, even if total mineralisation of K was achieved.

A question that remains is the suitability of reactor effluents for reinsertion in the aquaponic system, as our analysis revealed the presence of important concentrations of TS, COD, VFA and TAN. Post-treatment might be necessary prior to plant delivery. Previous studies have reported that organic compounds in commercially available hydroponic solutions generally have phytotoxic effects that lead to poor plant growth [212–214]. As such, TS and COD concentrations in effluent should be reduced for proper use in aquaponic solutions. While sludge reduction and mineralisation in the EGSBs was not undertaken, the measurement of TS and COD in their effluents did not demonstrate sufficient removal of the TS and COD from the UASB effluents to allow the safe use as hydroponic solution. Although removal of the VFAs was successful (VFAs are reported to be phytotoxic) [215], EGSBs may not be the best posttreatment solution for sludge digestion in aquaponics, and an aerobic posttreatment of the anaerobic effluent would potentially be a better solution to reduce the potential phytotoxicity of the effluents [212,216]. As shown in our results, such a solution would also remove the VFA adequately, and if nitrification is promoted, would also reduce the TAN and increase the nitrate concentrations, with subsequent benefits of increasing the TSS and COD removal, while also removing other phytotoxic anaerobic secondary metabolites [213]. It is however important to experiment further in order to examine the desired dilution rate of the effluent in an aquaponic solution, and the ability of the plants to directly assimilate or cope with the effluents. It is possible that the hydroponic beds are sufficient as posttreatment.

7.5. Conclusion

The present study aimed to assess the organic sludge reduction and macro/microelement mineralisation performances in UASB-EGSB reactors. The suitability of the effluents to complement a commercially available hydroponics solution was also examined. Our results show that aerobic and UASB reactors were superior for organic sludge reduction, but prior studies have shown the best performance of the UASB reactors occurs over a longer time frame and is the best solution for organic sludge reduction in aquaponics. Our findings clearly indicate that the mineralisation performance of P, K, Ca and Mg is enhanced under acidic conditions, however these conditions are not suitable for sludge organic reduction. Also, N is better mineralised after organic matter degradation occurs. Therefore, a two-stage digestion process seems necessary, with (a) the first stage organic sludge reduction, N mineralisation and biogas production, and (b) a second acidic stage to mineralise the macro and microelements contained in the outputs from the first stage.

While EGSB were efficient in removing the VFA, they were not able to substantially remove the TS and COD from UASB effluents, and appeared thus to be not the best posttreatment solution for sludge digestion in aquaponics. Because of very low mineralisation rates in UASB, microelements in their effluents were low and far from hydroponic concentrations. K concentrations were also lower than hydroponics because fish sludge did not contain enough initial K. However, in this study the concentrations of S, Mg, Ca and P obtained in UASB effluents were close to the concentrations found in commercial hydroponic solutions. This shows the potential ability of a two-stage digestion system including UASB to recover the nutrients from fish sludge in aquaponics. Finally, the high concentrations of TAN and the absence of nitrate in all anaerobic effluents data highlight the presumably necessity of aerobic post-treatment, which could also reduce the TS and COD of UASB effluents.

It is recommended that further research be undertaken on the suggested two-stage digestion setup to determine potential performance, while also experimenting to identify the best posttreatment solutions by examining plant growth in the treated effluents.

7.6. Acknowledgements

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8. General discussion

The first objective of the thesis addressed in chapter 3 was to highlight the challenges to make aquaponics (AP) a sustainable breakthrough technology. It has been identified in the literature that nutrient concentrations in aquaponic solution are less than optimal for plant growth. Hence, the consistency of plant growth in aquaponics should be accurately verified. The following question ensued: given the shortfall in nutrient concentrations in aquaponic compared to hydroponic solution, to what extent does this affect plant growth? This thesis addressed this and the results are discussed below. In addition, the review assumed that an important part of the nutrients input were unavailable and were lost from the aquaponic system via sludge spillage. Phosphorus is known to be released by fish in undissolved form and thus accumulates in sludge, for example [18]. This cemented the need to determine what proportion of mineral element is recycled in aquaponics since its main aim is to close the loop and reduce the impact on the environment from both fish and plant production. A consistent way to characterise its impact on the environment is to determine the mineral elements mass balances in order to see what proportions of elements are trapped in the system and what proportions are released in the environment. These considerations meet the second objective of the thesis that was to determine the impact of aquaponics on the environment in relation to the performance of the system. Therefore, a one loop aquaponic system named the plant and fish farming box (PAFF Box) was built in Gembloux and has been studied during one season of production in order to study its impact on the environment and especially its mineral elements mass balances (Chapter 4).

The analysis of the nutrient mass balances in the PAFF Box gave interesting information. First, regarding the total mass of all nutrient input (via fish feed and tap water) and their ratio to total N mass inputted, only K, Fe, B and Mo were inputted in lower quantity than required (i.e. if similar ratio to N as hydroponic formulation would be obtained). K and Fe being inputted around 60 % less than required and 80 % less for B. Mo was below detection limit in inputs. In an ideal aquaponic system all nutrients inputted would end-up in water in soluble plant-assimilable forms, however nutrient accumulation in PAFF Box water showed that this ideal was not attained. Indeed, only N and Ca accumulated quickly in the water and influenced EC levels the most. The other nutrients accumulated at variable lower rate in solution giving a nutrient concentration profile with a totally different profile than in the input and with a ratio to N far from the hydroponic standard. Ratio K:N and P:N in the PAFF Box were 0.15 and 0.05, respectively while 1.1 and 0.3 are recommended in hydroponics for lettuce in NFT or DWC. This clearly indicates that the nutrient content and ratio to N in fish feed does not lead to a balanced nutrient content in water. Thus, in such aquaponic systems the nutrient content in feed does not directly control the nutrient content in water, and suggests that even if the nutrient profile in the fish feed is well balanced, there is no guarantee that the nutrient profile in water will match.

