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PHYTOREMEDIATION CAPACITY AND PHOSPHORUS MASS BALANCE IN A BASIL-STURGEONS AQUAPONICS INTEGRATED RECIRCULATING SYSTEM

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Abstract

The long-term increased demand for animal-based protein had determined the practice of intensive farming technologies, strategy that may rise environmental sustainability issues. Aquaculture food production industry manages both to satisfy market demand for fish and contributes to restocking programs conducted in order to support biodiversity conservation programs. Integrating aquaponics techniques into already existing recirculating aquaculture production systems may represent a solution for limiting the environmental impact, while maintain a high production intensity. The present study targets to identify the basil phytoremediation potential by evaluating its phosphorus removal capacity from the technological water resulted by practicing intensive sturgeons aquaculture. The P mass balance is identified in order to determine the sustainability of the tested, aquaponics based, water treatment solution, by considering the P concentrations of fish feed, solid waste, wastewater, technological water, fish and plants biomass. The results indicate that basil presents high P removal capacity and both hydraulic parameters and regimes are important parameters to be considered in the phytoremediation technology optimization process. Similar studies are recommended to be performed by testing both aquaponics deep water and nutrient film techniques.

***Key words**: phytoremediation, basil, phosphorus, aquaponics, mass balance.*

INTRODUCTION

According to recent study (White, 2017), the aquaculture industry is predicted to continue increasing production by intensifying the existing aquaculture practices and increasing the number of farms.

Then negative effects of wastes discharges, from aquaculture, to aquatic environment are a subject of high interest, although they represent only a small part to land-based pollutants. According to some authors (Boyd et al., 2015), the aquaculture effluents are associated to a range between 2 and 3% of total anthropogenic nitrogen (N) and phosphorus (P) entering natural waters. Cao et al. (2007) emphasized that properly planned use of aquaculture waste alleviates water pollution problems and not only conserves valuable water resources but also takes advantage of the nutrients contained in effluent. Some authors (Cochard, 2017) pointed out that the discharges from intensively operated fish farms are known for their high concentration of nutrients, suspended solids, oxygen-demanding substances and chlorophyll a and these high levels of nutrients may cause eutrophication and affect fisheries adversely (White, 2017).

The aquaculture feed inputs are the main source of the above-mentioned structure of the effluents of intensive aquaculture facilities. Recent study (Prüter et al., 2020) emphasized that up to 80% of carbon (C), 76% N and 82% of P from total feed input in aquaculture can be lost to the environment.

The new European Union (EU) requirements in terms of increasing the sustainability of aquaculture generates restrictions in intensive fish rearing technologies in terms of fish stocking densities and feed input, solutions that are meant to keep the pollution loadings under environmental capacity. Although, according to Martins et al. (2010), intensive recirculation aquaculture systems (RAS) have the potential to become one of the most sustainable animal protein production systems, improvements are required for better discharges management.

According to Herath & Satoh (2015), discharges of N and phosphorous P have received a great deal of attention, during the last decade, from scientists and environmentalists as they disturb the natural balance of aquatic ecosystems. Lazzari et al. (2008) revealed that these two nutrients (N and P) are considered the main end-products of fish loading, and can affect not only the rearing water, but also the environment as a whole. The N and P originate, within aquaculture production systems, from fertilizers, feeds, and metabolic processes, such as uneaten feed, ingested but undigested food (faeces), or food ingested and eliminated as excretion during production (Wang et al., 2020). However, according to Stratful et al. (2001), phosphorus is often the most critical nutrient in eutrophication of freshwater.

Barak & van Rijn (2000) revealed that P is supplied together with feed, particularly compound feeds and its accumulation in the aquaculture technological water results from it not being fully assimilated by fish. It is pointed out that phosphorus compounds accumulate in the water during intensive fish culture in recirculation systems (Zarski et al., 2008).

Several efforts to reduce P concentrations in aquaculture systems had been made, mostly focused on improving the bioavailability of phosphorus in fish feed (Barak & van Rijn, 2000). The P excretion in RAS are usually 69-86% of dietary P, therefore, some authors suggest of phytase in fish feeds, in order to reduce P waste (Lazzari et al., 2008). Recycling of P from feed input in aquaculture systems gains increasing importance (Schröder, 2005), especially relating to the requirement of EU related to shifting to sustainable aquaculture practices.

