INTRODUCTION OF ALGAE TECHNOLOGIES IN AQUAPONIC FOOD PRODUCTION SYSTEM

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SUMMARY: In this paper some results of the project »Integration of algal and aquaponic technologies« will be presented and discussed. Growth performance of microalgae suitable for aquaculture nutrition cultivated in a medium derived from aquaponic solids mineralization was tested. *Spirulina* showed the best results and was further cultivated in a pilot photo-bioreactor integrated with the aquaponic system. Cultivated *Spirulina* culture was used as a foliar fertilizer for the plants. Radicchio plants (*Cichorium intybus*) treated with foliar application of algae showed 40% better average mass gain in comparison to control treatment radicchio plants.

Key words*: aquaponic, algae, aquaculture solid mineralization, foliar biofertilizer*

INTRODUCTION

Aquaponics is an engineered food production ecosystem which integrates two most high-tech farming technologies: hydroponics and recirculating aquaculture. Smart integration of elements of recirculating aquaculture and hydroponics, results in a closed loop system based on bio-filtration, which enables effective cultivation of fish and plants.

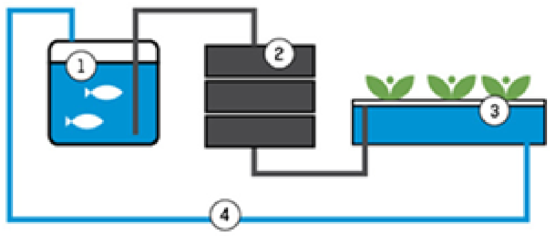

Fig. 1: Simplified schematic diagram of an aquaponic system (Leskovec, 2013)

Fish produce waste metabolites (in general ammonia and organic solid waste) (1). These waste metabolites are decomposed and converted by microorganism (2) to forms that are less toxic to fish and are available nutrients for plant uptake (3) (Jeršin, 2014).

Bacteria are the vital element in aquaponics, as they perform mineralization of organic matter from fish waste and the conversion of toxic ammonia to nitrate. Nitrification is the most important microbial process and most system design calculations (bio filter design, fish to plant ratios) are based on nitrification. Nonetheless from the perspective of availability of other plant nutrients, mineralization should get more attention. In reality, fish feed as the only input in aquaponics cannot provide adequate concentrations of some essential plant nutrients (P, K, Fe) for commercially viable plant production. Solids have to be effectively removed after the aquaculture part with mechanical filtration. Accumulation of solids in the system causes oxygen depletion and can lead to formation of anaerobic zones (toxic H_2S, de-nitrification). High organic loads also have an inhibitory effect on nitrification efficiency (Michaud et al., 2014). Mineralization of aquaponics solids is therefore often performed in a bioreactor separated from the aquaponics system in the process called off-line mineralization. This way we can safely and effectively extract and use plant nutrients from the waste solids, which are otherwise discharged. Off-line mineralization is fairly often used among aquaponics practitioners and has also been the topic of some research papers (Rakocy et al., 2005).

In this project the concept of off-line mineralization was upgraded with the usage of mineralized solids supernatant as a nutrient solution for cultivation and integration of microalgae in aquaponics. Microalgae can potentially be used as an alternative fish feed source and as an organic fertilizer for hydroponics. In this manner, otherwise unwanted algae can be incorporated in aquaponics and potentially furthermore increase the sustainability of such an engineered production ecosystem.

Microalgae already have diverse uses in aquaculture. Their applications are mainly to provide the main nutrition for larvae and to enhance the color of the flesh of salmonids (Hemaiswarya et al, 2011). Recently there has been a lot of interest in using microalgae as *Chlorella* and *Spirulina* to partially or totally replace the unsustainable use of fish meal in commercial feeds (Taelman et al., 2013). The production and formulation of pelleted fish feed from harvested algae is complex and expensive as well are the nutritional tests on fish. Therefore the primary focus of this paper is the use of algae as a bio fertilizer. Using microalgae is a promising natural fertilizer because of high macro and micronutrient content in addition to the natural enzymes and hormones that have stimulative effect on plant growth. (Shaaban et al, 2010).

MATERIALS AND METHODS

In the scope of the project two identical pilot scale aquaponic systems (total volume 1,350 L) were constructed each composed from: fish tank, radial sedimentation tank, ebb-flow grow bed and hydroponic DWC channel. Aquaponic solids were collected from both pilot systems and transferred to an off-line mineralization bioreactor where the algae cultivation medium was made. A small open photo-bioreactor for growing *Spirulina* was also built in the greenhouse (Fig. 2).

