GEOFOOD – RAS and heating installation in The Netherlands

Half-yearly update report no. 3 – June 2020

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The RAS- and aquaponic research facilities for the GEOFOOD project, located in Bleiswijk, the Netherlands, were constructed between January and May 2019. After completion the facilities were used, among other functions, to gather data by running fish- and lettuce production trials until June 2020. This half-yearly update report summarizes the overall progress and suggested best practices for the period December 2019 until June 2020.

For a full description of the RAS facilities please refer to the design report by Landing Aquaculture, 2019. For more information on the aquaponic design and heating installation please refer to the previous update reports in this series (Boedijn, Poot, *et al.* 2019a, 2019b).

1 Progress in the research facilities

1.1 Timeline

The timeline in figure 1 summarizes the key processes and milestones during the operation of the aquaponic research facilities. The main goal of the period December 2019 until June 2020 was to increase fish stocking density and steadily ramp up the feed load towards the system's design target. In November 2019 it was decided to stock the system only with tilapia after several failed attempts with pikeperch (Boedijn, Poot, *et al.* 2019b). The third batch of tilapia was introduced to the RAS on December 4th and consisted of 1500 fingerlings with a weight of 0.2 grams each. At this point the system held a total of approximately 3000 fish.

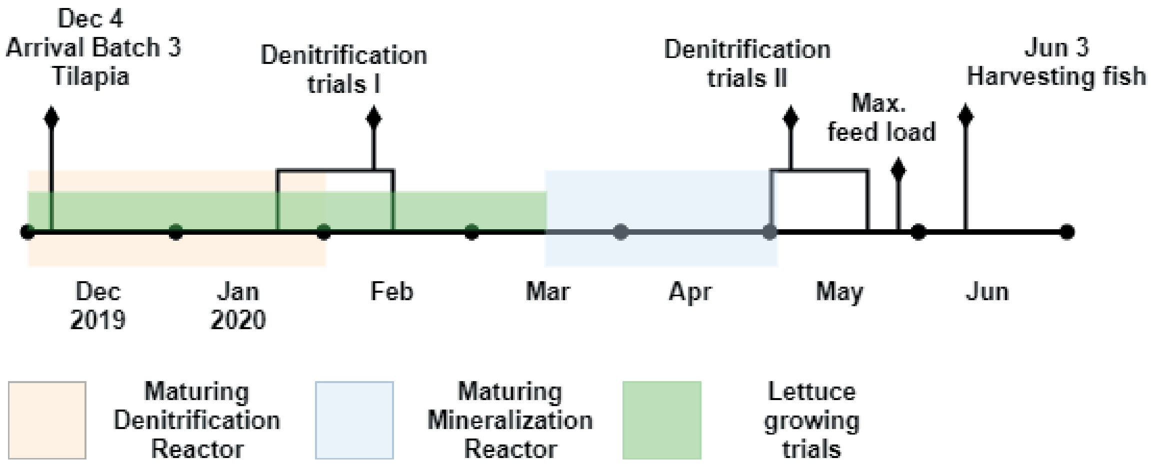

Figure 1 *Timeline summarizing the progress of the aquaponic research facilities in Bleiswijk, The Netherlands, during December 2019 – June 2020.*

The denitrification unit was matured over a three-month period as fish sludge production increased. The performance of the reactor was determined and improved upon in several trials which were carried out as part of a Validation Voucher provided by the EU-project VIDA (INNOSUP Call of Horizon 2020). More detailed information on the function and performance of the denitrification reactor can be found in Section 2.5 of this report.

The mineralization unit was also started up successfully once fish sludge production was sufficient. Unfortunately, maturation of the reactor took longer than planned due to limitations caused by the COVID-19 pandemic. Still, some samples for nutrient profile analysis were taken to estimate performance. More detailed information on the function and performance of the mineralization reactor can be found in Section 2.6 of this report.

Lettuce was grown on RAS effluents in a deep water culture system for two growth cycles. The first cycle ran from September 2019 until October 2019. The second cycle started at the end of November 2019 and ran until the beginning of March 2020. Results are presented in Section 1.3 of this report.

Despite some minor issues that temporarily impacted the fish feeding schedule, a feed load for the tilapia of about 20 kg/day was achieved towards the end of May 2020. This was an important milestone to reach because it confirmed the calculated limits of the RAS facility and provided insights regarding future improvements for system design as well as RAS management. Furthermore, an increase in feed load resulted in a higher intake of fresh (colder) water in order to keep the dissolved nitrate concentration at an acceptable level for fish health and -growth. Therefore, the feed load influences RAS heat demand, which is a key parameter for the validation of the energy model that has been developed within the GEOFOOD project. RAS fish production ended when the tilapia were harvested on the 3rd of June and sent to Diergaarde Blijdorp Rotterdam Zoo to be used as feed.

