

Feasibility Report on Innovative Aquaponic Systems production (Deliverable 5.1)

by
University of Rostock
Professorship for Aquaculture and Sea-Ranching
Justus-von-Liebig-Weg 6
18059 Rostock
Germany

and

Danish Technological Institute
AgroTech
Gregersensvej
2630 Taastrup
Denmark

Table of contents

1.	General introduction to aquaponics	2
2.	Thematic background of InnoAquaTech	3
3.	Aims of the pilots “Aquaponics” and “Integrated System”	4
4.	Nutrient balance of aquaculture	4
	Dissolved nutrients	4
	Particulate nutrients	5
A.	Integration of microalgae into aquaculture production	5
	Introduction	5
	Material and methods	5
	Results	7
	Discussion and Conclusions	9
B.	Integration of vermifiltration into aquaculture production	10
	Introduction	10
	System Design & experimental set up	11
	Analysis & Sampling	12
	Results, discussion & conclusions	12
5.	General discussion	13
6.	Outlook	14
7.	References	14

1. General introduction to aquaponics

By 2050, the world population is expected to grow to 9.6 billion people. The largest growth is expected in the already overcrowded urban coastal regions. Moreover, more than 800 million people are already suffering from chronic malnutrition (FAO et al. 2018).

This enormous growth alone is the biggest challenge for global agricultural markets. Additionally, the income of a globally growing middle class is rising, which increases food demand, especially in developing countries. This trend is further compounded by the fundamental change in eating habits towards a prosperity diet with significantly higher proportions of resource-intensive animal food (UNEP 2009). A convergence of the nutritional habits of the developing countries with those of the industrialized countries would require two to three times more land for the production of food necessary to sustain the global population.

At the same time, there is a growing demand for land for the production of renewable resources for renewable energy, such as biogas facilities (FAO 2006). This development leads inevitably to increasing competition between the forms of land use. Additionally, the world acreage can hardly be increased. The rapidly growing world population, new eating habits, and land use conflicts are therefore the biggest challenges currently facing global food security (Koerber et al. 2008).

Food insecurity is already a major humanitarian problem today, which will be accelerated in the future due to the expected demographic development. The prevailing conflicts and nutritional trends spur this insecurity, where those affected have no access to enough safe and nutritious food. In addition to a required reduction in the proportion of animal foods in our diet, these conflicts can only be remedied through a consistently sustainable food production. Here, all health, environmental, social and economic aspects of sustainability must be taken into account.

The food production systems of the future will undoubtedly have to deal with all these issues, although it will be difficult to deal with them all at the same time. One promising method could be aquaponics as a modern and most sustainable form of food production. This refers to the combination of two production methods, i.e. aquaculture and hydroponics. The integrated production has some advantage over conventional agriculture. Although both individual methods represent very efficient production methods, aquaculture in recirculation systems faces the problem of exchanging water filled with metabolic products of aquatic animals (usually fish). The disposal of the wastewater is often expensive, and hydroponics requires special and cost increasing fertilizers. In aquaponics the nutrient-enriched aquaculture process water is directed to the hydroponic system. The plants absorb nitrate, phosphate and other nutrients, thus cleaning the water, which in turn benefits the aquaculture system. This form of agriculture production is both environmentally friendly as well as sustainable (Stout 2013). The fish farming thus provides the nutrients for the plants, which in turn treats the water as a form of natural filter. Both methods benefit mutually.

Aquaponics offers the possibility to maximize the production of the outputs "fish" and "plants", whereby on the one hand the water treatment technology within the aquaculture

unit is a water saving process and on the other hand hydroponics in crop production unit is a soil saving process. Since the process water is treated and recycled, overall relatively little water is needed, and aquaponics facilities are mainly independent of natural water sources. In addition, since soil is not required, the condition of the soil is irrelevant (Tokunaga et al. 2015). The systems are very variable, and the locations can be chosen freely. This also provides the opportunity to set up production sites close to the markets in order to save transport costs and to operate more ecologically. Growing cities and the trend towards regional products also speak in favour of aquaponics operations in urban areas. As scarcely any additional fertilizers or pesticides are used, the outputs produced during subsequent marketing could possibly also be certified as organically or biologically grown, thus achieving higher market prices.

Regarding world's future challenges of rapid population growth and accompanied protein/food demand, water scarcity and desertification, urbanization and overfishing, sustainable food production systems such as aquaponics are an option for addressing these.

However, as the production of farmed animals continues to grow in general, treating effluent streams sustainably becomes increasingly important. In many countries the nitrate load of soil and groundwater is already at a critical level or exceeds the official guideline values. The random distribution of manure as fertilizer accounts for large parts of this load. As a countermeasure aquaponic producers recycle nutrients within their system. Though, usually only the dissolved nutrients are being used at the moment, whereby some nutrients are often lacking in the nutrient solution for the plants. The nutrient-rich solids are disposed within the municipal sewage system or being spread on agricultural grounds.

Nutrients derived from fish sludge have not been used in aquaponics *sensu strictu* (s.s.). (Palm et al. 2018) yet. However, considerations have been made using fish sediments for aquaponics (*sensu latu* or s.l.) farming with barley and wheat (Brod et al. 2017). In recirculating aquaculture systems (RAS) for the production of African catfish, solids are primarily separated by gravitation (sedimentation units). The accumulated solids are discarded during regular management and cleaning intervals, which results in nutrient as well as water loss. The status quo of solid waste treatment in RAS catfish production does not conform to optimal and sustainable resource use, and therefore could be adapted in future.