Basically, the mass balances analysis confirmed that P and all the microelements ended-up in sludge instead of accumulating in water. Except N and B that accumulated in the same proportion in water and sludge. Surprisingly, an important proportion of each nutrient was lost (i.e. 50 to 90 %). It is presumed most of this loss was by spillage when cleaning filters and by water exchange. A daily average water exchange of 3.5% was applied resulting of 278 L of water needed to produce 1 kg of tilapia or corresponding to 243 L of water exchange per kg of feed added per day. RAS are renowned for excellent performance regarding water consumption [10]. It is, therefore, surprising that even

with the PAFF systems low water exchange, such an important proportion of nutrients were lost from the system. Such loss meant that only a small proportion of each nutrient were trapped in plants (i.e. < 4 %). This nutrient budget suggests that, counter theoretical expectation, the PAFF Box one loop aquaponic system was not efficient at recycling nutrients contained in fish waste, in fact, most nutrients were either lost, or trapped in the sludge (i.e. mainly fish solid excretions removed out of the system).

The third objective was to determine if aquaponics can assure consistent plant growth compared to conventional systems. Lettuce and basil growth was first studied in the PAFF Box and then lettuce growth was studied in AeroFlo in controlled conditions and compared to a hydroponic control. In these experiments the mineral element concentrations in solution were closely recorded.

The study in chapter 4 showed that sustained growth of lettuce (*Lactuca sativa* var. *capitata* cv. 'Grosse Blonde Paresseuse') and basil (*Ocimum basilicum* cv. 'Grand Vert') was achieved in the PAFF Box. No visual nutrient deficiency was noticed, although dissolved iron (FeSO_4) was sprayed on the leaves once per crop in order to prevent iron deficiency that was reported to occur in one loop aquaponic system [45,217]. In our study no fertilizer or any kind of salt were added to the water. This facilitated following the dynamics of mineral elements (i.e. macroelements as N, P, K, Ca, Mg and S, plus microelements as Fe, B, Zn, Cu, Mn, and Mo) released by fish excretions in water. Mostly soluble excretions accumulated in the aquaponic water solution as the solids were daily removed by a sieve filter. However, fish excretions were not the only nutrient source; tap water was used to fill up the system. Its content in macro- and microelement ions was quite favourable in this case because it had consistent and well balanced concentrations of them (P, K, Ca, Mg, and S were 0.5, 0.6, 103, 17, and 31 mg/L, respectively and Cu, Mn, Zn, B were 13, 3, 2000 and 14 $\mu\text{g/L}$, respectively). Unlike in aquaponics, in hydroponics mineral elements are fully controlled in solutions aiming at specific ratios between them and a suitable electro-conductivity (EC) in order to optimise their absorption by the plants and so their growth [11]. EC used in hydroponics for lettuce with their roots dipping in water (i.e. deep water culture (DWC) and nutrient film technique (NFT)) is 1.8-2 mS/cm [12,145]. The EC recommended for basil is not clearly established in literature because its culture in hydroponics is quite a recent subject (Most authors adopt hydroponic solution formulated for lettuce [218]). The EC in PAFF Box water solution went never above 1.3 mS/cm. This lower EC indicates fewer ions in the PAFF Box than in standard hydroponic solution. Indeed, regarding macroelements, K and P were the less concentrated with in average 9.2 and 3.3 mg/L, respectively. This representing a ratio to N of 0.15 and 0.05 for K and P, respectively which is far from the recommended ratio used in hydroponics (i.e. 1.1 and 0.26 for K:N and P:N, respectively). Moreover, the recommended concentrations in hydroponics are 210 and 50 mg/L for K and P, respectively. For microelements the situation was quite similar; for Zn, Cu, Mn and B with a concentration 10 to 20-times lower to the one recommended in hydroponics. Fe was 500 to 1000 times lower than the recommended concentration. This confirms the low level of iron in aquaponic water and especially its restricted release in soluble form by fish. Mo was not detected with ICP-OES that has a limit of detection of 0.005 mg/L.

Then it could have been supposed that lower macro- and microelement concentrations and ratios would give less favourable plant growth condition in PAFF Box solution leading to lower crop yield. But surprisingly, the yield of lettuce and basil obtained did not validate this supposition. No visual nutrient deficiency was noticed, and thus it was presumed that all nutrients were present in sufficient quantity. Higher lettuce head mass was obtained than the one found in literature indicating

good growth conditions in the PAFF Box. However, although the variety was the same (i.e. var. capitata), head mass is also dependent on cultivar, which differed from that found in the scarce literature on the subject. For basil, the average shoot fresh biomass harvested in the PAFF Box was 125.41 g which is higher than the average 96.6 g obtained in hydroponic treatment reported in a recent study [218]. The dry mass of basil obtained in hydroponics in this study corresponded itself to higher yield (in kg/ha) of 1.6 to 5.3 fold compared to 38 basil accessions grown in soil [219]. Again the growing conditions and varieties used in the PAFF Box and these studies were not identical. But because basil achieved a consistent biomass it gives the important information that the PAFF Box one loop aquaponic system offered suitable growth conditions also for basil crops. This undeniable sustained growth of lettuce and basil showed the reliability of one loop aquaponic system for lettuce and basil production in a temperate oceanic climate as Rakocy et al. [56] did for the tropics.