1.2 RAS tilapia production

The system was stocked with a total of 3000 Red NMT tilapia from Til-Aqua International, Someren. The first batch of 1500 fingerlings entered the RAS on August 14th 2019 and the second batch of 1500 fingerlings arrived on December 4th 2020. The fingerlings in both batches had a starting weight of 0.2 grams each. Commercial pellets with a protein content of 42% were fed at a rate of 1 to 1.5% body weight/day, on average, across the production cycle.

Table 1 shows an overview of the different fish tanks that were used in the RAS. The fingerlings started out in the system's smallest tanks (500 l in volume). As the fish grew, it became apparent that not the stocking density (i.e. kg of fish per m^3 of tank water) but the feed load determined the carrying capacity of a tank. If the maximum feed load of a tank (see Table 1) was exceeded, water quality parameters started to deteriorate. Therefore, whenever maximum feed load was reached, the fish population in that tank was graded (i.e. sorted based on size) and split into two tanks.

Table 1
Available fish tanks within the RAS.

Fish tanks	Function	Volume [m^3]	# of tanks	Maximum feed load per tank [kg/day]
Small	Fingerlings	0.5	2	0.6
Medium	On-growing	1.5	3	1.2
Large	Grow-out	10	3	9.5

As an example, an incoming batch containing 1500 fish started out in a single small tank. Once the batch required more than 0.6 kg of feed per day, the fish were split into two groups based on their size. Each group, containing roughly 750 fish each, was then placed into two separate small tanks. When both tanks again needed more than 0.6 kg/day, the fish were transported to medium tanks. When the fish outgrew the medium tanks, both groups would first be merged in a large tank and later separated again into two large tanks where the fish grew to their final size.

In the final phase of the project, two large tanks for grow-out each contained 1500 fish with an average weight of 550 g, resulting in a stocking density of around 80-85 kg/m^3. A higher stocking density may have been feasible and has been reported for other (commercial) RAS systems growing tilapia (DeLong *et al.* 2009). However, to mitigate risks to animal welfare, the system was not pushed any further and the water quality parameters in Table 2 were maintained as much as possible. Especially oxygenation of system water became increasingly unstable as feeding load and stocking density approached the design limit.

Table 2

Water quality parameters and monitoring management.

Parameter	Unit	Target value	Frequency of measurement	Method of measurement
Oxygen	mg/l sat. %	7 – 9.5 80 – 100	Continuous	Probes in tanks
TAN	mg/l	<1	Daily	Cuvette test
Nitrite	mg/l	<1	Daily	Test strips
Nitrate	mg/l	<500	Daily	Test strips
Alkalinity	mg/l $CaCO_3$	70 – 150	Weekly	Test strips
pH	-	7.0 – 7.8	Continuous	Probe in sump
EC	mS/cm	0.5 – 1.0	Weekly	Handheld EC sensor
Temperature	°C	28	Continuous	Probes in tanks

1.3 Greenhouse lettuce production

Lettuce (Exaudio RZ) was grown hydroponically in a deep water culture system (DWC), using water from two sources. Half of the ponds were filled with RAS effluent and the other half was filled with UV disinfected rain water. Both were supplemented with nutrients to get a good growing medium for lettuce with the same pH and EC values (see Table 3). Ponds were aerated continuously and water in the ponds was circulated once a day to maintain a uniform water quality. Nutrient composition of both growth media was determined several times and O_2 level, EC and pH were determined weekly. If needed, adjustments were made based on these data. Iron was added weekly because it is depleted very fast in hydroponics.

Table 3

Nutrient analyses of growing media for lettuce production based on two sources: rainwater or RAS effluent. Analyses were done one week after transplantation to production floaters.

Parameter	Unit	RAS effluent	Rainwater +nutrients	RAS effluent + nutrients
pH		7.4	5.4	5.2
EC	mS/cm	0.7	1.8	1.7
NH_4	mmol/l	<0.1	1.0	0.1
K	mmol/l	4.9	6.7	6.7
Na	mmol/l	0.7	0.3	0.5
Ca	mmol/l	0.1	2.9	2.6
Mg	mmol/l	<0.1	1.0	1.1
Si	mmol/l	<0.1	<0.1	<0.1
NO_3	mmol/l	1.9	11.8	9.8
Cl	mmol/l	1.5	0.3	0.7
SO_4	mmol/l	<0.1	0.9	1.0
HCO_3	mmol/l	1.5	<0.1	<0.1
P	mmol/l	0.3	1.1	1.4
Fe	µmol/l	<0.4	57.2	25.8
Mn	µmol/l	<0.1	6.5	6.3
Zn	µmol/l	0.2	5.5	5.1
B	µmol/l	5.0	34.0	33.0
Cu	µmol/l	<0.1	0.6	0.7
Mo	µmol/l	<0.1	0.29	0.34

Lettuce seeds were sown on press clods (peat), placed in a starting floater and then stratified for 4 days at 4°C. Thereafter the starting floaters were moved to ponds in the greenhouse and clods were kept wet by overhead watering. When plants had a root of reasonable length, overhead watering was gradually reduced. When plants started to overlap they were transplanted from starting floaters to production floaters. Two types of production floaters were tested; type 1 contained 8 plants per floater (16.7 plants/m^2), and type 2 contained 10 plants per floater (20.8 plants/m^2). Both floaters were designed for dry-hydroponics meaning that the press clod did not touch the water surface. Floaters of each type were used in two ponds with RAS effluent as a growing media basis and two ponds with rainwater as growing media basis (see Figure 2).