Phytoremediation is also an environmentally friendly and cost-efficient method compared to other remediation technologies which target the reduction of P discharges from aquaculture (Khan et al., 2004). According to Pilon-Smits (2005), there are six types of phytoremediation methods: phytoextraction, phytodegradation, rhizofiltration, phytostabilisation, phytovolatilization and using plants to remove pollutants from the air. The phytoextraction method is based on the use of plants to remove metals or organic matter from the soil by accumulating them in the harvestable parts of the plant (Pilon-Smits, 2005). Also, Pilon-Smits (2005) defines phytoremediation as the removal or neutralisation of pollutants from the environment by using plants and pointed out that some plant species are known to have a high ability to concentrate elements in their biomass tissue.

Therefore, this P removal method can be adapted to already existing RAS systems by integrating aquaponics modules. Also, Ju et al. (2014) emphasized that, in constructed wetlands, P is removed primarily by plant and microbial uptake during growth, precipitation, and sediment adsorption. Considering phytoremediation, it must be revealed that these P is one essential element for organism growth and a key factor limiting the primary production of plants in various ecosystems, including aquaponics (Elser et al., 2007).

However, the P removal by using plant biomass process optimization depends on a multitude of variables as plant root system capacity, effluent flow rate (hydraulic loading rate - HLR and hydraulic retention time - HRT of the effluents within the plants biomass surface) (Tyson et al., 2004), light quality and quantity and water quality matrix. It is known that leafy greens as basil (*O. basilicum*), spinach (*S. oleracea*) or lettuce (*L. sativa*) have high potential in accumulation nutrients and are suitable plant species for aquaponics. Also, since most aquaculture investors/farmers targets a dual purpose by implementing phytoremediation techniques within their farms, namely the increase of both environmental and economic sustainability, basil seems to accomplish these desiderata due to both high nutrient absorption rate and economic value.

The present study aims to reveal opportunity to implement P phytoremediation solutions, based on aquaponics techniques, to an already existing sturgeon farm, considering a variety of technological scenarios which implies different P concentrations in the aquaculture effluent and different hydraulic regimes, respectively.

MATERIALS AND METHODS

Experimental design

The experimental trial lasts 29 days and was performed by integrating of the aquaponics modules based on light expended clay aggregate (LECA) substrate techniques in an already existing sturgeon RAS (Figure 1 and

Figure 2). The detailed description of integrated aquaponic system is presented in a previous study (Petrea et al., 2021.).

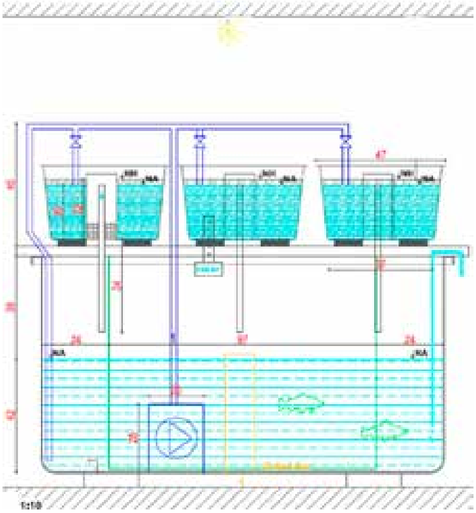

Figure 1. The side-view of the integrated aquaponics solution (IAS)

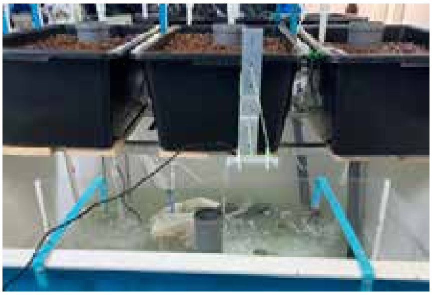

Figure 2. The front view of IAS

The aquaponic modules, in triplicate, are placed above each of the rearing units. Two hydraulic regimes were used (flood and drain - FD and continuous flow - CF, respectively). Before starting the experiment, the activation of LECA substrate was made as described by Petrea et al. (2021).

A constant luminous power of 5800 lm, measured with TESTO 545 light meter, was assured during the entire experimental period (Petrea et al., 2021).