Given the sustained growth results of the PAFF box, it remains unclear, however, what importance should be ascribed to the variation in dissolved mineral element concentrations and ratios between aquaponics and hydroponics. In this case it seems that lower concentrations did not affect the crop yields. To test the claim that lettuce and basil yields achieved in PAFF Box were similar to hydroponics, it became necessary for some lettuce and basil to be grown in the same greenhouse (i.e. same environmental conditions) but in conventional hydroponic solution. These kinds of comparisons have been achieved in the study of chapter 5.

For this study, the experiment took place in a climate-controlled greenhouse. The hydroponic growing systems were DWC AeroFlo systems. Lettuces (*Lactuca sativa* var. capitata cv. Sucrine) were exposed to 3 different solutions and their fresh shoot and root weights obtained after 36 days were recorder and statistically analysed. The three solutions were the hydroponic solution (HP), aquaponic solution (AP) and the complemented aquaponic solution (CAP). The HP solution and the CAP solution were formulated to have their macro and microelement concentrations equal to conventional NFT lettuce nutrient solutions based on Resh [12]. The AP was formulated for having the same macro and microelement concentrations found in the single loop aquaponic system of the University of Virgin Islands (UVI) published by Rakocy et al. [40]. Rain water was used for the HP while RAS water was used for the CAP and AP (for AP the RAS water was diluted 10 times in rain water). The result showed that the yields in fresh lettuce heads obtained in the AP and HP were significantly equivalent while they were 39% higher in CAP, in both trials. Roots fresh mass were equivalent in AP and CAP while statistically lower in HP.

Important information has been highlighted thanks to these results. First, they confirmed that lettuce can thrive in low concentrated AP solution to achieve same yields as in HP solution. Buzby and al. [220] obtained similar lettuce growth for Butterhead, Bibb and Romaine lettuce subtypes in cold fish water with very lower mineral element concentrations and an EC of 130 $\mu\text{S}/\text{cm}$. Other authors obtained plant growth comparable to control in low concentrated solution [88,162]. Similar growth for plant standing in low and high nutrient concentrated solutions can be explained by previous observations made by Olsen [149]. He demonstrated that the rate of ion absorption for a given ion is independent of the concentration of the ion in the nutrient solution, except for concentrations under 0.003 mg/L. This suggests that even elements not detected by ICP-OES might have not been deficient (e.g. Mo in PAFF Box solution). Ion absorption by plants is an active process requiring energy since it occurs against the concentration gradients and is thus not dependent of the ion concentration as soon as there is no ion depletion in the roots zone (i.e. concentration inferior to 0.003 mg/L). Ion absorption rates can be altered if ions are depleted but in NFT and DWC hydroponic culture the

solution constantly flows around the roots making depletion impossible. Olsen also showed that it is the proportions between the concentrations of the different ions that control the uptake rate either than the concentrations themselves. So, as soon as the proportions between the concentrations of the different ions stay the same the rate of ion absorption for a given ion is independent of the concentration of the ion. In other words, the rate at which the individual cations or anions are absorbed from the solution is determined by the ratio between the concentrations of these ions, but not their absolute concentration. If one ion suddenly increases in proportion its uptake rate will increase to the detriment of the other ions uptake rate. To sustain ion uptake, ratios between ions need to stay constant and therefore they have to be similar to the natural plant ratio uptake. Then if ions are removed out of the solution by plant in the same proportion as ratios in solution, these stay constant in solution and ion uptake rates stay optimal. These ratios are respected in hydroponic solution formulation, but in aquaponic water this is not necessarily the case. In the study presented in chapter 5, ratios were different between AP and HP and AP ratios were far from the HP optimal ones. In AP, the TAN/NO₃ ratio varied greatly which may have perturbed the nitrogen absorption rate in this treatment. Moreover, pH was higher in AP (7.3-7.5) than in HP treatment (5.7-5.8) and pH has also an important effect on ion absorption rate with an optimal pH in NFT and DWC conditions of 5.5-6 [12].

These findings indicate that the nutrient absorption rates were less favourable in the AP treatment. Indeed, the nutrient leaf content in AP was statistically lower than in HP with an average reduction of 35 % revealing lower nutrient uptake in AP treatment. This is also comforted by a different shoot to root ratio and more root production in the AP treatment. Lettuces in AP were able to achieve the same shoot mass as HP presumably thanks to their root mass increase. Indeed, the larger the root system, the larger the absorbing surface, greater is the number of ions absorbed per unit of time (Kreyzi, 1932 in [149]). It is surprising that the lettuces in AP were able to produce more biomass in total (because of the roots) than HP while they were in less favourable nutrient uptake conditions. They were grown in the same system (i.e. AeroFlo) and in the same greenhouse (i.e. in same light and climate condition) but AP had some RAS water containing factors that were absent in rain water. These factors must have been plant growth stimulating agents. More precisely, it could be either dissolved organic matter (DOM) or microorganisms. It is not clear yet if they promoted only the root growth or also the nutrient absorption rates. Their promoting effect is comforted by the result obtained in CAP treatment. Indeed, while CAP lettuces were in equivalent pH, EC and nutrient ratio conditions as in HP, their shoot and root mass were increased in average by 39% and 47%, respectively. This result showed that an increase in nutrient in aquaponic solution led to higher crop yields than in hydroponics.