2. Thematic background of InnoAquaTech

The European Commission's Blue Growth Agenda for the Baltic Sea Region identifies aquaculture as one of the most promising sectors of the region's maritime economy in terms of growth and job potential. In the South Baltic area, however, aquaculture is not a widely established sector yet. There is a clear territorial disparity in introducing innovative and environmentally friendly production technologies that could help to create added value and increase the sector's international competitiveness.

InnoAquaTech has contributed to the cross-border development and transfer of such innovative and sustainable aquaculture technologies and offered SMEs all over the region access to state-of-the-art technology, know-how, expertise and financing models. Special emphasis was given to Recirculating Aquaculture Systems (RAS) and innovative

combinations of RAS with e.g. plant production (aquaponic systems) and/or renewable energy. In general, InnoAquaTech was initiated in order to boost the innovation potential of the project's target group, which consists of SMEs along the aquaculture and aquaponic value chain as well as related support organisations. By directly involving them in the project implementation, InnoAquaTech helped SMEs to develop and implement cross-border value chains that strengthen the South Baltic area's aquaculture sector as a whole.

Specifically, the project was designed to:

- identify best practices of integrated aquaculture systems and to evaluate their agro-economic and environmental impact
- develop and implement an SME service package (consisting of e.g. matchmaking events, trainings, study visits and an innovation check tool) that shall be sustained by a South Baltic aquaculture alliance beyond the project lifetime
- implement four aquaculture pilot cases to gain hands-on experience on the actual regional potential of different innovative and sustainable aquaculture systems.

This document presents results and conclusions from two of these pilots, namely pilot 3: "Aquaponics" and Pilot 4: "Integrated System".

3. Aims of the pilots

As a true cross-border activity, by a close cooperation between Rostock University and the Danish Technological Institute, the two pilots focused on the development of aquaponics as a new technology, the nutrient load under different fish/plant combinations and respective plant growth in order to achieve the economically most feasible results. The possibilities and potentials of RAS aquaculture technologies, microalgae biomass cultivation with aquaculture effluent water in an innovative integration of existing systems were explored and evaluated. The work covered microalgae cultivation and aquaponic technologies, vermifiltration, microalgae culture and fish selection, mass balances, environmental impact, energy and economic feasibility of these combined and integrated technologies, nutrient remediation and resource efficiency.

4. Nutrient balance of aquaculture

During the commercial culture of fish and other aquatic organisms employing conventional feeds, nutrient excretion always occurs due to the metabolism of the cultured animals. These nutrient excretions are either excreted in dissolved form via the gills and urine to the surrounding water, or as undigested solids, which also contain excess bacteria of the digestive tract.

Dissolved nutrients

The concentrations of the excreted dissolved nutrients vary depending on the nutrient type. The most important and quantitatively largest part of the dissolved nutrients is caused by the nitrogen metabolism of the culture animals, since nitrogen occurs to about 16% in proteins and proteins are particularly important for the formation of biomass (muscle mass). Depending on the cultivated livestock species, different fractions are excreted as ammonia by animals, but as a rule of thumb it can be assumed that about 50% of the applied nitrogen is excreted as ammonia via the gills. The fractions for other nutrients, such as phosphorus or

other plant-relevant nutrients, such as calcium, magnesium, potassium, iron, etc., are significantly lower, but verifiable quantities are still released into the culture water.

Particulate nutrients

The concentrations of the excreted particulate nutrients also vary according to the nutrient type. Nitrogen plays a minor role here. Depending on the type of crop, the feed used and the feeding regime, about 10 - 20% of the fed nitrogen is released via the particulate excretions. The fractions for other nutrients, such as phosphorus or the other plant-relevant nutrients calcium, magnesium, potassium, iron, etc., are much more abundant in the particulate fraction. Again, depending on the type of culture, up to 60% of the phosphorus applied through the fish feed can be released into the surrounding culture water by means of particulate excretions.

In order to increase the resource efficiency and sustainability of aquaculture production, and thus improving the economic balance, it is necessary to recycle the excreted nutrients in the sense of circulatory management, since feed in aquaculture represents the largest cost group, with up to 50% of the total production costs. This was investigated in the following two approaches and presented here.

A. Integration of microalgae biomass cultivation into aquaculture production

Introduction

Microalgae are considered an ecologically important group of organisms, used industrially for numerous applications including the production of pharmaceutical ingredients and chemical raw materials, food and dietary supplements, feed, biofertilizers and bioactive compounds, biofuels and bioplastics, biosensors as well as bioremediation, e.g. CO₂-fixation and wastewater treatment (Harun et al. 2010; Chapman et al. 2013).

Modern RAS production facilities create considerable amounts of nutrient-enriched wastewater. To recycle valuable nutrients and prevent eutrophication, terrestrial plants can be grown in soilless cultivation systems, a principle widely known as hydroponics or aquaponics. Alternatively, microalgae can be cultivated using wastewater as the culture medium. Nutrients contained in wastewater are hereby recovered while the water is biologically remediated (Sriram et al. 2012; Queiroz et al. 2013; Chiu et al. 2015).

In this InnoAquaTech study we aimed to assess the potential of green microalgae to extract nutrients from wastewater deriving from a production of African catfish (*Clarias gariepinus*), developing a robust biomass suitable for possible further applications under controlled and pilot scale conditions.

