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A Study of Nitrogen Cycle in an Integrated Aquaponic System with Different Plant Densities

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Abstract. There is no doubt that a fundamental element like nitrogen is one of the most important components in both animal and plants cells. Within an integrated aquaponic system, nitrogen cycle has highest priority because it converts fish wastes into nutrients for plants. The main goal of this study is to quantify the nitrogen budget for an integrated rainbow trout –spinach aquaponic system, where three plants densities were used. The second objective is to determine a balanced plant density for optimal nitrogen removal rate and hydroponic vegetable production. The experimental design consists in a recirculating aquaculture system with 12 growing units, mechanical and biological water treatment units and four aquaponic units. Three plants densities were used (V1-59 plants/m^2, V2-48 plants/m^2 and V3-39 plants/m^2 and a control variant V4). Fish were fed with two types of feed (41% and 50% protein), using 3 different feeding regimes. Water samples were taken and analyzed by using Merck kits so that nitrate, ammonium and TAN retention rates will be observed. Water oxygen, pH and conductivity levels were also monitored. The meat, plants and faeces nitrogen content was determined by Kjeldahl method. The feed protein content was verified using the same method.

The amount of nitrogen removed from integrated aquaponic system through biological filtration and also by each of the three tested spinach biomass densities was determined apart. The nitrate, ammonium and TAN retention rates, as water passes through mechanical filter, were found to be insignificant ($p>0,05$), compared to ones from the biological filter that were higher. Differences between the retention rates for each of the three variants of tested plants densities were significant higher ($p<0,05$) at V1 compared to V3 and also higher at all three variants comparing them to the control variant. Also differences between plants nitrogen composition from V3 compared to V1 were found significant higher ($p<0,05$). The content of nitrogen from fish meat and fish faeces was found to be according to literature. In the present study, nitrate, ammonium and TAN retention rates were found to being related in a certain way with plants density and total nitrogen input in the aquaponic system, facts that also influence the plants nitrogen content. It is recommended higher plants densities to be used and also a better light intensity can be also used.

Keywords: nitrogen cycle, nitrogen storage, aquaponic systems, retention rate, rainbow trout meat, spinach

INTRODUCTION

The aquaponics concept implies nutrients balance within a given integrated system. Endut *et al.* (2010) and Timmons (1996) stated that the amount of nitrate produced in a fish culture system is directly proportional to two factors: the amount or density of fish in the system and the amount and protein content of the food, as different fish species require different protein content in their respective diets. Nutrient levels from fish aquaculture are suitable for plant growth and can be manipulated by increasing fish biomass and feed rate or by increasing the protein levels in the feed (Licamele, 2009). Aquaponic systems are

categorized by AL-Hafedh *et al.* (2008) as very productive and ecologically food production systems, where fish waste provides a nutrient source for nitrifying bacteria, which in turn convert toxic waste of the fish to useful nutrients for plants. Also, he mentioned that this systems work by balancing nutrient generation from fish waste with nutrient uptake by plants to achieve proper water quality.

Dediu *et al.* (2011) pointed out that, although they are highly efficient, the new technologies of water treatment within recirculating aquaculture systems prove to be very expensive and difficult to manage. This fact is one of the major reasons because of which the implementation of integrated aquaponic systems should be future encouraged. The second reason can be obtaining an extra profit from a second crop culture (plants), generated by the valorization of by-products (wastes). Oomen *et al.* (1998) pointed out the postulations that suggested a reintegration of agronomic production forms, nowadays separated in monocultures, to combined production systems. For a given integrated system operating at steady state with no additional nutrient supplementation, nutrient concentrations will increase, decrease, or remain constant over time if nutrient production by fish is greater than, less than, or equal to nutrient assimilation by plants and nutrient losses, respectively (Seawright *et al.*, 1998).

Over time, a strong interest regarding the integrated aquaponic systems was given by the need of normalize the ratios between plants, fish, daily input feed, as well as the kind of integrated type of biofilter used (McMurtry *et al.*, 1990). Regarding the evolution differences of nitrogen concentrations in integrated aquaponic systems, it was demonstrated that are due to relative proportions of available nitrogen generated by fish and absorbed by plants. Graber and Junge (2009) state that in contrast to bacterial degradation, nutrient assimilation by plants is limited by surface, as photosynthesis is dependent on solar radiation.