Lettuces appear as a crop being successfully grown as well in one loop aquaponic as in CAP solution. Our results show that complementing the aquaponic solutions to obtain the right ratios between nutrients and lowering pH will assure the best yields. Since these results have been published, improved growths by macro- and microelements complementation in aquaponic water have also been observed by other authors. Pak choy yield were improved by 83.6% compared to an aquaponic control [221]. Basil fresh weight was increased by 56% compared to hydroponic control [218]. For fruity plants as tomato, the improving yield of complementation has not yet been shown. Identical tomato yields between complemented RAS water and hydroponic control have been reported but some crucial ratio were not respected in the complemented treatment [151].

These findings highlight the fact that aquaponic water complementation for improving crops growth in NFT or DWC is rather important to adjust the nutrient ratios between them instead of only increasing nutrient concentrations. Adjusting the pH to assure optimal uptake seems also important. The very promising results obtained motivates the importance to make more fundamental research in such growing conditions on optimal EC linked to nutrient concentrations and ratios in order to optimise plant growth in aquaponics. The identification of the promoting agents present in aquaponic water and their mode of action should also be further studied.

So in brief, the results presented above showed that while some nutrients in aquaponic solution were below the optimal concentrations and ratio, sustained lettuce growth was achieved in DWC with yields similar to hydroponics. But most of the nutrients inputted in the single loop aquaponic system were still discharged in the environment because of water exchange and sludge spillage. Moreover, valuable mineral elements ended up in the sludge instead of accumulating in solution. Our results also showed that lettuce yields can be greatly improved by increasing the concentration of macro and microelements and adjusting their ratios in AP solution. Hence, a solution to improve crop yields in aquaponics while reducing the nutrients release in the environment might be developed. Such a solution could be to treat the sludge with the aim of recovering the water and the nutrients contained within it, and then reinsert this back into the AP solution. Indeed, the sludge could be processed in the aim to mineralise mineral elements (i.e. macroelements as N, P, K, Ca, Mg and S plus microelements as Fe, B, Zn, Mn, Cu and Mo) trapped in the solid matter in order to recover them in dissolved forms (e.g. anions, cations, chelates...) in water. This water rich in elements solubilised into plant-assimilable forms could then be reintroduced in the AP solution. This solution meets the fourth and last objective of the thesis which was to analyse the potential of improvement of nutrient recycling by sludge digestion onsite in aquaponic systems.

To treat the sludge onsite in the most sustainable and simple manner, the use of aerobic (AE) or anaerobic (AN) biological digestion seems the most convenient techniques [37,74,222]. These techniques have been used and studied mostly for improving the reduction of the total suspended solids (TSS) or total solids (TS) and chemical oxygen demand (COD) of waste water [186]. The use of these techniques to solubilise mineral elements contained in sludge in order to recover them in their effluent is not usual. In the opposite, in water treatment fields specific technologies are developed to trap the mineral elements in the aim to obtain clean effluents [210]. In the field of aquaponics there is now a debate over which type of digestion, AE or AN, is the most suitable [178]. Therefore, an evaluation of the mineralisation performance in AE and AN in simple bioreactors was carried out. An adaptation of the equations necessary to determine their performance was also necessary. All these have been addressed in chapter 6 (short communication).

The experiment of chapter 6 compared the performance between an anaerobic reactor (ANR) and an aerobic reactor (AER). In the ANR the water was slowly recirculated to induce a slow mixing of the sludge while in the AER air was constantly injected with an air pump for oxygenating and mixing the sludge. Three times per week, fresh sludge coming from a tilapia RAS was inputted in reactors while the equivalent volume of supernatant was removed. This gave an influent HRT of 15 days. After 42 days, the TSS, COD and nutrient mineralisation was analysed. Unfortunately, no repetition was achieved giving only exploratory results. The results were however very promising and interesting. First, no treatment stands out over the other. Close performance in aerobic and anaerobic digestion were achieved while aerobic showed slightly higher TSS reduction, COD oxidation and mineral elements mineralisation performance for most of the nutrients. In both treatments, around 50% of

TSS reduction and COD oxidation was achieved. Mineralisation of all macro and microelements was comprised in range of 10 to 60 %. These exploratory results indicate that at least 50 % of the sludge can be reduced onsite while recovering substantial amount of nutrients to complement the aquaponic solution. This was obtained with very simple low tech reactors and it can be assumed that with more efficient design better results could easily be obtained. Especially, literature reported TSS and COD reduction of brackish fish sludge with up-flow anaerobic sludge blanket reactor (UASB) higher than 90% on long run experiments [185,186]. It can be assumed that the higher the TSS removal the higher the nutrient mineralisation will be. But this assumption should be investigated. The use of UASB seems promising since they are considered a low-tech reactor with a low running costs and can produce biogas that can be converted to electricity and heat [187]. As the anaerobic secondary metabolites are known to be phytotoxic [212,213], an efficient posttreatment of the effluent needs to be envisaged. Ratanatamskul and Siritiewski [190] obtained promising results with expanded granular sludge bed (EGSB) reactors for further treatment of UASB effluents. Because of their ease of use and simplicity they seemed to be a potentially suitable posttreatment solution for UASB in aquaponics.