Materials and methods

During this study, the aquaculture wastewater from the FishGlassHouse (FGH) of the University of Rostock i) pre-biofilter and ii) post-biofilter was initially analysed for its nutrient contents and suitability as culture medium (phase 1). Based on the results of this lab scale study, a polyculture with a suitable profile was adapted and pre-cultured in order to achieve a stock ready for upscaling at the pilot facility (phase 2, May - June 2018) in Denmark and later at the FGH facility (phase 3, July 2018 – May 2019) in Germany. The polyculture was used in phase 2 in connection with the pilot RAS facility at Guldborgsund Zoo in an open pond system (1000L) (see pictures 1 & 2).



Picture 1: The pilot RAS facility at Guldborgsund Zoo (the fish tanks, including automated feeders in front and the water treatment in the back.

Picture 2: The integrated outdoor culture of microalgae for nutrient recovery from the RAS effluent.

In phase 3 the polyculture was cultivated in a closed photobioreactor system (140L). In phase 2, the algal growth in the pilot aquaculture water was analysed by students from Copenhagen University, who tested the suitability of the sump water and sediments for nutrients as growth media for microalgae. The sump water was shown to contain high amounts of nitrate and a moderate amount of phosphate and was well suited as a growth medium for microalgae. The sediment, however, contained no nitrate and only little ammonium, both before and after autoclaving, and therefore could not be used to enrich the media water with nutrients.

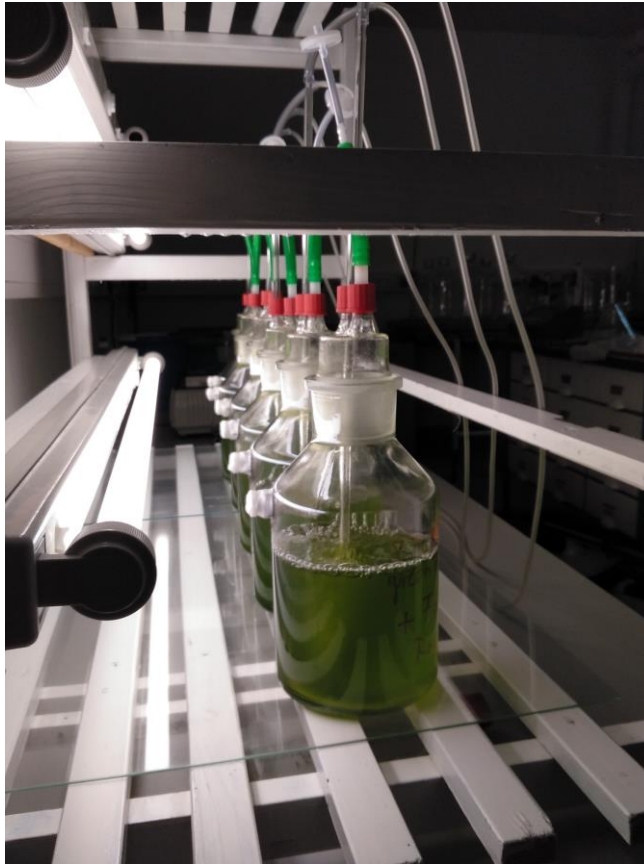
In phase 3, the DTI stock of freshwater green microalgae polyculture predominated by *Scenedesmus* sp. and *Chlorella* sp. was maintained in cell culture flasks with ventilation caps utilizing F/2 medium as nutrient source. To create pre-cultures used for actual cultivation stock, cultures were transferred to aerated 2L glass chemostats and nutrient-enrich fish aquaculture wastewater from the FGH, which had been filtered threefold, was added (see picture 3).

Pre-cultures were kept in a fed-batch cultivation process with artificial illumination set at a 16/8h light/dark rhythm until reaching an OD₆₆₅ nm-value (optical density obtained at an absorption wavelength of 665 nm as proxy for concentration of algal biomass) of approximately 1.0. Pre-cultures were diluted if OD₆₆₅ nm-values exceeded the desired value.

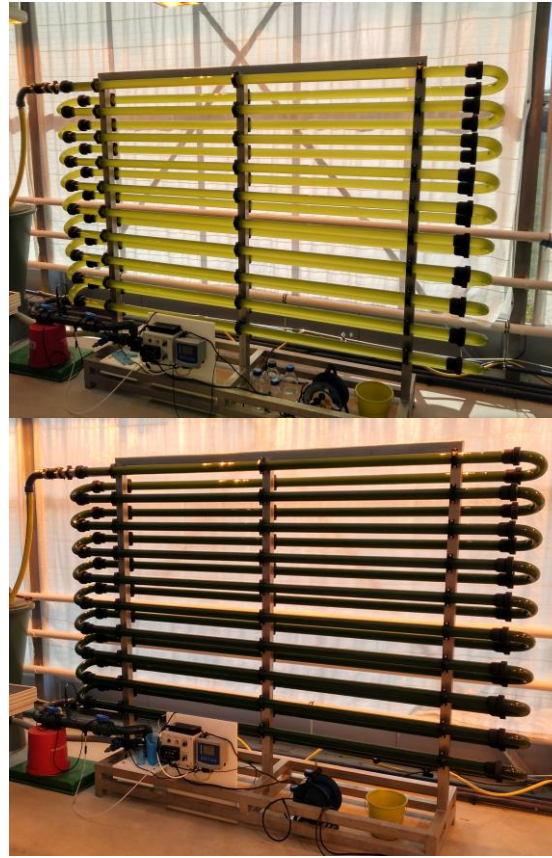
Cultivation took place in a chamber of the FGH aided by artificial illumination set at a 16/8h light/dark rhythm (lights on: 6 AM until 10 PM) using sodium-vapor lamps (Philips Master SON-T PIA Plus 400W). To run a cultivation cycle, 7L (i.e. 5% of total volume) of pre-culture were transferred and inoculated in a 140L tubular photobioreactor (PBR) containing double filtered fish aquaculture wastewater from the same source as the pre-cultures' (see picture 4). The set point for temperature in the chamber was 25°C, a value shown to be well-suited for the cultivation of *Scenedesmus* spp. (Hodaifa et al. 2010; Chalifour and Juneau 2011; Guedes et al. 2011). To assure optimum pH-levels for sufficient algal growth not surpassing 7.8, a device to automatically supply CO₂ gas was connected to the PBR. The flow rate in the PBR was set at approximately 4800 L/h.