Figure 2 *Overview of the hydroponic growing facility consisting of 10 deep water culture ponds. Left: schematic overview and layout of the experiment. Right: Lettuce production towards the end of the second cycle.*

Lettuce was grown under natural light conditions, supplemented with 5 hours of LED (red and blue, 135 µmol/m^2/s). LED lighting was started 30 minutes after sunset. In the first cultivation cycle temperatures at night were between 15 and 17°C, and during the days temperature ranged between 18 and 22°C. In the second cultivation cycle at night temperatures were between 8 and 11°C, and during the days the temperatures ranged between 10 and 14°C.

Lettuce was grown for two growth cycles. The first cycle ran from September 2019 until October 2019. The second cycle started at the end of November 2019 and ran until the beginning of March 2020. In between the two crop cycles, growing medium was renewed. But to keep the microbial community 30 liters of growing media (either based on RAS effluent or rainwater) from well performing ponds of the first experiment was added to each pond at the start of the second cycle.

Lettuce was harvested when the lowest leaves started to become yellow. Moment of harvest was not equal for both floater types. For yet unknown reasons in some ponds roots were dying at a certain moment (beginning October 2019), but fortunately were regrown.

Lettuce heads harvested from floaters of type 1 were larger than type 2 (see Figures 3A and 3C). This was expected because plant density was lower for type 1. The difference between the two types of floaters was larger in the second trial when temperatures were lower and less light was available. Production per m^2 was not equal for both floater types (see Figures 3B and 3D). In the first experiment, floater type 2 gave a higher production in rainwater based growing medium. In the second experiment, floater type 1 gave a higher production in both RAS effluent- and rainwater based growing medium.

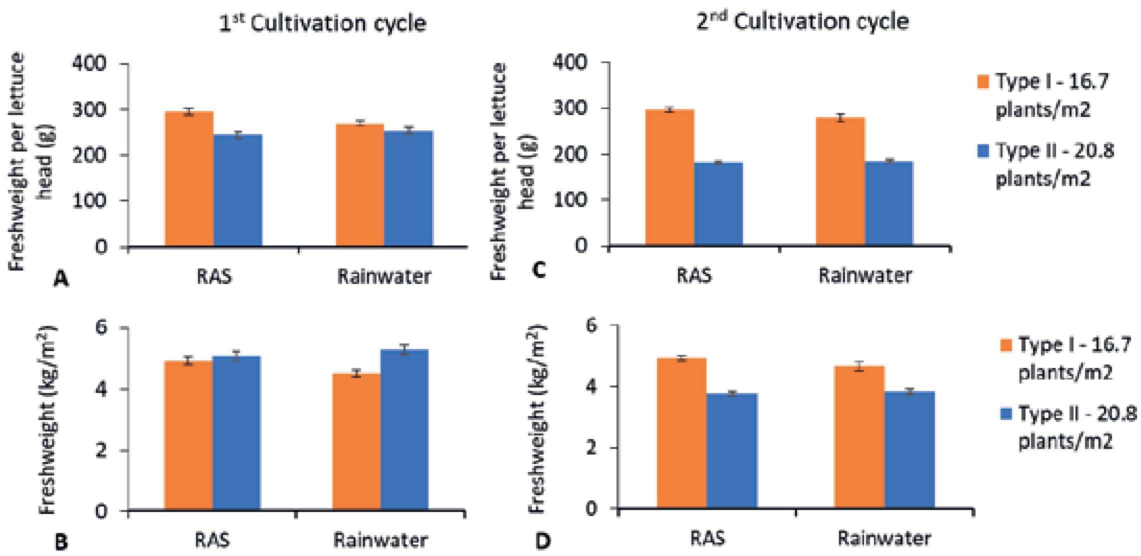

Figure 3 *Production data of lettuce on RAS effluent- and rainwater based growing media. (A) and (C) Fresh weight per lettuce head (g). (B) and (D) Fresh weight of marketable product (kg/m²). Data is given for both types of floaters, type 1 has 16.7 plants/m² and type 2 has 20.8 plants/m².*

Though lettuce yield was influenced by the type of floater used, the findings of a previous set of trials by Goddek & Vermeulen, 2018, were not repeated. In those experiments lettuce grown on RAS-derived water performed better than a standard hydroponic nutrient solution, despite a higher sodium level. As a possible explanation the authors indicated that the beneficial interaction between microorganisms from the RAS water and the plant roots could play a role. The results from the trials for the GEOFOOD project do not suggest the same conclusion. Then again, the fish stocking density was relatively low (i.e. 30-40 kg/m³) when the lettuce trials were performed. It could be the case that a more developed RAS microbiome and a higher concentration of sludge particles does have an effect on plant growth performance. Still, a more elaborate study that includes investigation of the metabolome and microbiome is needed to properly test such hypotheses.