Therefore, the last chapter of this thesis (chapter 7) aimed to assess the macro and microelement mineralisation efficiency in a UASB-EGSB reactors set treating freshwater RAS-sludge. As it was needed to investigate whether nutrient mineralisation was correlated to the organic reduction performance, the performance for reducing the total solids (TS), the chemical oxygen demand (COD), volatile fatty acids (VFA), and lignocellulosic compounds (i.e. hemicellulose, cellulose and lignin) were also assessed. The quality of the effluents in terms of TS, COD, macro and microelements content was also studied in order to evaluate their suitability to reinsert them directly into the plant hydroponic beds. The experiment was realised in collaboration with Wageningen University and Research (WUR, The Netherlands) that operated two UASB-EGSB reactor sets. One other UASB-EGSB reactor set was operated in Gembloux (ULg, Belgium). This allowed to record the performances in different condition and multiplied the numbers of UASB-EGSB reactor sets studied. An anaerobic and aerobic reactor similar as the one used in chapter 6 were used as control. The reactors were conducted during 3 weeks in repetition to enable statistical analysis.

During the experiment conducted at WUR, an interesting phenomenon occurred in one of two UASB-EGSB set studied. The pH in WUR UASB II decreased from 6.5 to 5.8 while the concentration of VFA dramatically increased and the methanogenic fermentation stopped. This acidifying UASB achieved then poor organic reduction performances but surprisingly carried the best mineralisation performances for P, K, Ca and Mg. This situation highlighted the fact that the mineralisation performance of these elements is not correlated to the organic reduction performance as it was assumed. The mineralisation dynamic here seems best described by an equilibrium model based on the solubility of calcium orthophosphates which starts to dissolve in water when pH drops under 6.5 [206,207]. Most likely, these elements are present in sludge in the form of undissolved minerals rather than trapped in the organic matter. Our results showed also that WUR UASB II had a lower N mineralisation than the other UASBs. This seems to indicate that N mineralisation unlike the other macroelements is dependent of the organic reduction of the sludge. Indeed, ammonia is released mainly by the degradation of proteins [187].

Regarding the TS reduction performances, they were similar between the UASBs with high pH (i.e. 6.5 to 7) and the AERs. The AERs had the best COD reduction. However, the AERs tended to accumulate N, P, Ca and Mg instead of mineralising it. The UASBs with the high pH had an average

mineralisation of 53, 7, 15, -1, and 14% for N, P, K, Ca and Mg respectively and the acidic UASB (WUR UASB II) had 20, 22, 59, 22 and 53 % mineralisation for N, P, K, Ca and Mg respectively. These results showed an advantage for the use of UASB in AP. Especially because literature reports the highest TS and COD performance in UASB on long term operations [185,187,209]. The mineralisation for microelements was very low (i.e. < 1.7 %) in all reactors, even in WUR UASB II. But previous authors obtained very high mineralisation of macro and microelements and other heavy metals from sludge by lowering the pH until 4 with glucose or organic acids [74,223]. Presumably, the best heavy metal mineralisation can be achieved with organic acids because these are offering a chelating capacity when complexed with the metals [74].

All these findings indicate an existing antagonism between nutrient mineralisation and organic reduction. In brief, the lower the pH inside the UASB the higher the macro and microelements mineralisation but the lower the organic reduction and N mineralisation. This indicates that sludge digestion on aquaponic system sites for reducing sludge and recovering nutrients should be done in separated reactors operated in different conditions. A two stages digestion seems the most convenient. A first stage, receiving the fresh sludge and consisting in an UASB conducted at neutral pH for organic reduction, N mineralisation and methane production. This stage will considerably reduce the amount of sludge to be treated in the second stage. The stabilised sludge out of this first stage will be composed mainly by insoluble minerals and recalcitrant organic matter. The second stage would be the mineralisation stage conducted at low pH for macro and microelements mineralisation. Acids (mineral or organic) would need to be added in this reactor. It is therefore more advantageous to treat the sludge that has been reduced first as it will require less acid. Also, the effluents enriched in dissolved macro and microelements will be at a low pH which meets well the hydroponic fertiliser requirements. The opposite situation (i.e. first stage acidic and second stage sludge reduction) would require more acids in first stage and then base addition in the second stage to higher the pH. This implicates also the potential risk of re-precipitation of mineral elements inside the UASB and produce unsuitable effluents out of it for fertilising hydroponic plants. This two stage digestion technique presented above (i.e. UASB then acidic reactor) seems to be the best solution for aquaponic sludge treatment onsite. Further research should be undertaken to determine its performance and test its implementation.

While they removed most of the VFA (e.g. EII_WUR removed 97%), EGSB were able to remove the TS and COD only by 25 and 50 % on average, respectively. EGSB might not be an adapted posttreatment because of this low TS and COD removal. But further experimental investigations are needed to identify if a posttreatment is needed after the two stage digestion. A way to test the necessity of posttreatment is to study the plant growth in hydroponic beds receiving the effluents. The impact on crop yield can be compared to a hydroponic control. The hydroponic beds could themselves be a method of posttreatment, in which case it would mainly depend upon the dilution rate of the effluent in the hydroponic beds. These are further research subjects to investigate.