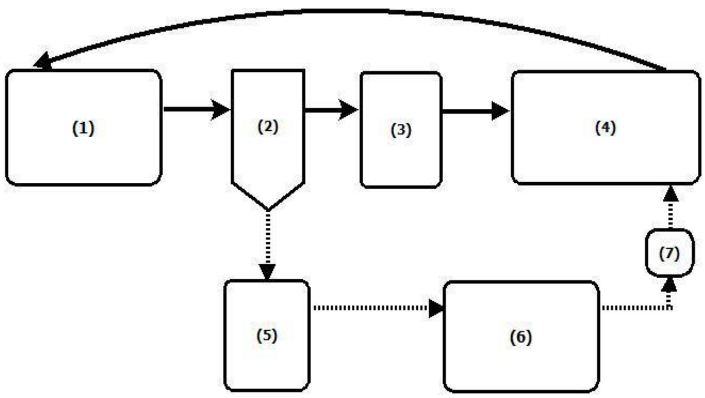

Fig. 2: Schematic diagram of the pilot integrated system: (1) fish tank; (2) sedimentation for solids collection; (3) biofiltration; (4) hydroponic DWC; (5) off-line mineralization; (6) *Spirulina* cultivation; (7) foliar application of *Spirulina* (Hartman, 2015)

A) Cultivation of algae in the supernatant of mineralized aquaponic solids

For the algae cultivation experiment sufficient quantity of pure cultures of *Chlorella*, *Spirulina* (*Arthrospira platensis*), *Hematococcus* and Mix of algae from the Algen company were prepared. *Spirulina* in Algen company was grown in the Zarrouk medium, others in the U-medium. The pilot scale cultivation system was composed from 13 PET bottles (5 L). Mixing and CO_2 input in the improvised photo-bioreactors was achieved with two membrane air pumps, distribution system and ceramic diffusers in each bottle. For lighting Spudnik reflector with 200 W metal-halide bulb was installed and reflective foil was placed on the surrounding walls. To assess the suitability of selected algae for cultivation in the supernatant of mineralized aquaponic solids a pre-experiment was first conducted. An aquaponics mineralized solids medium (M.S. medium), which was obtained by off-line mineralization was tested. In the pre-experiment all cultures were grown in mix medium of M.S. medium and algae culture specific commercial medium, because it was predicted that 100 % M.S. media will not support successful growth. Detailed methodology and result of the pre-experiment will not be presented in this publication. Based on the results of the pre-experiment the growth of *Spirulina* and Mix algae culture in the 100% M.S. medium were observed. There were 3 repeats (3 reactors) for each algae culture, M.S. medium was used as control. Each reactor was filled with 2 L of M.S. medium and 10 % inoculum (200 mL) was added. Photoperiod was set on 12 hours of light and 12 dark, experimental reactors were continuously aerated and the average temperature of the culture was 20.6 °C. Samples were taken for 2 weeks in a 24 hours interval. Algae growth was monitored by measuring optical density (OD) at 650 nm and by measuring pH of the samples. Furthermore samples were taken on the beginning and the end of experiment for dry biomass estimation. The experiment was up-scaled with an open photo-bioreactor integrated with the aquaponic system in the greenhouse. The morphological properties of cultivated *Spirulina* culture were not appropriate for OD 650 nm biomass estimation, thus only visual observations were made.

B) Foliar application of *Spirulina* culture on radicchio (*Cichorium intybus*)

For fertilizer evaluation purposes, two different varieties of radicchio were used: ‘Leonardo’ and ‘Corelli’. Seedlings were transferred from adjacent greenhouse on 23.06.2015. Seedlings were weighted for starting weight and planted in rockwool substrate in DWC part of aquaponic system. Seedlings were planted using statistical block design (Fig. 2) where block is defined as one channel. During 1 month growing period, plants were sprayed with either mineralized solids supernatant, algae supernatant or rain water acting (control treatment) on 2 day period. After one month growth period, plants were weighed and the results were analyzed for statistical significance using R-statistical computing software.

<u>Hypothesis:</u>

Mass of radicchio treated with foliar application of either mineralized solids supernatant or algae supernatant will be statistically different from the control group.

Left canal

L30	C29	L28	C27	L26	C25	C24	L23	C22	L21	C20	L19	C18	L17	C16	S	S	S
C30	L29	C28	L27	C26	L25	L24	C23	L22	C21	L20	C19	L18	C17	L16	S	S	S

Right canal

C15	L14	C13	L12	C11	L10	C9	L8	C7	L6	C5	L4	C3	L2	C1	S	S	S
L15	C14	L13	C12	L11	C10	L9	C8	L7	C6	L5	C4	L3	C2	L1	S	S	S

Legend:
S - Spirulina
M - Mineralized solids
C - Control
S-salad

Fig. 2: Schematic presentation of a foliar application to the radicchio plants in the aquaponic system (Mazinjanin, 2015)

RESULTS AND DISCUSSION

A) Cultivation of algae in the supernatant of mineralized aquaponic solids

Results of optical density (OD) showed that *Spirulina* did not reach starvation phase after 13 days of cultivation in the M. S. medium (Fig. 3). OD measurements indicate a drop of biomass after day 12 in reactors 1 and 3, which is consistent with the visual appearance of cultures in days after daily measurements (only until day 13). The pH was increasing proportionally with OD and it rose from 9.3 to around 11 at day 13 in all cultures.