1.4 Heating system

The GEOFOOD RAS was heated using a plate heat exchanger connected to the greenhouse's central heating network. The 'cold' side of the heat exchanger was installed as a side stream loop connected to the aquaculture system. The 'hot' side of the heat exchanger was connected to the central heating network. Because the water flows in both loops were fixed, the main way to control heating energy input into the GEOFOOD RAS was by controlling the water temperature on the hot side of the heat exchanger.

Figure 4 shows the daily mean RAS system water temperature in the period November 2019 to June 2020. Before November, water temperature could be controlled accurately (i.e. target temperature ± 0.2°C), but as the fish stocking density increased, so did the daily water changes that were needed to keep nitrate concentration at an acceptable level for fish health. Since the fresh water that entered the RAS during a water exchange had a temperature of 10 to 15°C (during the winter months), the system water temperature started fluctuating between 26 and 27.5°C. Temperature would drop in the morning when fresh water was added to the system and slowly rise over the course of the day as the heating system warmed it back up again to a target temperature of 28°C. For the period December and January it was found that the heating capacity was insufficient to avoid the temperature fluctuations, nor was the heating system able to reach the desired setpoint. Of course, heat loss of the RAS to the outdoor environment also plays a significant role during the colder months.

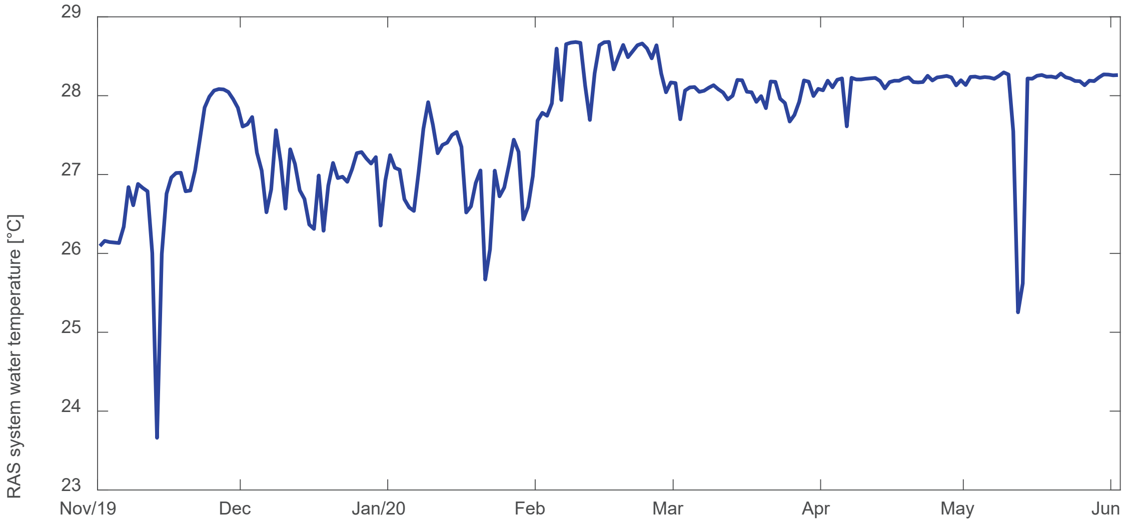

Figure 4 *Daily mean RAS system water temperature.*

Heating capacity was increased in February by adjusting the maximum heating temperature on the hot side of the heat exchanger from 35 to 45°C. This adjustment underlines that the peak heat demand of a RAS facility is a key design parameter, especially when employing low-temperature heat. More information on the reuse of residual heat from a geothermally heated greenhouse for RAS production can be found in the GEOFOOD reports by Boedijn, Baeza, *et al.* 2019a, 2019b.

The system water temperature stabilized once heating capacity increased, but a discrepancy was found between several sensors all measuring water temperature. After sensor recalibration, the heating setpoint was changed in March to achieve 28°C. In February water temperature had been slightly too high (about 28.8°C) due to the difference in sensor outputs.