An experiment to test the effect of highly diluted AER and ANR effluents on lettuce growth in NFT has been achieved at the ZHAW institute in Zurich. The results have been published in the journal *Agronomy* (MDPI) with this thesis author as co-author [224]. The experiment consisted in the comparison of yields of lettuce grown in 3 treatments: an aerobic treatment with a solution composed of 85 % tap water, 15 % RAS water and 0.25 % (i.e. one litre) of aerobic reactor effluent; an anaerobic treatment with the same solution composition but with one litre of anaerobic reactor effluent instead; and a control with the same solution composition but with one litre of RAS water

instead. Every week one litre of aerobic, anaerobic or RAS water effluent was added to each respective treatment. The AER and ANR effluents were so diluted in a 0.25 - 3.75 % range (the proportion increased during the experiment as effluents were added 3 times per week). The effluents were coming from the reactors used in the experiment presented in chapter 6.

The mineral element content in concentrations in AER and ANR effluents were quite close to each other confirming the result obtained in chapter 6 showing that none of both conditions (i.e. AE and AN) stand out remarkably to each other. The nitrogen molecule forms were different in AE and AN. Indeed, in AE effluent nitrate was the dominant molecule form while it was absent in AN effluent. AN effluent had ammonium as dominant nitrogen molecule form.

The main results of the study were that lettuce growth was significantly higher in the treatment with AN effluent and that the control and the AE treatment had similar growth. The root mass was lower in the AN treatment leading to a higher shoot to root ratio than the other treatments. These results indicated the suitability of AER and ANR effluents to complement aquaponic solution when diluted in a 0.25 - 3.75 % range. With this dilution rate, no phytotoxic effect was noticed and even significant promoting effect occurred with the ANR effluent.

Several assumptions can be made to explain the best growth observed in the ANR effluent. The ANR effluent contained N only under the form of ammonia. The addition of ammonia in the NFT system solution composed with nitrate as only N source, might have promoted lettuce growth. Indeed, an enhanced NO_3^- uptake when the hydroponic nutrient solution's N source contained between 5% and 25% NH_4^+ have been reported by several authors [145,152,153]. Also the pH in solution was relatively high (i.e. 8.2 - 8.7) and in these pH conditions ammonium uptake is preferred than the nitrate uptake [145]. The significantly lower root mass in the ANR treatment indicated a better nitrogen uptake than in the other treatments as root mass is known to be mainly controlled by nitrogen availability in hydroponic solutions [11]. Another assumption is that DOM and microorganisms present in the AN effluent are different than in the AE effluent and RAS water and have a more pronounced growth promoting effect. It was quite surprising to observe the best growth in ANR treatment as during AN digestion volatile fatty acids are produced and their phytotoxic effect have been often reported in literature [215]. Presumably, the dilution rate of the effluent diluted the VFA under their toxic threshold while the growth promoting compounds were still in sufficient concentration to impact the growth.

Another experiment was achieved in Gembloux (ULg, Belgium) to test lettuce growth in UASB effluents. The effluents were diluted only three times in rain water. In this situation, the phytotoxic effect of the effluents was evident as the lettuce had a significant lower growth compared to the one grown in the hydroponic control. The results are not published yet. The importance of the dilution rate of AN effluent is highlighted by these two experiments. Further investigation is needed to determine one hand, the dilution rate that would occur in an aquaponic system integrating sludge digestion. And on the other hand, until what dilution rate the AN effluents present a plant promoting effect. These would help to determine if a posttreatment following AN digestion is required in aquaponics.

So far, the results of this thesis have highlighted four key points: 1) that simple one loop aquaponic systems discharge a high proportion of mineral elements to the environment, but that 2) aquaponic water undeniably promotes plant growth. Indeed, 3) when the aquaponic solution is complemented, plant yields have been demonstrated to be higher than in hydroponics. Further to this, 4) fish sludge

can efficiently be anaerobically digested onsite to better close the loop for water saving and for mineral element recovery to complement the aquaponic solution.

The design of aquaponic systems should be revisited in order to integrate these findings to improve the production performance while reducing aquaponics environmental impact. A paper addressing this design issue has been published in Water (MDPI) with this thesis author as co-author [150]. The authors proposed the theoretical concept of decoupled aquaponic systems (DAPS) design that integrates these findings. DAPS was modelled with a specific software and the manuscript presents the results that it generated.

The DAPS design (Fig 8.1) proposed in the paper is composed of 3 water loops. Indeed, in DAPS the fish and plant components have their own recirculating water loop. The fish part consists in a recirculating water loop similar to a RAS and the plant part is another recirculating loop similar to a recirculating hydroponic system. In this DAPS concept, all the nutrient rich water that needs to be exchanged from the fish part is discharged into the hydroponic part. But the water from the hydroponic part will not return in the fish part. Clear fresh water will enter the fish part to assure good water quality. The water should leave the plant part only under the form of vapour i.e. by evaporation and evapotranspiration carried by plants. The mineral elements contained in water should be uptaken in plant tissues. The sludge leaving the RAS is treated in a third loop called the sludge mineralisation loop. Sludge is treated using an up-flow anaerobic sludge blanket reactor (UASB). The UASB effluent composed of recovered macro and microelements and water is sent to the hydroponic parts. The hydroponic plant loop has then water input from the fish part and from the mineralisation loop. Hence, all the water and the nutrients are supposed to end up in the hydroponic part and not released anymore in the environment.