Within an integrated aquaponic system, nitrogen cycle has highest priority, fact revealed also by Licamele (2009) who mention that this cycle is critical for sustaining life in an integrated aquaponic system. Therefore, the main aim of present study is to quantify the nitrogen budget for an integrated rainbow trout (*Onchorhynchus mykiss*) –Nores spinach *(Spinacia oleracea)* aquaponic system, where three plants densities were used. The second objective is to determine a balanced plant density for obtaining an optimal nitrogen removal rate. Furthermore, the experimental data obtained will be used for a new projection of already existing integrated aquaponic system, so that its level of crop productivity and also water treatment capacity to be maximized.

MATERIALS AND METHODS

Integrated aquaponic system description. The present experiment took place between 20th February and 4th April 2013 at the pilot recirculating system station of Aquaculture, Environmental Science and Engineering Department from Food Science Faculty, "Dunarea de Jos" University of Galati. The recirculating system consists in 12 rectangular shape rearing units with a volume of 0.15 m^3/unit, 2 rectangular sump units with a volume of 0.29 m^3/unit, 1 mechanical-quartz sand water conditioning unit with backwash, 1 biological trickling filtration unit, 1 sterilization UV filter (TETRA POND, Type UV-C 35000 and 36 Watt), recirculating pumps, oxygenation unit (compressor Resun Air-Pump, Model: ACO-018 A with a flow of 260 l/min) and water quality control sensors. The aquaponic modules consist in 4 rectangular glass made units (900x600x200mm), placed high above the recirculating system, on a metal support. A lighting system made of 4 fluorescent lamps, with reddish wavelength and a luminous power of 1080 lm was placed above the hydroponic units.

Regarding the water cycle inside the integrated system, it must be said that water that flow out from the rearing units pass first through mechanical filter and after that, by using a recirculating pump, it goes through the biological filtration unit and then gravitational to aquaponic modules, that flow out the treated water back to rearing units. The total volume of water from the integrated system is around the value of 2.5-2.7 m^3. An equal water flow of 6 L/minute was set for the inlet of all 4 hydroponic units. The support media of spinach consisted of polystyrene plates with holes for plastic special supports. Plants were placed in plastic supports and then, the supports were filled with a few hydroton balls to ensure their stability. The distance between plants was equal with 15 cm, both for aquaponic and conventional cultured ones. The maximum capacity of an aquaponic unit is 32 plants.

The current integrated aquaponic system exists for several years and it had been concluded that a new design is imposed for improving both crop productivity and water treatment capacity. So, the real experimental data obtained in present study, a new design of this already existing integrated system will be created, in the future.

Experimental design. Before starting the experiment, the activation of biological trickling filtration unit was made as described by Dediu *et al.* (2012). Daily ammonia, nitrite and nitrate levels were monitored to determine the degree of ammonia oxidation to nitrate and therefore to observe when a stable state of bacterial biomass is obtained. For the 44 days experiment, a total number of 228 rainbow trout (*Oncorhynchus mykiss*), with an average initial weight of 111.77 grams, was used in parallel with spinach (*Spinacia oleracea*), Nores variety, at an age of 25 days. The total fish biomass from the recirculating aquaculture system, at the beginning of the experiment, had 25.51 kg. Fish were divided in six subgroups, in duplicate. Three of them were fed with Classic Extra 1 P–41% brute protein and formed F1 group and the other three with Nutra PRO-MP-T–50% brute protein –F2 group, as in the protocol described by Hayward *et al.* (1997). A total amount of 12 363.32 grams of Classic Extra 1 P feed and 11 579.54 Nutra PRO-MP-T was administrated during all 44 experimental days. Nores variety spinach was placed in the hydroponic units with the following stocking densities: (V1-59 plants/m^2, V2-48 plants/m^2, V3-39 plants/m^2 and V4–control variant–without plants). The seedlings were obtained at Natural Sciences Museum Complex Galați, Botanical Garden.