The sharp temperature drop in November (see Figure 4) was caused by a leaking valve. The heat exchanger had to be disconnected from the rest of the system as repairs were done. A second sharp temperature drop, in May, was the result of an operational error. The freshwater input valve was unintentionally left open for too long, thereby replacing about 30% of total system volume with colder water. Target water temperature is a key parameter that influences heat use, the temperature fluctuations as described above will therefore be accounted for when analyzing the data and validating the GEOFOOD energy flow model on geothermal aquaponics (Boedijn, Baeza, *et al.* 2019a)

To get a first indication of the RAS energy model's performance a load duration curve was created based on the daily heat demand. Figure 5 shows both measured and simulated heat use. Though the model overestimates heat demand, the results are promising and explainable. Some input parameters of the RAS still have to be fine-tuned based on further analysis such as the R-value as well as infiltration- and ventilation rates. Furthermore, the model enforces ventilation based on a relative humidity threshold which was not the case in the GEOFOOD RAS. Finally, the actual weather data must be used as an input rather than values from a dataset containing yearly averages.

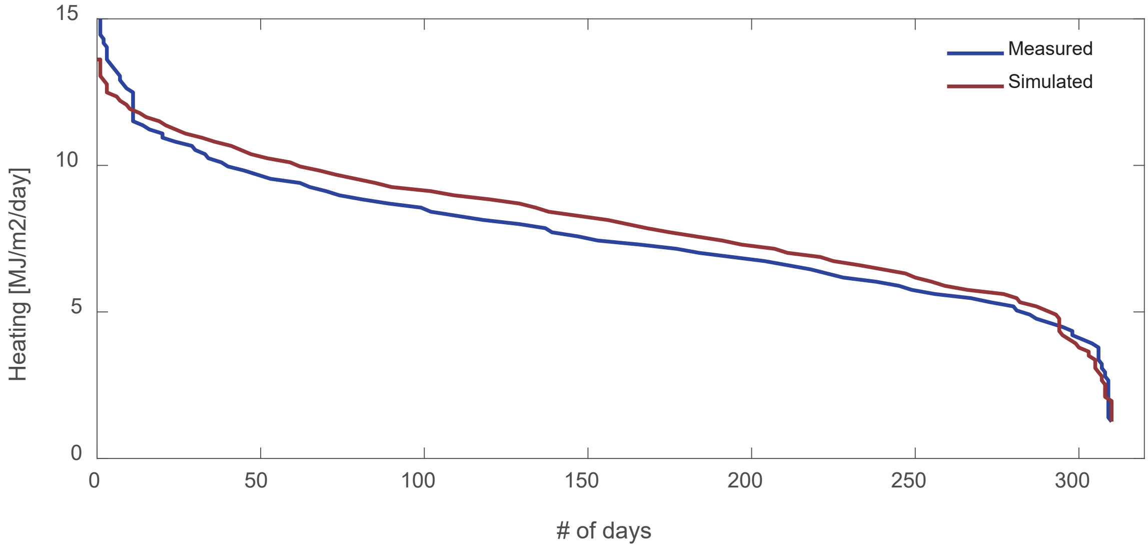

Figure 5 *Measured and simulated load duration curves of the RAS daily heat demand.*

2 Lessons learned and best practices

2.1 RAS management: tilapia versus pike-perch

A few issues arose because the GEOFOOD RAS was originally designed for pike-perch, but was used to grow tilapia. First, we started with 0.2 g tilapia fish instead of 30 g pike-perch. Smaller fish eat more relative to their body weight, and the stocking density for tilapia is higher than for pike-perch. Therefore the maximum carrying capacity of especially the small and medium tanks were more of a bottleneck than planned. This issue was solved by splitting the fish population over multiple tanks as is described in Section 1.2.

Second, the automatic feeding system had to be altered to accommodate the tilapia. The feeders on the small tanks were not suitable for the fine feed and many small doses required for the tilapia fry. Also, because pike-perch is fed around the clock and tilapia are not, the control system for the feeders had to be changed.

Tank outlets must consider the type of feces produced by the fish as well as the behavior of the fish. Tilapia produce stringy, floating feces that clog screens. This means tanks need surface drains that can remove feces without clogging. As a result, more manual labor than planned was needed to avoid clogging.

Higher water temperatures mean lower dissolved oxygen in the water because oxygen transfer becomes more difficult. With tilapia, significantly lowering the temperature to slow down the fish, increase dissolved oxygen or reduce the toxicity of ammonia is not possible. This became especially apparent towards the end of the trials when stocking density was highest.

An observation worth mentioning is that tilapia can indeed reach high swimming speeds in a tank with a rotating flow of 1 to 2 body lengths per second. In a properly stocked tank with a rotating flow, aggression was pretty much non-existent. A rotating flow also helps in distributing fish feed, which can make up for limitations on the feeding system and reduce competition when feeding. When using floating feed and sidewall drains in combination with a rotating flow, feed loss through the drain(s) needs to be monitored and flow adjusted if required.