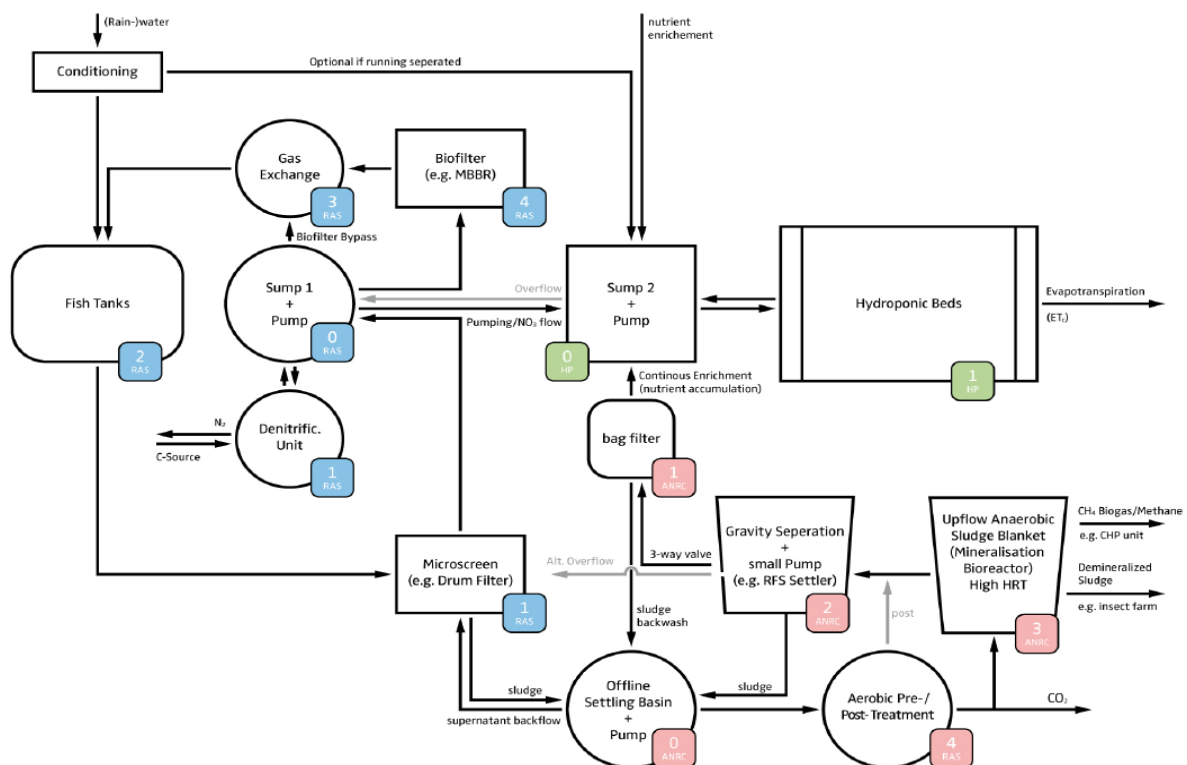


Fig 8.1. Decoupled aquaponic system (DAPS) layout. The blue tags comprise the RAS component, the green tags the hydroponic component, and the red tags the sludge mineralisation components. The level of each component is illustrated numerically in the small box and refers to the vertical direction the flow needs to

travel to; whereas high numbers refer to high positioning and low numbers to low positioning. Gravity flow occurs, when water flows from high levels to low levels, and pressurized flow is required when the flow goes from low to high numbers.

DAPS design allows also avoiding compromises in water quality (i.e. pH, temperature, DO, EC, etc.). In one loop aquaponic systems the water is recirculated from one part of the system to the others. When fish, plants and nitrifying bacteria are in the same water loop a compromise on water quality has to be made [42]. On the plant side, nutrient uptake in hydroponic condition is optimal at a pH of 5.5 - 6 and water temperature of 18 - 25°C is recommended for most of hydroponic crops [12,225]. Optimal hydroponic solutions have a high EC (i.e. > 1.8 mS/cm) and so elements in substantial concentration (e.g. nitrate, iron...) that can be harmful for certain species of fish [146]. On the fish side, nitrifying bacteria located in the biofilter which has always to be connected to the fish tanks perform better with pH higher than 7 and temperature of 20-30 °C [61,70]. Most of fishes do not thrive in pH lower than 6.5 [111]. If tropical fish are reared water temperature needs definitely to be higher than 25°C to assure optimal growth (e.g. optimal tilapia growth is reached at 28°C [60]). But it is warmer than plant optimal. Hence, decoupling enables optimal environmental conditions for each biological process. The right pH, water temperature, dissolved oxygen and EC can then be set in each loop to optimise fish and plant growth. Because the water does not go back from the hydroponic part to the RAS part, it can be complemented without any risks for fish. Lacking or low concentrated macro- and microelement in RAS water can be added in order to obtain an EC and nutrient ratio optimal for plant growth. In contrast to one loop system with DAPS no compromises on water quality has to be made. DAPS allows producing fish and plant in optimal growing conditions and thus assures competitiveness with conventional production systems.