A daily percentage of 10% water exchange was applied. The technological water was analyzed in terms of temperature, pH, dissolved oxygen, nitrates, nitrites and ammonium concentration. The temperature and dissolved oxygen were monitored with a portable WTW ProfiLine Oxi 3205 Dissolved Oxygen Meter. The pH was measured with WTW inoLab Multi 720 SET ph/Cond/Oxygen Meter and nitrogen compounds were determined by Spectroquant Nova 400 spectrophotometer, with Merk compatible kits twice a week. Samples of water were taken from the inlet of mechanical filter (inlet of biological filter), outlet of biological filter (inlet of hydroponic units) and outlet of each hydroponic unit. The luminous intensity was measured with TESTO 545 light meter. The SGR and FCR fish production indicators were determined by using the formulas described by Ridha and Cruz (2001): Specific growth rate (SGR) [(ln mean final weight–ln mean initial weight) x100]/culture days, (%BW/day); feed conversion ratio (FCR)=total weight of dry feed given/total wet weight gain (g/g). The values obtained were 16m/day for hydraulic loading rate and 0.008 h for hydraulic retention time. The total ammonia nitrogen, generated per a certain period of time, in the integrated aquaponic system, is calculated upon the feeding rate (Timmons *et al.*, 2002): P_{TAN}=Fx PC x 0.092, where: PTAN=Production rate of total ammonia nitrogen, (kg/period); F=Feed rate (kg/period); PC=Protein concentration in feed (decimal value). The nitrification performance of a biofilter is usually reported in literature as surface specific TAN removal or volumetric TAN removal rate, so nitrification rate has been calculated in terms of Volumetric

TAN Removal (VTR), using the equation (Díaz *et al.,* 2012): VTR=[([NH4+-N]in−[NH4+-N]out) · Q]/Vmedia, where VTR=amount of TAN removed per m^3 of filter media per day; [NH4-N]in and [NH4-N]out=ammonia concentration measured at the inlet and the outlet of the trickling filters system (g/m^3); Q=flow rate through the filters (m^3/day) and Vmedia is the volume of the filter media (m^3). The TAN removal rate in hyroponic units was calculated with the following formula (Dediu *et al.,* 2012): TAN retained (g/m^2/day)=((Q/V*(Cin-Cout)–dCout/dt)*d, where, Q=the flow rate (m^3/day), V=system volume (m^3), C=concentration of TAN (g/m^3), d=depth (m), t=time (d). The obtained results were then expressed in m^2. The biochemical determination of nitrogen content from spinach dry matter (leaf and root), fresh rainbow trout meat, fish feed and dry fish faeces was made by using Kjeldahl method (HACH, Cat. No. 23130-18 Instruction Manual). Faeces collection was made with a special EHEIM water vacuum cleaner with a mesh compartment for vacuum collection.

Statistical methods. Statistical analysis was performed using the IBM SPSS Statistics 20 for Windows. Statistical differences between treatments were tested using T test (α=0.05) after a normality test (Kolmogorov-Smirnov). Comparisons between variants were assessed using post-hoc Duncan test for multiple comparisons (ANOVA).

RESULTS AND DISCUSSIONS

Fish and plants growth performance. The growth performance indicators reveals that the group of fish fed with Nutra PRO-MP-T–50% brute protein had a higher total weight gain comparing with the second group, fed with Classic Extra 1 P–41% brute protein. The difference is resulted from the use of a higher brute protein level feed. Also, the F2 group registered better values for both FCR and SGR (*Tab. 1*).

Tab. 1

Fish growth performance indicators for F1 and F2 experimental groups (mean ± S.E.)

Growth indicator	F1 group (feed with Classic Extra 1 P–41% brute protein)	F2 group (feed with Nutra PRO-MP-T–50% brute protein)
Total initial biomass (g)	12 768 ± 7.01	12 746 ± 3.53
Total final biomass (g)	25 208 ± 208	27 684 ± 302
Total weight gain (g)	12 440 ± 292	14 938 ± 288
Average feed conversion ratio (g/g)	1.1 ± 0.05	0.84 ± 0.04
Average specific growth rate (%BW/day)	1.5 ± 0.07	1.66 ± 0.08

Regarding plant growth performance, a higher value for total weight gain is registered in V1, compared with V2 and V3, which is explained by the number of plants per each variant (*Tab. 2*). The first variant (V1) has 32 plants, compared with the second variant (V2) that has 26 plants and the third variant (V3) that has 21 plants.

Tab. 2

Plant growth performance indicators for V1, V2 and V3 experimental variants (mean ± S.E.)

Plant growth indicator	V1-59 plants/m^2	V2-48 plants/m^2	V3-39 plants/m^2
Total initial biomass (g)	23.74 ± 0.3	16.52 ± 0.2	15.59 ± 0.31
Total final biomass (g)	112.88 ± 1.79	101.32 ± 1.63	89.47 ± 1.64
Total weight gain (g)	89.14 ± 1.68	84.8 ± 1.57	73.88 ± 1.13

The total nitrogen input quantity and total ammonia nitrogen production rate. A total quantity of 12 363.33 g of Classic Extra 1 P–41% brute protein feed and 11579.54 g of Nutra PRO-MP-T–50% brute protein was distributed in the integrated system among 44 experimental days. The biochemical analysis of feed was made and a content of 6.74 g% was found at Classic Extra 1 P and 8.11 g% at Nutra PRO-MP-T. This means a total nitrogen input of 1772.68 g with a daily average of 40.28 g/day. Also, a total ammonia nitrogen production rate of 351.91 g was registered, with a daily average of 8g/day, depending on feeding rate.