Best practice: A more versatile, modular RAS design that can accommodate multiple fish species was discussed, but having that flexibility also adds costs to the system. Given the specific needs of each species, we think most commercial systems perform best when optimized for those needs. Research systems could benefit from a more versatile design in order to extend its lifetime. One general recommendation would be that overcapacity, by having some extra tanks, can offer flexibility when a change in species is required. Similarly, slightly oversizing the drainage side of a RAS may help to avoid limitations caused by insufficient water flow and renewal.

2.2 RAS water supply

An issue that was also addressed in the previous update report is supply water quality. Good water quality starts with selecting a source. It can be rainwater, groundwater or tap water but in all cases a list of water quality parameters has to be checked for both the production of fish and plants. At the start of the project rainwater was used after UV and ozone treatment. Unfortunately the rainwater contained traces of aluminum (Al), copper (Cu) and zinc (Zn) that started to accumulate in the RAS. The main supply of water was then changed to groundwater that was treated using reverse osmosis (RO). This did solve the issue of potential build-up of heavy metals, but the RO water contained so few minerals that (sea) salt had to be added on a regular basis to avoid osmoregulatory related fish health- and growth issues (Timmons *et al.* 2018). Apart from that, the RO installation stopped functioning a couple of times which meant the RAS had to go without the input of fresh water for about a day or temporarily rely on the rainwater.

Best practice: When relying on RO water, make sure the salinity is still within the optimum range for the species of fish that will be reared. In general it is recommended to include two sources of water in the design; a primary source and back-up. Of each source it should be known which treatments (e.g. UV, ozone, RO, filtering) are needed to ensure the required quality and which actions should be taken (e.g. increase/decrease salinity or alkalinity) whenever a switch from primary to back-up water source occurs.

2.3 RAS water quality management within aquaponics

In particular two practices in water quality management for fish production cause potential issues whenever that water is reused for the production of plants. First, a minimum salinity of 0.5 ppt must be maintained to reduce osmoregulatory related fish health- and growth issues. In aquaculture, sodium chloride (NaCl) or sea salt is added to the system if salinity is too low. Salinity is also increased by salts in the fish feed. Most crops on the other hand require much lower sodium and chloride concentrations for optimum growth. This is especially the case for recirculating hydroponic plant cultivation because sodium will accumulate in the system as plants do not take up sodium. Sodium and chloride concentration are therefore key parameters that determine the quality of irrigation water for greenhouse horticulture. Quality is considered high if water has a sodium concentration of less than 1 mmol/l or 0.023 ppt (Raaphorst & Benninga, 2019).

Second, alkalinity supplements are added to most RAS to maintain a certain pH-buffering capacity. All sodium based supplements such as sodium hydroxide and sodium (bi)carbonate should be avoided since most crops require a low sodium concentration. During the tilapia trials for the GEOFOOD project a potassium based buffer was used. However, towards the end of the trials potassium concentration exceeded lettuce nutrient uptake requirements. And it would have been beneficial to combine or alternate with for instance a calcium based buffer (PCG, 2017).

Best practice: When regulating salinity or alkalinity of a RAS that is part of an aquaponic system, supplements should be selected that have no negative effect on plant production. Especially supplements containing sodium or chloride should be avoided. Proper selection of and alternation between supplements can however contribute positively to the required nutrient profile of a crop. It is advisable to install a pH-buffer dosing system that can cope with different types of supplement.

2.4 Food safety measures in aquaponics and hydroponics

In January an article was published by Wang *et al.* 2020, who investigated the occurrence of Shiga toxin-producing *E. coli* in aquaponic and hydroponic systems. Their results indicated that the potential of food safety hazards is higher for these types of food production systems than previously perceived. Therefore, as a response to the publication, water samples from both the RAS and hydroponic compartments were sent in for analysis. Table 4 shows the results as well as the quality requirements that are stated in the proposal for EU regulation on water reuse (European Commission, 2018). Compared to those quality requirements, the outcome of the analysis did not indicate a food safety hazard regarding *E.coli.*

Best practice: Though aquaponic and hydroponic systems may offer a more controlled food production environment, it should be assumed that pathogens can still occur and even contaminate edible plant parts and fish products. It is therefore advisable to implement measures that mitigate food safety risks such as handling and harvesting protocols, sanitizing equipment and regular water sample analysis.

Table 4

Occurrence of E.coli in the water from the RAS and hydroponic facilities.

Sample	E.coli	Quality requirement*
	[cfu/100 ml]	[cfu/100 ml]
RAS water	<1	≤10
RAS water in hydroponics (i.e. aquaponics)	<10	≤10
Standard water in hydroponics	<1	≤10

* The requirement of ≤ 10 cfu/100 ml is considered for high quality applications and applies to water reuse for all food crops, including root crops consumed raw and food crops where the edible part is in direct contact with reclaimed water.