The plants culture density was 74 crops/m^2 (Figure 3), while fish stocking density was 93 kg/m^3.

Fish were divided in two groups and a feeding rate of 1% (LR) and 2% (HR), respectively, of their total biomass weight (BW) were applied. Therefore, a number of 4 experimental variants were defined, in triplicate, as follows: *HR - FD* (the variant with high feeding rate - high P input in RAS effluent and flood and drain hydraulic regime), *HR - CF* (the variant with high feeding rate and continuous flow hydaulic regime), *LR - FD* (the variant with low feeding rate - low P input in RAS effluent and flood and drain hydraulic regime) and *LR - CF* (the variant with low feeding rate and continuous flow hydraulic regime).

Figure 3. The upper-view of IAS

Water quality matrix

The water quality matrix of the effluents presents the following mean values, during the experimental trial:

Table 1. Water quality matrix during the experimental trial

Water quality parameter	**Aquaculture effluents**	
	LR	***HR***
N-NH$_4$ (mg/L)	0.14±0.05	0.16±0.06
N-NO$_2$ (mg/L)	0.07±0.02	0.09±0.03
N-NO$_3$ (mg/L)	126.2±33.6	173.2±45.2
pH (upH)	7.44±0,88	6.95±0.94
Turbidity (NTU)	2.83±0.54	3.35±0.79
EC (µS/cm)	1194.4±184.23	1248.9±200.54
Cl (mg/L)	70.08±5.95	69.34±2.76
Mn (mg/L)	0.59±0.23	0.81±0.16
Fe (mg/L)	0.53±0.12	0.77±0.10
K (mg/L)	15.56±2.85	16.58±3.97
Mg (mg/L)	31.74±11.84	35.5±15.35
Ca (mg/L)	88.46±20.45	90.56±21.45
BOD5 (%)	27.74±4.24	43.64±7.95
DO (mg/L)	8.44±1.34	7.81±0.85
COD (mg/L)	72.41±15.97	80.52±25.76
TSS (mg/L)	0.031±0.01	0.042±0.02
Temperature (°C)	19.49±1.21	19.44±1.23

Sampling and analysing methods

Technological water analysis was performed by using Spectroquant Nova 400 spectrophotometer, with Merk compatible kits. Samples of water were collected once a week from both the outlet and inlet of each aquaponic unit.

The phosphorus removal rates for each experimental variant were presented as average of the triplicate aquaponic units. The following equation was used in order to determine phosphorus removal rates (Petrea et al., n.d.), (eq. 1):

$$PR = \left[\frac{Q}{V} \times (C_{in} - C_{out}) - \frac{\Delta C_{out}}{\Delta t}\right] \times d.. \quad (1)$$

where: *PR* is phosphorus removal rate (g/m^2/day), *Q* is the flow rate (m^3/day), *V* is the system volume (m^3), *C* is the concentration of phosphorus (g/m^3), *d* is the water depth (m) and *t* is the time (days).

The fish faeces were collected as described in (Petrea et al., n.d.) by using a EHEIM water vacuum cleaner provided with a mesh compartment for solids retention. Phosphorus concentration in fish muscle tissues and basil biomass and fish faeces was determined by using the SR ISO 2294:2009 reference method.

Statistical methods

The software IBM SPSS Statistics 20 for Windows was used for the statistical analysis revealed in present paper.

The T test (α=0.05) was applied in order to identify the statistical differences between treatments, after the Kolmogorov-Smirnov normality test was performed. The ANOVA test (post-hoc Duncan test) was performed in order to compare variants

The P balance stream diagrams were performed by using Python's Plotly function.

RESULTS AND DISCUSSIONS

Technological water P_2O_5 concentration

The P_2O_5 concetration in technological water emphasizes high values attributed to HR experimental variants, durring the first part of the trial, due to higher feed input (a 2% BW feeding rate) and, therefore, higher P input into the integrated multi-trophic system (Figure 4). Also, it seems that, in case of both LR and HR technological scenarios, the FD hydraulic regime is recommended to be used as it performs better during long-period production cycles, compared to CF (Figure 4). However, the LR-CF registers the highest P_2O_5 concetration in technological water, at the aquaponics units outlet. These findings emphasizes that the prediction of P_2O_5 concetration in technological water, as dependent variable, should imply both independent data variables related to both RAS effluent quality matrix and technological scenario peculiarities related to feed input.