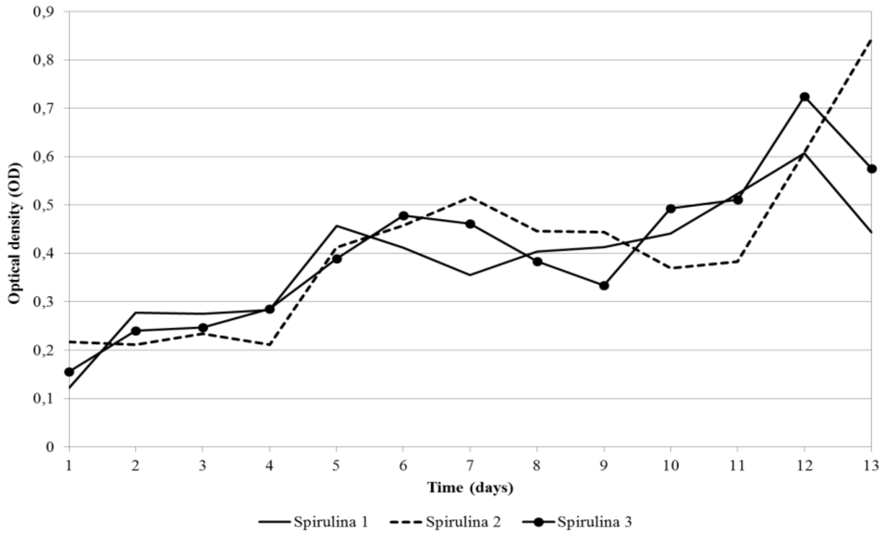

Fig. 3: The results of OD (optical density) measurements in three different cultures of *Spirulina* cultivated in the supernatant of mineralized aquaponic solids.

Upscale cultivation of *Spirulina* in the 100 % M.S. medium in the open photo-bioreactor showed promising results as the cultures did not visually show any nutrient deficiency until around 12 days of cultivation in summer greenhouse conditions. Average dry mass of *Spirulina* culture immediately after inoculation was 0.78 g/L and it increased to an average of 2.1 g/L after 13 days of cultivation in the 5L pilot reactors. No publications were found with comparable cultivation conditions from which the comparison of dry mass yields of *Spirulina* could be made. For better assessment of M.S medium performance a comparison of *Spirulina* growth in Zarrouk medium at same conditions should be made.

B) Foliar application

The average weight gain of two varieties of radicchio was compared using the analysis of variance (Table 1). Because of uniform performance of both systems, inclusion of block was not necessary as it did not improve statistical model. Included below is a print of null hypothesis test for average mass gain by variety and foliar application treatment.

Table 1: Results of the analysis of variance for the average mass of raddichio plants

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Variety	1	192	192	0.083	0.77394
Treatment	2	25227	12613	5.483	0.00669**

The null hypothesis which states that the average mass gain by variety is the same is confirmed. But the null hypothesis for average mass gain by foliar treatment must be rejected, which means that foliar application has statistically significant influence on the average mass gain ($p<0,01$ $n = 90$).

Furthermore the comparison between mineralized solids supernatant and algae supernatant was made. Included below is a print of multiple comparison HSD test between different types of foliar application and their average mass gain.

Table 2: The results of multiple comparisons HSD test

trt		Means
1 Supernatant	118.8790	A
2 Mineralized aquaponic solids	95.9350	B
3 Control	68.7135	C

Multiple comparison test showed that the average mass gain between different types of foliar application is statistically significant. The best result can be seen with the application of algae supernatant, where average mass gain is approximately 40% better than in the case of control treatment.

CONCLUSION

Successful cultivation of *Spirulina* in the M.S. medium showed promising results, but growth performance comparison test with commercial substrates should be made for quantification of the M.S. medium performance. Big scale commercially viable microalgae production from aquaculture effluents is already optimized for integrated production of microalgae as feed for some larvae and juvenile fish and crustaceans (Knuckey et al., 2006). Thus production of fish fry with microalgae cultivated from supernatant of mineralized aquaponic solids seems feasible for aquaponic production of fish like Tilapia. Foliar application of mineralized solids supernatant resulted in increased growth of radicchio grown in aquaponic system. This was expected as bacterial mineralization of fish waste solids increases availability of nutrients (Rakocy et al., 2005). Surprisingly foliar application of *Spirulina* culture showed increased growth of radicchio even compared to application of mineralized solids supernatant. Possible reasons for this are: more complete macro and micronutrient mineralization by algal-bacterial culture than bacterial culture alone (Mulbry, 2005), production of amino acids that are beneficial for plant culture and can possibly increase nutrient uptake and stress response. (Rai, 2002). For more complete understanding of here provided results detailed nutrient analysis of algae supernatant and mineralized solids supernatant is needed. Microbiological food safety of vegetables sprayed with *Spirulina* culture grown on medium derived from fish waste is of course questionable, although very high pH (up to 12) in intense *Spirulina* culture has inhibitory effect on potential pathogens (Bhuvaneswari et al., 2012). Thermal processing could resolve the food safety problem and microbiological and chemical safety analysis should be made to assess the risks. Overall results of the project showed that further work on the integration of algae in aquaponics is reasonable and it can further increase the nutrient efficiency of aquaponics.

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