Several growth and nutrient parameters including OD₆₆₅ nm, cell concentration via flow cytometry, chlorophyll a content, dissolved organic and inorganic matters, as well as particulate organic nutrient contents (nitrogen=N, phosphorus=P) were assessed over the course of 14 days. Samples were taken every second day. Additionally, samples for the

analyses of the fatty acid profile of the obtained algal biomass were taken. To verify the results, a total of four cultivation cycles were conducted.



Picture 3: Pre-culture of the microalgae stock for the PBR at the microalgae lab of the University of Rostock.



Picture 4: The PBR stocked with the pre-cultures (above) and at the end of a cultivation period (below).

Results

All results shown here in the report were taken from the third cultivation cycle, as this one resulted in average values for the assessed parameters and illustrate in the best way the findings of the four experiments.

OD665-values as proxy for cell concentrations had increased from approximately 0.1 at day zero (D0) to more than 4.6 at the final D14 and hence, multiplied numerous times which indicated that under the given conditions the green microalgae polyculture had grown strongly (Figure 1).

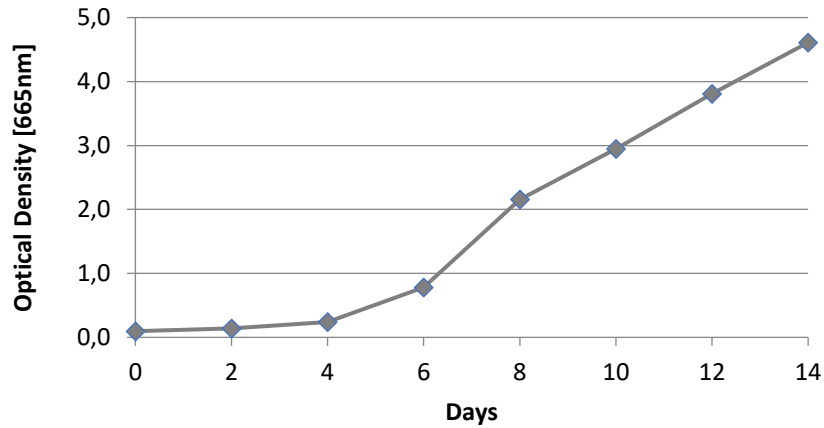


Figure 1: Development of optical density over time measured at a wavelength of 665 nm of a green algae polyculture in wastewater deriving from a production site of African catfish (*Clarias gariepinus*).

Chlorophyll a content had developed from initial $9.69 \text{ mg} \cdot \text{m}^{-3}$ (D0) to $327.83 \text{ mg} \cdot \text{m}^{-3}$ on D14 meaning that concentrations had increased almost 34-fold and indicating high metabolic activity of the algal cells (Figure 2).

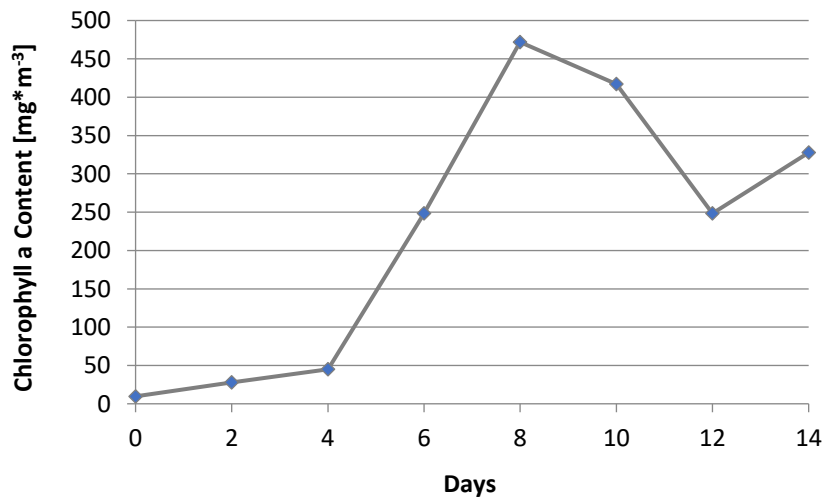


Figure 2: Development of chlorophyll a content over time of a green algae polyculture in wastewater deriving from a production site of African catfish (*Clarias gariepinus*).

As indicated by OD665-values, cell concentrations had strongly grown from initial $0.167 \cdot 10^6 \text{ cells} \cdot \mu\text{L}^{-1}$ at D0 to a final $14.324 \cdot 10^6 \text{ cells} \cdot \mu\text{L}^{-1}$ (D14), resulting in an almost 86-fold multiplication (Figure 3).