2.5 Denitrification reactor

A denitrification reactor can be installed in RAS to remove nitrate from the fish rearing water in order to keep water exchanges to a minimum and avoid discharge of nitrate-rich water to the environment. Within aquaponics nitrate is removed from the water by the plants, which take it up as a nutrient. Denitrification reactors convert nitrate to nitrogen gas after which it dissipates from the system into the air. Because denitrification reactors in principle remove plant nutrients, they are not commonly used within aquaponic systems. Within the GEOFOOD project the nitrate rich water, produced by the RAS, was far too much for the available greenhouse lettuce production area. To mitigate loss of surplus water to the sewer, a novel denitrification reactor was installed (see Figure 6).

The aim was to run a simple, low-cost reactor, using fish sludge as an endogenous source of carbon to fuel the denitrification reaction. Ideally the reactor would require minimal input by an operator and run in-line, meaning that it automatically and continuously takes in nitrate rich RAS water and returns water with a lower nitrate concentration directly to the system. To monitor the performance of the reactor and improve upon its design, additional funding was obtained in the form of a Validation Voucher provided by the EU project VIDA.

Key findings include that the reactor effectively removed nitrate up to 22 mg NO_3-N/l/h. Such a removal rate could only be achieved when the reactor was not continuously fed water, but was operated as a batch-fed system. Best results were obtained if the retention time of a batch was at least 1.5 hour. This processing time was needed because the denitrification reaction in the reactor occurs under anaerobic conditions and input of fresh water also introduced oxygen into the reactor. Recycling the water coming out of the reactor back to the RAS meant the net solids discharge of the system was zero, creating issues with maintenance and water quality. More information on the trials and performance of the denitrification reactor can be requested by contacting Landing Aquaculture.

__Figure 6__ The installed denitrification reactor, designed by Landing Aquaculture.

Best practice: A denitrification step impacts the nitrogen balance in a RAS and/or aquaponic system because nitrate is removed and, if the reactor works suboptimal, nitrite may be produced. Furthermore, alkalinity is broken down by the nitrification process in the biofilter, but is restored by a denitrification process. It is advisable to monitor the performance of the denitrification reactor on a regular basis and adjust water quality management protocols, such as the dosing of pH-buffer. If a sludge-based denitrification reactor is used to recycle water back to the RAS, a solids capture step which takes solids out of the system regularly must be installed.

2.6 Mineralization reactor

A mineralization reactor was included in the GEOFOOD aquaponic design that can aerobically digest fish sludge (i.e. feces and uneaten feed), in order to recover nutrients such as phosphorus, potassium and calcium. The working principle is based on a community of heterotrophic bacteria, living in the reactor, that feeds on the sludge. During the digestion process, a part of the nutrients that are captured in the sludge become soluble and bioavailable for plant uptake.

Unfortunately, COVID-19 restrictions delayed the start-up of the reactor and forestalled efforts to closely monitor its performance. Still, some samples could be taken to assess the nutrient profile of reactor water before and after a 6 week digestion period. Figures 7 and 8 show the results for macro- and micro nutrients respectively. Though a limited amount of data could be gathered and the experiment was not repeated, it seems that the aerobic digestion did extract nutrients from the sludge since almost all elements increased in concentration.
It also looks as if the process is not equally effective for all elements. Figure 7 shows that a potential negative side effect of aerobic digestion within aquaponics could be that along with useful elements, sodium and chloride concentrations also increase.

Apart from aeration of the sludge with an air pump and air stones, conditions such as temperature, pH, EC and dissolved oxygen were not monitored or actively controlled. Recent studies show that controlling these parameters can improve performance and especially control of pH can increase the recovery of nutrients (Goddek *et al.* 2018; Inagro, 2019; Panana *et al.* 2021). Based on literature and experiences during the GEOFOOD project, the mineralization reactor can be adapted and its design can be improved for future research projects.