The evolution of water dissolved oxygen, temperature, pH, ammonium, nitrite and nitrate nitrogen. The water temperature among the experiment was almost constant, without large fluctuations, with a minimum value of 16.16^{o}C and a maximum value of 17.8^{o}C (*Fig. 1*). The values were suitable for both rainbow trout and Nores spinach proper growth and development. The pH ranged between 6.6 and 7.96, with an almost constant evolution throughout experiment, except the first two experimental days, when the values were a bit higher (*Fig. 1*). The value of pH among the experimental period was proper for assuring a normal activity for nitrifying bacteria from biological trickling filtration unit. Dissolved oxygen concentration varied between 6.65 and 9.41 mg/L, with a downward trend in first 9 experimental days, but after that its evolution was relatively constant (*Fig. 1*).

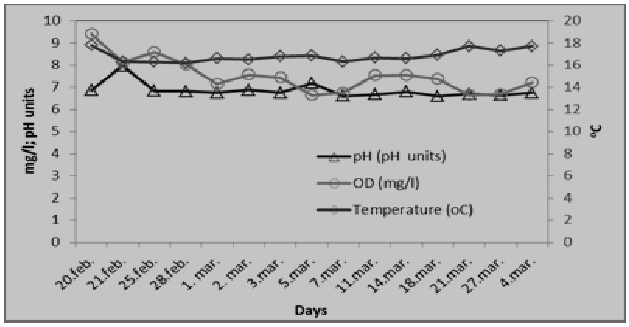

Fig. 1. The evolution of dissolved oxygen, temperature and pH
in the integrated aquaponic system among the experimental period

The evolution of ammonium nitrogen concentration throughout the experimental period has an upward trend. It can be also said that the evolution is influenced by the quantity of feed administrated in a certain period, plant nitrogen absorption and also biological filter water treatment performance. In case of V1 outlet, the variation range of N-NH_4^+ is between 0 and 0.27 mg/L, with an average concentration of 0.05 mg/L, for V2 outlet the N-NH_4^+ variation range is between 0 and 0.28 mg/L with an average of 0.06 mg/L and for V3 outlet we had a variation range between 0 and 0.34 mg/L, with an average of 0.11 mg/L (*Fig. 2*). At the control variant outlet the N-NH_4^+ concentration range between 0 and 0.43 mg/L with an average of 0.14 mg/L (*Fig. 2*). The N-$NH4^+$ concentration registered for biological filter inlet (mechanical filter outlet) were between 0.03 and 0.71 mg/L with an average of 0.23 mg/L and the ones for biological filter outlet (aquaponic module inlet) were between 0 and 0.46 mg/L, with an average of 0.12 mg/L. Also, the concentration from water samples taken from mechanical filter inlet ranged between 0.02 and 0.66 mg/L N-NH_4^+, with an average of 0.218mg/L. By applying post-hoc Duncan test for multiple comparisons (ANOVA), it was found that differences between V1 outlet and V3 outlet are statistically significant ($p<0.05$), also the differences between V1, V2 and V3 experimental variants outlet and V4 control variant outlet and differences between biological filter inlet and biological filter outlet N-NH_4^+ concentration are statistically significant ($p<0.05$). Difference between biological filter

outlet and V1, V2, V3 experimental variants outlet are statistically significant ($p<0.05$). Differences between mechanical filter inlet –mechanical filter outlet and V1 outlet–V2 outlet are not statistically significant ($p>0.05$). Tukey and Duncan tests divided the values in three homogeneous subsets: V1 outlet+V2outlet+V3outlet; Biological filter outlet + Control variant outlet; Mechanical filter inlet + Biological filter inlet.