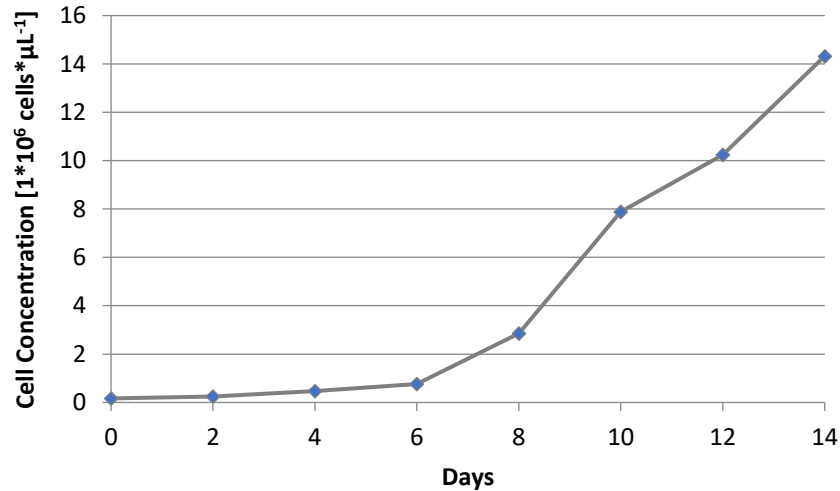


Figure 3: Development of cell concentrations over time of a green algae polyculture in wastewater deriving from a production site of African catfish (*Clarias gariepinus*).

At this point in time, analysis of nutrient parameters, including the fatty acid analysis of the sampling materials has not been completed, but will be shortly and the respective data submitted in the form of a scientific publication as soon as possible.

Based on the growth curve in Figure 3 algal growth from day 6 to 14 has a specific growth rate of 0.366 with 0.529 divisions per day and a doubling time of 1.89 days.

Discussion and Conclusion

Members of the microalgal genus *Scenedesmus* have shown to remove nutrients, including N and P, efficiently from artificial and real wastewater from African catfish aquaculture alike (Voltolina et al. 1999; Martinez et al. 2000; Ruiz-Marin et al. 2010; Ji et al. 2013).

Although there are no nutrient parameter data available yet, the exhibited growth and algal biomass production during four subsequent experiments indicate a substantial nutrient removal from the culture waters. The growth curves of these algae were similar to the ones presented here indicating that the utilised polyculture was able to thrive and develop quantities of biomass suitable for further applications (Xin et al., 2010).

We therefore conclude that it is possible to grow green freshwater microalgae of the genus *Scenedesmus* in substantial amounts in wastewaters deriving directly from fish aquaculture facilities of African catfish under pilot scale conditions. No further pre-treatment of the wastewater was required, showing the high ability of the utilized polyculture to adapt to nutrient enriched water or even eutrophicated conditions, making it a feasible and promising opportunity for practitioners to improve their economic and environmental profile. Even higher yields of algal biomass appear to be achievable if cultivation conditions are further optimized.

B. Integration of vermifiltration into aquaculture production

Introduction

Nutrients in effluent sludge are carbon-bound and must be treated in order to make them available for crop production. Many different filtration methods have been tested in aquaculture systems to reduce organic loads and enhancing water quality by treatment of solids. However, filtration often requires costly, sophisticated technology and may be very energy consuming (Turcios et al. 2014). A very promising on-site and low-energy treatment option for aquacultural sludge could be vermifiltration, hence worm digestion of effluents from fish production. Former studies showed high potential of this technique regarding physical and biological filtration and mineralization of non-solvent plant nutrients. Nevertheless, most studies applied vermifiltration as treatment for municipal sewage sludge and land-based livestock manure (Sinha et al. 2008; Jicong et al. 2005).

Regarding the research about innovative aquaponics in the course of InnoAquaTech it was decided to dedicate to vermifiltration as a main objective and analyze the suitability of aquaculture sludge from African catfish production for worm feed. This fish species is successfully produced in modern recirculation aquaculture systems in Mecklenburg-Western Pomerania and could be a future target species also in other countries of the southern Baltic Sea region.

To analyze the potential of this treatment, scientists from Rostock University built a pilot-scale setup with worms that were fed with sludge from African catfish (*Clarias gariepinus*) production.

The aim was to investigate if:

- aquacultural sludge is suitable as worm feed
- worm digestion supports the mineralization of organic bound nutrients
- vermifiltration could be an effective method to supplement traditional filtration methods, remove organic contaminants and enhance the water quality for fish production

Previous studies have shown that vermifiltration effectively reduces biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total suspended solids (TSS) in sewage effluents (Sinha et al. 2008; Manyuchi et al. 2018). Furthermore some authors found earthworms being capable of enhancing growth conditions for phosphorus solubilizing (PSB) and other mineralizing bacteria (Wan and Wong 2004). Thus, the application of worms in the sense of vermifiltration can be seen as an additional low energy filtration device, which could transform aquaculture sludge into high quality biological fertilizer – a valuable by-product from aquaculture.

Preparatory studies at the University of Rostock have demonstrated that nutrient contents in aquaculture process water for hydroponic plant production is deficient, especially for the macronutrients potassium, nitrate and phosphorus and the micronutrient iron (Schneider 2017; Wenzel 2017; Strauch et al. 2018). Highly water soluble elements, e.g. potassium mostly accumulate in the water; whereas African catfish sludge was found to be rich in iron and phosphorus. Especially feed derived phosphorus accumulates to a large amount (9.7-19.3 %) within the sediments (Strauch et al. 2018).