As fish, bacteria and plant growth are dynamic processes depending themselves on dynamic variables a computed dynamic model is a required tool to size and predict DAPS productivity. The computed dynamic model written by Dr. Simon Goddek constituted also a valuable tool to understand DAPS dynamics and design boundaries. It enabled to study and to closely predict the N and P mass balances, fish and plant production, water consumption and evaporation and sludge mineralisation. The model is also of primary importance to size the system components. DAPS design complicated the sizing of the system because the size is not based only on the amount of fish feed input per hydroponic area, as it is for one loop system [48]. But it needs to be based on the evaporation potential of the hydroponic area and at the same time the nutrients input via fish feed. The sizing of the hydroponic part is a critical aspect because it needs to be able to treat the all water flow coming from the fish part (directly or via sludge mineralisation). Indeed, the plant area size determines the amount of water that can be evaporated and is the main factor for RAS water replacement. The water sent from the RAS to the hydroponic part is replaced by clean water which impacts positively the RAS water quality. The amount of water that can be replaced depends on evapotranspiration rate of plants that is controlled by net radiation, temperature, wind velocity, relative humidity, and crop species. Notably, there is a seasonal dependency with more water evaporated in sunny seasons.

The authors sized the plant part based on environmental condition in Central Europe and the phosphorus input (i.e. via fish feed) in the system in order to optimize its recycling and use. Indeed, as explained in chapter 3, phosphorus has been identified as one of the most valuable nutrients because it is formulated from an exhaustible ore resource. But nitrogen, unlike P, is the nutrient that is solubilised the quickest in fish water (see chapter 4) and might accumulate too much in the RAS

loop. Hence, the authors hypothesized that denitrification means might need to be implemented in this loop.

Another potential issue that could occur in DAPS is that some nutrients assimilated by plants at lower rate than the others could accumulate in the hydroponic loop. This would lead to unfavourable nutrient ratios. Some elements not preferably assimilated by plants (e.g. sodium) might also accumulate and eventually raise the EC until plant toxic level. These situations would conduct to the necessity of discharging all or part of the solution. But even in this case presumably lower water will be consumed compared to one loop aquaponic and conventional farming systems. Nutrient recycling would still also be greatly improved. However, this should be verified. Further studies should consist in building this system and test it to confront the field results to the one predicted by the model. The mass balances of the all macro- and microelements should be followed in DAPS in order to establish its nutrient recycling performance. Energy and water use should also be recorded for confronting its consumption to the model and to conventional equivalent farming systems. More research should determine the achievable plant yields in such system in relation to their evapotranspiration potential. Dilution rate of UASB effluents should further be studied in order to ascertain that potential phytotoxicity of UASB effluent is avoided or if posttreatment is required.

Other upgrades of the simple one loop aquaponic system, different that the DAPS presented above, could be imagined and tested. A hybrid DAPS design would be interesting to investigate. This would consist in the addition of hydroponic beds in the RAS loop of the DAPS for preventing nitrate accumulation. In this system two type of plant could be produced. Low feeder crops thriving on low concentrated solutions, such as leafy lettuce type plants could be grown in the RAS loop. Especially, studies have recently showed the ability of 34 food crops (lettuce, Asian greens, mustards, other greens, vegetables and herbs) to achieve totally satisfying yields and leaf nutrient content in flow-through fish water low in nutrients [220,226,227]. Heavy feeders crops more exigent on nutrient concentrations and ratios, EC and pH, such as fruiting plants could be grown in the decoupled hydroponic loop.

It is also important to notice that some recent authors have highlighted the potential to totally replace the biofilter by hydroponic beds in the RAS loop [228]. Hydroponic beds represent themselves already a considerable surface for nitrifying bacteria biofilm and for gas exchange. Plants are able to also directly uptake ammonia [228]. This could represent lower running costs since biofilters require considerable amounts of energy for their aeration via air blower (for moving bed) or high water pumping (for trickling filters) [14].

9. General conclusion

The present thesis aimed to investigate whether enough soluble mineral elements are released by fish to assure healthy and consistent plant growth in aquaponics compared to hydroponics. The thesis also aimed to determine the impact on plant productivity when the concentrations of soluble mineral elements in the aquaponic solution were increased. The proportions of mineral elements recycled in a simple one loop system were assessed and a solution to improve the recycling of these elements was explored.

In the term of this work, it appeared that lettuce could achieve similar growth performance in DWC in aquaponic and hydroponic solution but significantly higher growth (i.e. 39% fresh mass increase) in complemented aquaponic solution. This indicates that lower mineral elements concentrations do not

impact negatively plant growth and with an increase of their concentrations the yields can overtake the conventional hydroponics ones. Also the microorganisms and dissolved organic matter may play an important role for promoting plant roots and shoots growth in aquaponics. Other results showed that aquaponics consumed and discharged less water to produce fish and plant but required more energy than conventional farming systems. The mass balances analysis of the mineral elements indicated that an important proportion of the elements accumulated in fish sludge and were lost by water and sludge spillage. A solution to prevent this is digesting the sludge onsite to recover the mineral elements and water. Especially, anaerobic digestion of sludge with UASB showed promising results to reduce the sludge. To improve the mineral element mineralisation in available form for plants, a two stage digestion including an alkaline and an acidic stage seems the best solution for aquaponic sludge treatment onsite.

Regarding these results an amelioration of the one loop aquaponic system was suggested as a decoupled aquaponic system. Such system is assumed to reduce water spillage, recycle mineral elements and improve fish and plant yields. DAPS has the potential to improve productivity while reducing impact on the environment which meets well the goals of eco-intensification of the fish and plants production.

Further research should be undertaken to determine DAPS performances and test its implementation. Further experimental investigations are also needed on the two-stage sludge mineralisation process proposed. It remains to determine if posttreatment is needed and assess the plant growth in the diluted effluents.

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