Thus, a average P_2O_5 concentration in technological water output of the aquaponics modules revealed a 15.66±7.37 mg/L at LR-FD, 17.14±9.76 mg/L at LR-CF, 15.96±7.47 mg/L at HR-FD and 16.06±8.21 mg/L at HR-CF, respectively (Figure 4).

Statistically significant differences ($p<0.05$) were recorded between the LR-CF and LR-FD experimental variants.

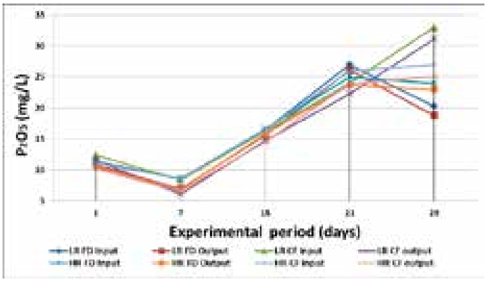

Figure 4. The dynamics of P_2O_5 concentration in water inlet and water outlet, during the experimental period

The dynamics of technological water P_2O_5 concentration reveals an accumulation trend during the experimental trial for all variants, except LR-FD, emphasizing the limiting phytoremediation capacity of the aquaponics system used in present study. Therefore, by analyzing the water quality matrix (Table 1) and the P dynamics (Figure 4), it can be stated that a decision to increase the aquaponics culture surface is suitable to be adopted in order to improve the environmental sustainability of already existing RAS and to limit its environmental impact in terms of P discharges, especially if HR technological scenarios are applied.

The P_2O_5 removal rate

The dynamics of P_2O_5 removal rate for each trial, during the experimental period (Figure 5), emphasizes highest values after the first 7 days of the trial, most probably due to the increase demand for phosphorus manifested by leafy greens, especially basil, in the preliminary stage of the growing cycle. The HR-CF experiment is the most consistent in terms of P removal rate during the entire trial period (Figure 5), with an average of 17.35±4.32 g/m^2/day. Also, upward trends, in the second part of the experiment trial period, can be also observed for both LR-CF and LR-FD, although the first variant records lower P removal performance (10.51±4.37 g/m^2/day), compared to the second variant (16.15±5.03 g/m^2/day). Statistically significant differences ($p<0.05$) were recorded between the group formed from LR-CF + HR-FD and LR-FD + HR-CF, respectively.

Therefore, the results related to P removal rate confirms that the FD aquaponic technique is recommended for maximizing the P phytoremediation capacity of basil for LR, while the CF offers better results in HR technological scenarios.

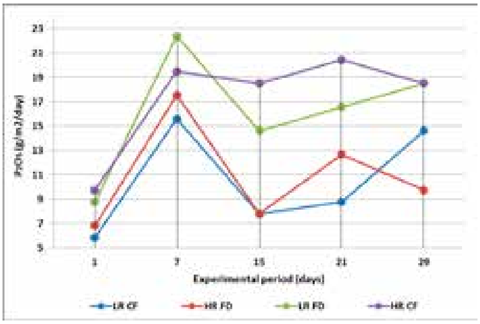

Figure 5. The dynamics of P_2O_5 removal rate for each trial, during the experimental period

The dynamics of P_2O_5 input by administrated feed

During the experimental trial, an input of 25.44 g P was recorded in each of the LR experimental variants, while 54.51g were attributed to each HR experimental variants (Figure 6). The input was provided by administrating fish feed at a feeding rate of 1% BW (LR) and 2% BW (HR), respectively. The administrated feed had a protein content of 41% and a P concentration of 0.9%. After the first 13 days of the experimental trial the feed input was increased due to sturgeons biomass gain since feeding rate in aquaculture production systems must be adjusted in relation to biomass weight.