System Design & experimental set up

Nine individual vermifiltration devices were set up at the laboratories of the Faculty of Agricultural and Environmental Sciences at the University of Rostock (see picture 5). The devices were made out of plastic boxes (36x36x12 cm) and arranged in a drawer system style. Each system had 4 drawers and a liquid catch tank. Three drawers per device were filled with 12 liters (\pm 10cm thick) of beech wood chips each as worm substrate and the remaining drawer, inaccessible to the worms, was filled with “Kaldnes K1 Micro” Moving Bed Filter medium. The fish sludge was applied from the top and trickled through the filter under gravity. The catch tank was holding a 5 W (Tunze 5024.040 Universalpumpe Mini) pump for recirculation of input wastewater/sludge liquid remains.



Picture 5: Vermifiltration unit stocked with worms prior to the first feeding.

The sludge/water was recirculated twice a day and spread again onto the top drawer, from where it took three hours to trickle down to the catch tank. For uniform distribution of sludge and wastewater a plastic box fitting snugly into the top drawer was drilled with evenly distributed holes. The room temperature within the laboratory was kept constant at 21°C.

The nine devices were divided into three groups (each in triplicates) and stocked with different worm densities. The intensive group (H) was stocked with 25 g worm biomass per liter substrate volume. The extensive group (L) was stocked with 15 g/l and the third group (K) was left without worm biomass. Each filtration device was fed sludge from a recirculating aquaculture system (RAS) producing African catfish. Sludge was collected at the semi-intensive aquaculture unit in the FishGlassHouse at the Faculty of Agricultural and Environmental Sciences at the University of Rostock stocked with 630 African catfish with \pm 500 g*fish⁻¹*System⁻¹ at the beginning of the experiment. The experiment was carried out for four weeks and at the end the fish biomass within the system was \pm 441 kg. Dry matter

content within the fed sludge varied between 2.68 and 3.52%. The sludge was collected from the sedimentation units. Fish were fed with Coppens Meerval Special PRO EF feed.

Analysis & Sampling

Adapted to the weekly management routine in the aquaculture facility the sludge was taken six days after cleaning of the sedimentation unit. Inside the clarifier the solids were left to settle for 15 minutes before the water was pumped out from the surface leaving only the settled solids and some leftover supernatant. The sludge suspension was mixed extensively and 27 liters were taken out to feed the 9 vermifiltration systems. Thus, each filtration device was fed with 3 liters sludge per week. After one week the liquid fraction was collected from the catch tank and kept for sampling. Solids remained trapped within the filter substrate.

The chemo-physical water parameters (dissolved oxygen level (DO), pH-value, temperature, redox potential, conductivity and salinity) were measured with a multimeter (HACH LANGE HQ40d) in the input sludge and output effluent after one week of filtration. Supernatant from input sludge and output effluent were analyzed colorimetrically (Gallery™, Thermo Fisher Scientific, Dreieich, Germany) for plant macronutrients: Ammonia nitrogen ($\text{NH}_4^+\text{-N}$), nitrite nitrogen ($\text{NO}_2^-\text{-N}$), nitrate nitrogen ($\text{NO}_3^-\text{-N}$), phosphorus (PO_4^{3-}), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), iron (Fe^{2+}) and sulfur (SO_4^{3-}).

Chemical Oxygen Demand (COD), Total Organic Carbon (TOC) and Total Nitrogen bound (TNb) was measured for input sludge before weekly feeding, and output effluents after one week recirculation within the devices. LCK Cuvette Test System and Hach Lange DR3900 Spectrophotometer were used.

Results, discussion & conclusions

The results were very promising. The temperature within the vermisystems was on average ± 1.5 °C higher than in the control, which was statistically significant. This indicates a higher microbial activity inside the filters with worms included. Worm digestion seems to directly affect the number of bacteria and fungi degrading and decomposing the provided organic matter. Both, the intensive and extensive vermisystems, were able to reduce the organic load effectively. After one week of filtration both treatments showed a strong reduction in TOC values, whereas levels for control treatment without worms were approximately 25% higher. Over the course of the experiment TOC was reduced up to 84% and COD up to 79% compared to only 72% TOC respectively 63% COD in the control filters without worms.

Furthermore, the vermifiltration systems had strong pH regulating effects on input sludge/water. The pH in the vermisystems evened out at neutral levels around 7, whereas the control group filters were not able to significantly change input pH in the given time. Additionally, the substrate condition was severely impacted by worm burrowing activities. Substrate in vermifilters was loose and kept aerated, whereas the substrate in the control group appeared dense, compact and clogged over time.

Definite conclusions on the nutrient mineralizing effects of worm digestion and accompanied microbial activity could not be made within the course of this study. Unfortunately, the worms seemed to reduce the phosphorus content in the effluents. This resulted from the fact that young and not fully-grown worm were used, which incorporated nutrients in their biomass for growth. If more sludge would have been used as feed, results might be totally different. On the other hand, the vermisystem effluents still contained small amounts of nitrate, whereas in the control group those were not quantifiable with the applied methods. This was due to the fact that worm burrowing activities kept the substrate

well aerated and the water was able to trickle through the whole surface area preventing any anaerobic conditions and denitrification. In contrast to this, the control systems clogged and the water ran down on the very sides of the filters. Standing water in the liquid catch tank became anoxic and nitrate was reduced to molecular nitrogen (denitrification).