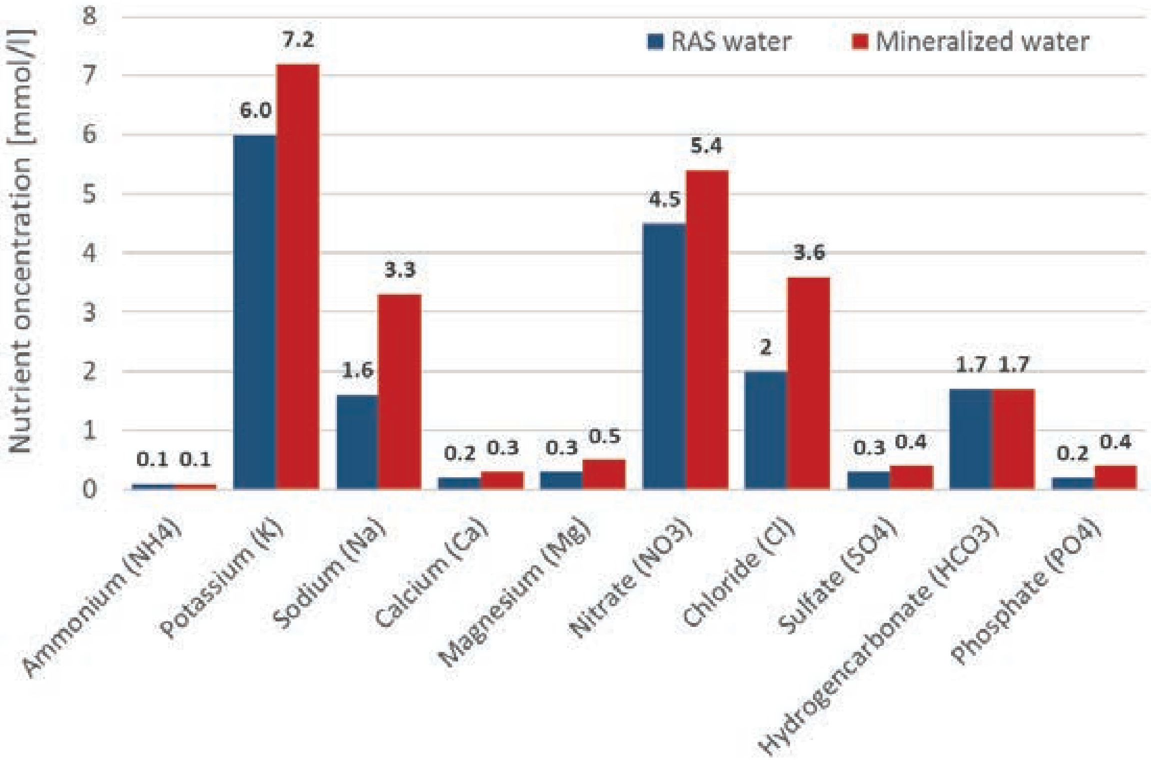

Figure 7 *Macro nutrient profile of the reactor water before and after 6 weeks of aerobic digestion.*

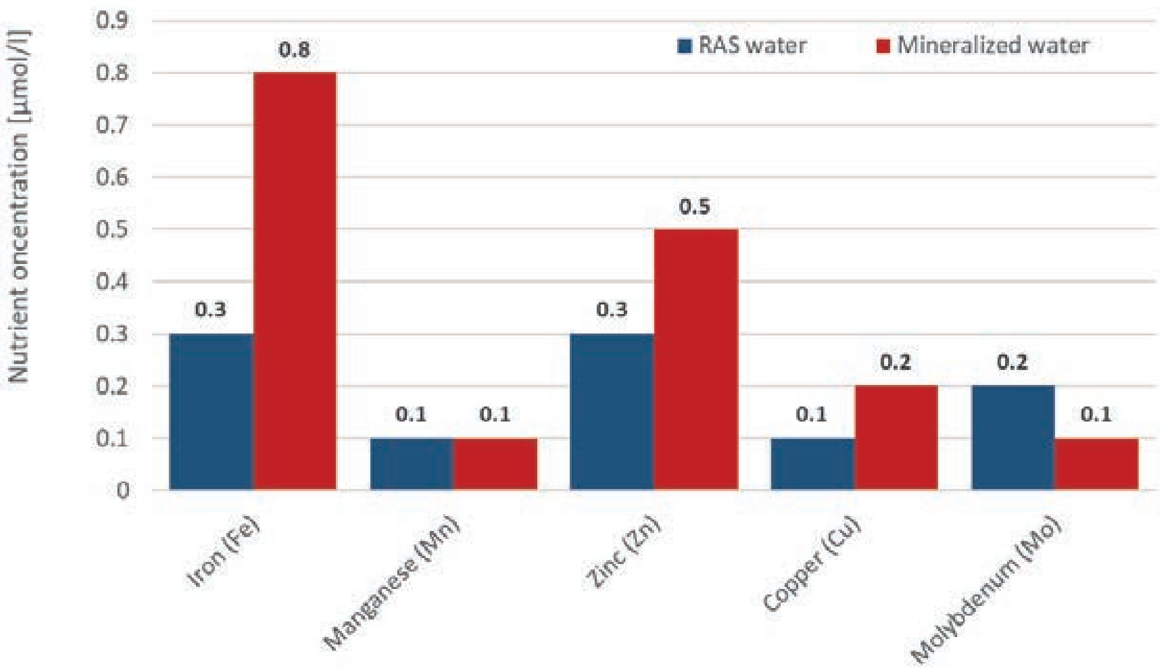

Figure 8 *Micro nutrient profile of the reactor water before and after 6 weeks of aerobic digestion.*

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To explore the potential of nature to improve the quality of life

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