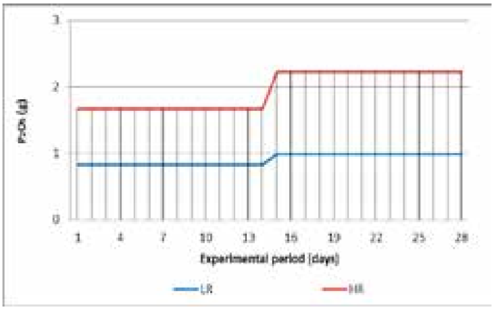

Figure 6. The dynamics of P_2O_5 inputs by feed

The P_2O_5 concentration in fish wastes and fish muscle tissue

The dynamics of P_2O_5 concentration in fish wastes revealed a higher upward trend at HR, compared to LR experimental variants (Figure 7). Also, statistically significant differences ($p<0.05$) were recorded between LR and HR in terms of P concentration in fish faeces.

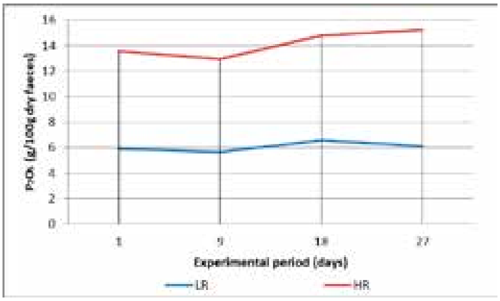

Figure 7. The dynamics of P_2O_5 concentration in fish wastes

Therefore, an average concentration of 6.08±0.39 g/100 g dry faeces was recorded at LR, while a 14.13±1.05 g/100 g dry faeces are attributed to HR experimental variants. The results are higher compared to the findings of other authors (Petrea et al., 2021.), which reports P_2O_5 concentration in fish wastes of 5.47±1.01 g/100 g dry faeces. However, feed chemical composition and fish metabolism can be considering determinant factors in terms of sturgeons P assimilation.

An increase of average P_2O_5 concentration in fish muscle tissue was observed for HR experimental variants, from an initial average concentration of 190.59±13.38 mg/100 g fresh muscle tissue, to 216.46±9.66 mg/100 g fresh muscle tissue (Figure 8). However, for LR variants, a decrease of 1.37% was recorded, to an actual average P_2O_5 concentration of 187.98±9.29 mg/100 g fresh muscle tissue.

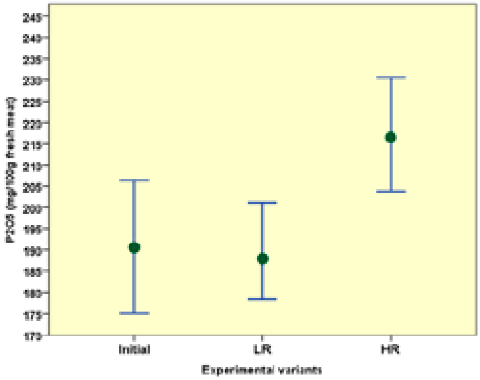

Figure 8. The P_2O_5 concentration in fish muscle tissue

At the end of the trial, the following average P_2O_5 concentrations in basil leaves were recorded: 58.73±3.56 mg/100 g fresh basil at HR-FD, 87.84±7.03 mg/100 g fresh basil at HR-CF, 84.10±4.48 mg/100 g fresh basil at LR-FD, 51.91±2.94 mg/100 g fresh basil at LR-CF. Before the experimental trial, the basil seedlings P_2O_5 concentration had an average of 25.23±1.76 mg/100 g fresh basil (Figure 9).

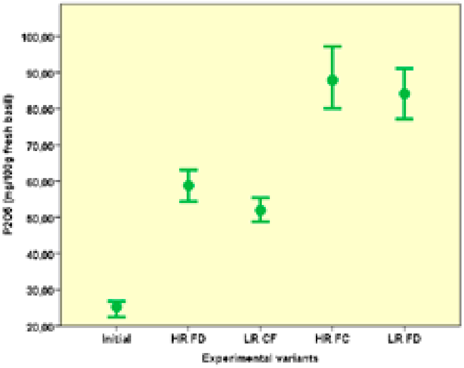

Figure 9. The P_2O_5 concentration in basil biomass

The basil P concentration confirms the P removal rate results and highlights the need of considering the relation between hydraulic regime and aquaculture effluents P concentrations, in order to maximize aquaculture environmental sustainability.