This study demonstrated that “worm filtration” effectively reduces organic loads in effluents from aquaculture. Chemical Oxygen Demand (COD), Total Organic Carbon (TOC) and Total Nitrogen bound (TNb) loads were significantly reduced. In this regard, the application of worms and microorganisms can be seen as a low energy additional filtration device for recirculating aquaculture operations. It also could be demonstrated that *Eisenia foetida* contains (up to 65%) high-quality lysine-rich proteins and may be used as a suitable source for substituting fishmeal in future fish diets. However, whether worms and microorganism together are able to transform aquacultural sludge and filter substrate into high quality biological fertilizer or worm digestion supports the mineralization of organic bound nutrients, has yet to be proved. Further research should be considered in order to fully understand the potential of vermifiltration to support sustainable operations of aquaculture and aquaponics systems in future.

Finally, it must be considered that according to the scientific literature worm filtration can be also used to remove contaminants from organic effluents through bioaccumulation (Sinha et al. 2010). Aquaculture sludge can contain undesired elements such as heavy metals (Wenzel 2017). Vermifiltration might also be a possibility to treat such effluents more effectively, instead of distributing them directly onto agricultural grounds or discarding them into the municipal sewage system. We can conclude that the proof of concept of incorporating vermifiltration techniques into successful aquaculture operations in the southern Baltic Sea region, as exemplified with African catfish, was demonstrated in the course of InnoAquaTech. However, the real potential of vermifiltration and – composting yet has to be discovered, and further research in this field should be encouraged.

5. General discussion

Since 2015, the FischGlassHaus at University of Rostock with a total research area of 1000 m² is one of the most modern experimental aquaponics research facilities in Europe. About 30% of the area is available for fish and 60% for plant production. During the InnoAquaTech project, our experiments focused on analyses and evaluation of the nutrient fluxes and possible reuse strategies of African catfish (*Clarias gariepinus*) effluents. The reuse of effluent waters and sediments from recirculation aquaculture systems requires a deeper understanding of the nutrient and energy flows as well as material pathways.

The highly water-soluble elements, e.g., potassium, accumulated in the water whereas iron, copper, chromium and uranium were found in the solids. Feed derived phosphorous was accounted for 58.3–64.2% inside the fish, 9.7–19.3% in sediments, and small amounts 9.6–15.5% in the process waters (Strauch et al. 2019). A total of 7.1–9.9% of the feed accumulated as dry matter in the sediments, comprising of 5.5–8.7% total organic carbon and 3.7–5.2% nitrogen. A total of 44.5–47.1% of the feed energy was found in the fish and 5.7–7.7% in the sediments. For the reuse of water and nutrients in hydroponics, the macro-nutrients potassium, nitrate, phosphorus and the micro-nutrient iron were deficient when compared with generalized recommendations for plant nutrition. Nevertheless, microalgae production was successful, applying the process waters without further treatment and the exhibited growth and algal biomass production indicates a substantial nutrient removal from the effluent waters. Low energy contents and the C/N-ratio restrict the solely use of African

catfish solids for vermiculture. Still, the use of the outputs as general fertilizer in aquaponics farming (s.l.) (combined with additional nutrients) appears possible.

6. Outlook

The implementation of the pilots in the framework of InnoAquaTech could demonstrate that an improvement of nutrient efficiency through the integration of microalgae cultures or vermiculture is possible and appears reasonable. The mechanically filtered process water from the production of African catfish could be used directly for subsequent cultivation of microalgae. The same applied to the production of the worms by means of the solid matter excretions from the fish. As a next step, experiments to upscale these systems together with an economic evaluation will be required.

7. References

- Brod, E., Oppen J., Kristoffersen A. Ø., Haraldsen T. K. and Krogstad T. "Drying or Anaerobic Digestion of Fish Sludge: Nitrogen Fertilisation Effects and Logistics." *Ambio* 46(8) (2017):852–64.
- Chalifour, A. and Juneau, P. "Temperature-dependent sensitivity of growth and photosynthesis of *Scenedesmus obliquus*, *Navicula pelliculosa* and two strains of *Microcystis aeruginosa* to the herbicide atrazine." *Aquatic Toxicology* 103.1-2 (2011): 9-17.
- Chapman, R. L. "Algae: the world's most important "plants"—an introduction." *Mitigation and Adaptation Strategies for Global Change* 18.1 (2013): 5-12.
- Chiu, S. Y., Kao, C. Y., Chen, T. Y., Chang, Y. B., Kuo, C. M. and Lin, C. S. "Cultivation of microalgal *Chlorella* for biomass and lipid production using wastewater as nutrient resource." *Bioresource technology* 184 (2015): 179-189.
- FAO. "Prospects for Food, Nutrition, Agriculture and Major Commodity Groups. Food and Agriculture Organization of the United Nations. Grobal Perspective Studies Unit, Rome." *World Agriculture: Towards 2030/2050 (June)(2006)*.
- FAO, IFAD, UNICEF, WFP, and WHO. *The State of Food Security and Nutrition in the World 2018. Building Climate Resilience for Food Security and Nutrition*. Rome, FAO. Licence: CC BY-NC-SA 3.0 IGO (2018).
- Guedes, A. C., Amaro, H. M., Pereira, R. D. and Malcata, F. X. "Effects of temperature and pH on growth and antioxidant content of the microalga *Scenedesmus obliquus*." *Biotechnology progress* 27.5 (2011): 1218-1224.
- Harun, R., Singh, M., Forde, G. M. and Danquah, M. K. "Bioprocess engineering of microalgae to produce a variety of consumer products." *Renewable and Sustainable Energy Reviews* 14.3 (2010): 1037-1047.
- Hodaifa, G., Martínez, M. E. and Sánchez, S. "Influence of temperature on growth of *Scenedesmus obliquus* in diluted olive mill wastewater as culture medium." *Engineering in Life Sciences* 10.3 (2010): 257-264.
- Hou, J., Yanyun, Q., Guangqing, L. and Dong, R. "The Influence of temperature, pH and C/N Ratio on the Growth and Survival of Earthworms in Municipal Solid Waste." *International Commission of Agricultural Engineering* 7(12)(2005):1–6.
- Ji, M. K., Abou-Shanab, R. A., Kim, S. H., Salama, E. S., Lee, S. H., Kabra, A. N., Lee, Y.-S., Hong, S. and Jeon, B. H. "Cultivation of microalgae species in tertiary municipal wastewater