The P_2O_5 mass balance

The analysis of P mass balance reveals the most efficient integrated multi-trophic production system from all 4 tested variants. This analysis was performed since it is important to reveal the utility of the findings related to phytoremediation potential of aquaponics basil production and to characterize the entire multi-trophic system (both fish and plants production modules) in order to identify the variant which can better valorize the symbiotic technological approach.

Therefore, it reveals that HR-CF is the most efficient experimental variant in terms of phytoremediation capacity (12.06% of total P content being recovered in basil biomass) (Figure 10).

However, the most significant percent of total P is recovered in fish wastes, situation which can rise long-term sustainability issues. This can be amplified by the high percentage of unrecorded P (43.77%).

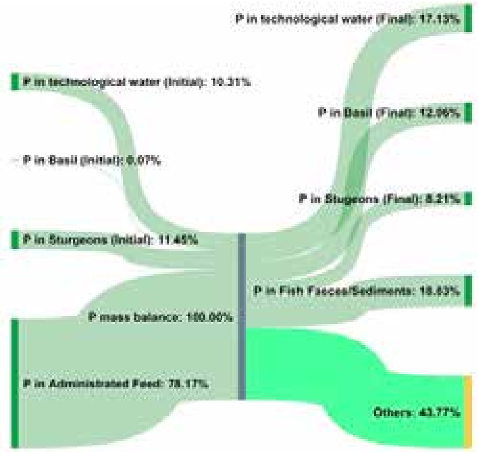

Figure 10. The P_2O_5 mass balance diagram from HR-CF

The LR-FD experimental variant reveals a 7.01% P recovery rate in aquaponics basil biomass (Figure 11). However, considering that the variant performs good in terms of P removal rate (Figure 5) and the percentage of unrecorded P is lower (40.15%) (Figure 11) compared to HR-CF (Figure 10), this variant can successfully compete as one of the most environmentally sustainable solution for an integrated multi-trophic system. However, as a drawback, the lower degree of production intensively will probably decrease the economic sustainability of LR-FD multi-trophic system.

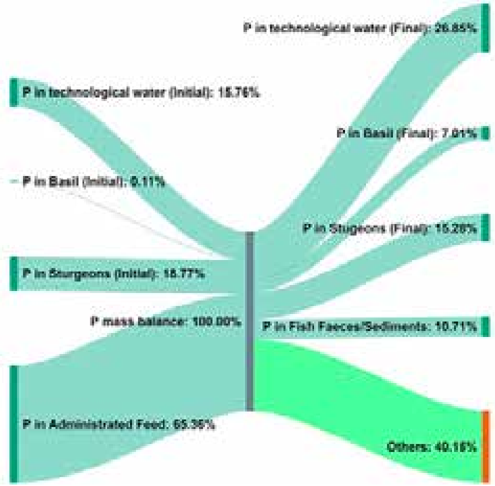

Figure 11. The P_2O_5 mass balance diagram from LR-FD

The HR-FD experimental variant reveals a 10.14% P recovery rate in aquaponics basil biomass (Figure 12), which places it on the second place among the studied scenarios and solutions. Also, the percentage of unrecorded P is the lowest among the analysed variants (37.63%).

However, the high percentage of P recovered in fish wastes decreases the environmental sustainability of HR-FD.

The LR-CF experimental variant reveals the lowest percentage of P recovery rate in aquaponics basil biomass (2.43%) (Figure 13). Considering the lowest P phytoremedion rate attributed to this variant (Figure 5) and low production intensivity, it can be stated that LR-CF is classed in the fourth place among the analysed scenarios, both in terms of economic and environmental sustainability.

Other authors (Chapman & Boucher, 2020) have tested plants as *Pteris vittata*, *Lemna minor*, *Rumex orbiculatus*, *Rumex verticillatus* and *Typha* × *glauca* for evaluating their P mitigation potential and reported a maximum P removal rate of 90% for *Rumex verticillatus* (0.46 g P/shoot, 1.93 g P/plant = root + shoot) and 84% for *T. glauca* (3.7-12.67 g P/plant). However, the experiments were performed in laboratory conditions, compared to the trial from present research paper, which can be considered as being performed in micro-production conditions.