- supplemented with CO₂ for nutrient removal and biomass production." *Ecological Engineering* 58 (2013): 142-148.
- Manyuchi, M. M., C. Mbohwa, and E. Muzenda. "Biological Treatment of Distillery Wastewater by Application of the Vermifiltration Technology." *South African Journal of Chemical Engineering* 25(2018):74–78.
- Martinez, M. E., Sánchez, S., Jimenez, J. M., El Yousfi, F. and Munoz, L. "Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*." *Bioresource technology* 73.3 (2000): 263-272.
- Palm, H. W., Goddek, S., Appelbaum, S., Kotzen, B., Knaus, U., Jijakli, M. H., Vermeulen, T. and Strauch, S. M. "Towards Commercial Aquaponics: A Review of Systems, Designs, Scales and Nomenclature." *Aquaculture International* 26(3)(2018):813–42.
- Queiroz, M. I., Hornes, M. O., da Silva Manetti, A. G., Zepka, L. Q. and Jacob-Lopes, E. "Fish processing wastewater as a platform of the microalgal biorefineries." *Biosystems Engineering* 115.2 (2013): 195-202.
- Ruiz-Marin, A., Mendoza-Espinosa, L. G. and Stephenson, T. "Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater." *Bioresource technology* 101.1 (2010): 58-64.
- Schneider, Philipp. "Wiederverwendung von partikulär gebundenem Phosphor aus der Wasseraufbereitung in rezirkulierenden Aquakultursystemen – eine Machbarkeitsstudie-„. Master thesis at the University of Rostock, Faculty of Agricultural and Environmental Sciences, Professorship Aquaculture and Sea-Ranching, 61 pp. (2018)
- Sinha, R. K., Bharambe, G. and Chaudhari, U. "Sewage Treatment by Vermifiltration with Synchronous Treatment of Sludge by Earthworms: A Low-Cost Sustainable Technology over Conventional Systems with Potential for Decentralization." *Environmentalist* 28(4)(2008):409–20.
- Sinha, R. K., Herat, S. Bharambe, G. and Brahmabhatt, A. "Vermistabilization of Sewage Sludge (Biosolids) by Earthworms: Converting a Potential Biohazard Destined for Landfill Disposal into a Pathogen-Free, Nutritive and Safe Biofertilizer for Farms." *Waste Management and Research* 28(10)(2019):872–81.
- Sriram, S. and Seenivasan, R. "Microalgae cultivation in wastewater for nutrient removal." *Algal Biomass Utiln* 3.2 (2012): 9-13.
- Stout, Meg. 2013. *Aquaponic Gardening*.
- Strauch, S. M., Wenzel, L. C., Bischoff, A., Dellwig, O., Klein, J., Schüch, A., Wasenitz, B. and Palm., H. W. "Commercial African Catfish (*Clarias gariepinus*) Recirculating Aquaculture Systems: Assessment of Element and Energy Pathways with Special Focus on the Phosphorus Cycle." *Sustainability (Switzerland)* 10(6)(2018).
- Tokunaga, K., Tamaru, C., Ako, H. and Leung, P. "Economics of Small-Scale Commercial Aquaponics in Hawai'i." *Journal of the World Aquaculture Society* 46(1)(2015):20–32.
- Turcios, A. and Papenbrock. "Sustainable Treatment of Aquaculture Effluents—What Can We Learn from the Past for the Future?" *Sustainability* 6(2)(2014):836–56.
- Voltolina, D., Cordero, B., Nieves, M. and Soto, L. P. "Growth of *Scenedesmus* sp. in artificial wastewater." *Bioresource technology* 68.3 (1999): 265-268.
- UNEP. "RAINWATER." *Water* (June)(2009):24–26.
- Wan, J. H. C. and Wong, M. H. "Effects of Earthworm Activity and P-Solubilizing Bacteria on P Availability in Soil." *Journal of Plant Nutrition and Soil Science* 167(2)(2004):209–13.
- Wenzel, L. C. "Partikuläre Nährstofffrachten bei der Zucht von Afrikanischen Raubwelsen *Clarias gariepinus* (Burchell 1822) unter verschiedenen Besatzdichten. Master thesis at

the University of Rostock, Faculty of Agricultural and Environmental Sciences, Professorship Aquaculture and Sea-Ranching, 81 pp. (2017)

Xin, L., Hong-Ying, H., Ke, G. and Ying-Xue, S. "Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp." *Bioresource technology* 101.14 (2010): 5494-5500.