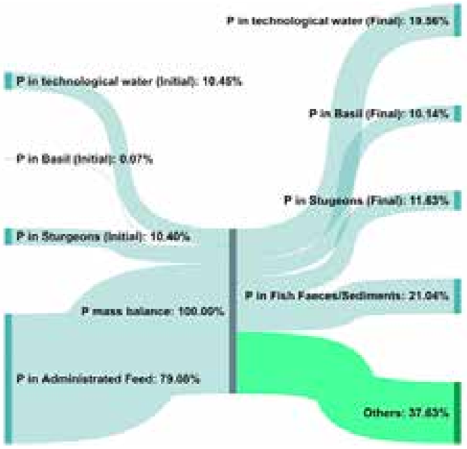

Figure 12. The P_2O_5 mass balance diagram from HR-FD

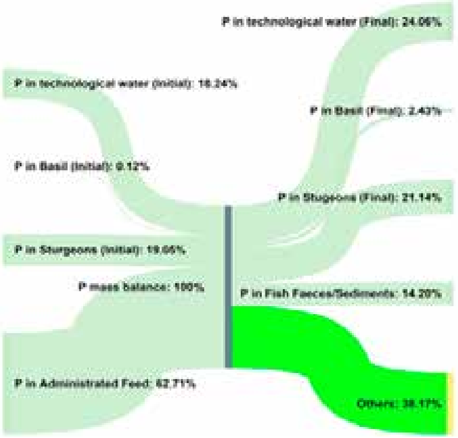

Figure 13. The P_2O_5 mass balance diagram from LR-CF

Future research can be performed during a longer period of time as P has a clear accumulation trend in RAS and, as emphasized by Li et al. (2021), high P concentrations, as well as N/P ratio in wastewater can directly decrease the nutrient uptake rate of plants.

On the other part, practicing phytoremediation within a low nutrients aquaculture facility can limit plants growth and, therefore, phosphorus removal (Adler et al., 2003).

According to (Tyson et al., 2004) optimum absorption of nutrients by plants depends on plant root system capacity, water pH - an important factor that impact the nitrogen fixing bacteria and the solubility of some nutrients in water (Mattson & Heinrich Lieth, 2019) and photoperiodism - should follow the temperature manipulation to achieve a healthy life cycle simulation of the plant (Raven et al., 2005). In addition, according to Stratful et al. (2001) the pH is an important factor for chemical precipitation of P when combined with Mg or other salts, as the solubility of their precipitates varies with pH.

Other authors (Endut et al., 2016) revealed the importance of the surface area of the roots for the removal of nutrients and concluded that leafy greens as spinach, known for their larger surface area roots) recorded a nutrients removal rate of 88.99 % for orthophosphates. Also, other authors (Hefni Effendi, Bagus A. Utomo, 2015) evaluated the decrease of nutrients in aquaponic systems of *Cherax quadricarinatus* - *I. aquatica* and identify a removal rate of 44.4% for orthophosphates.

Rakocy et al. (2004) revealed that a low HRL can cause a decrease of OD, while a high HRL can reduce the retention time of water, that can cause a decrease in the assimilation of nutrients by the crops' roots and the washing of the bacterial biofilm thus, the deterioration of water quality.

Previous studies related to basil culture in aquaponics conditions (Espinosa Moya et al., 2016) emphasized that a 57 g/day/m^2 of basil production can be sustainable in an aquaponic system, at a feed input ratio of 99.6 g/day/m^2.

Also, Adler et al. (2000) concluded that basil can be used as part of the biological filters and has the ability to remove significant concentration of N and P.

CONCLUSIONS

The present study reveals that basil presents high P removal capacity and the symbiotic approach of both hydraulic regimes and intensive feeding technological scenarios must be considerd in order to maximize the sustainability of multi-trofic aquaculture systems in terms of P discharges.

Also, it is recommended to practice continuous flow hidraulic regime is high the fish rearing technology requires high feed input and high P concetrations in effluents, respectively. However, is less intensive feeding technologies are applied, flood and drain hydraulic regime is recomanded for achieving high sustainability in terms of P discharges.

Similar studies are suggested to be performed by testing both aquaponics deep water and nutrient film techniques. Also, future studies which analysis the P mass balance within integrated multi-trophic RAS systems during multiple production cycles (long-term) are suggested to be conducted in order to reveal long-term sustainability of the recommended solutions.

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