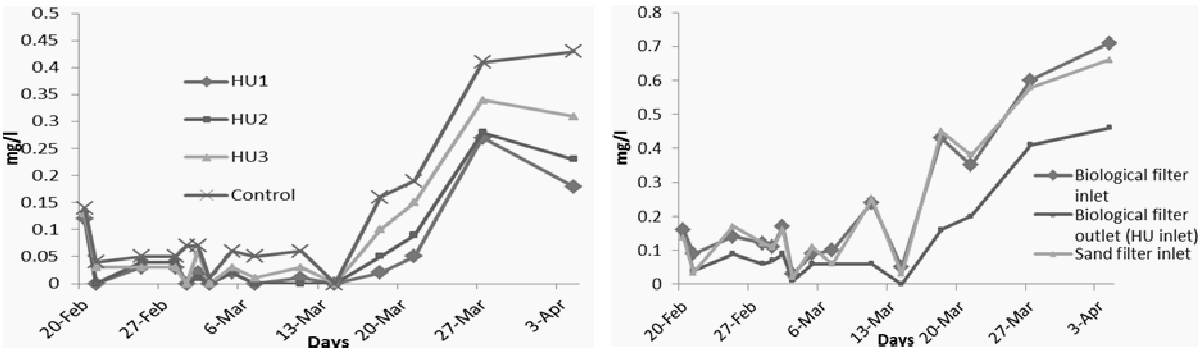

Fig. 2. Ammonium nitrogen evolution throughout the experimental period
(V1-HU1; V2-HU2; V3-HU3; V4 – Control; biological filter inlet/outlet, mechanical filter inlet)

The evolution of nitrite nitrogen concentration throughout the experimental period has a slight downward trend. It can be also said that the fact that influenced directly this trend is the biological filter water treatment performance and among indirect influence factors we can mention the temperature, pH and feed quantity administrated in a certain period. It can be observed that on the last 20 days of the experiment, the concentration of $N\text{-}NO_2^-$ from V3 outlet is almost identical with the one registered at V4–Control variant outlet. In case of V1 outlet, the variation range of $N\text{-}NO_2^-$ is between 0.01 and 0.09 mg/L, with an average concentration of 0.048 mg/L, for V2 outlet the $N\text{-}NO_2^-$ variation range is between 0.03 and 0.08 mg/L with an average of 0.054 mg/L and for V3 outlet we had a variation range between 0.03 and 0.11 mg/L, with an average of 0.06 mg/L (*Fig. 3*). At the control variant outlet, the $N\text{-}NO_2^-$ concentration range between 0.03 and 0.11 mg/L with an average of 0.062 mg/L (*Fig. 3*). The $N\text{-}NO_2^-$ concentration registered for biological filter inlet (mechanical filter outlet) were between 0.04 and 0.11mg/L with an average of 0.073 mg/L and the ones for biological filter outlet (aquaponic module inlet) were between 0.02 and 0.09 mg/L, with an average of 0.06 mg/L. Also, the concentration from water samples taken from mechanical filter inlet ranged between 0.04 and 0.09 mg/L $N\text{-}NO_2^-$, with an average of 0.063mg/L. By applying post-hoc Duncan test for multiple comparisons (ANOVA), it was found that differences between V1 outlet + V2 outlet and V3 outlet are statistically significant ($p<0.05$), also the differences between V1 and V2 experimental variants outlet and V4 control variant outlet and differences between biological filter inlet and biological filter outlet $N\text{-}NO_2^-$ concentration are statistically significant ($p<0.05$). Difference between biological filter outlet and V1 experimental variants outlet is statistically significant ($p<0.05$). Differences between mechanical filter inlet –mechanical filter outlet and V3 outlet– Control variant outlet are not statistically significant ($p>0.05$). Tukey and Duncan tests divided the values in three homogeneous subsets: V1 outlet + V2outlet + Biological filter outlet; V3 outlet + Control variant + Mechanical filter inlet; Biological filter inlet.

The nitrate nitrogen concentration throughout the experimental days has a relative alternating evolution, with a tendency to accumulate and some moments of quick downward and upward tends, generated by the nutritional requirements of plants and yield of nitrifying bacteria within the biological filter. It can be also said that the evolution is influenced by the

quantity of feed administrated in certain periods. In case of V1 outlet, the variation range of $N\text{-}NO_3^-$ is between 19.96 and 24.82 mg/L, with an average concentration of 21.02 mg/L, for V2 outlet the $N\text{-}NO_3^-$ variation range is between 20.23 and 25.11 mg/L with an average of 21.43 mg/L and for V3 outlet we had a variation range between 20.34 and 25.68 mg/L, with an average of 21.65 mg/L (*Fig. 4*). At the control variant outlet, the $N\text{-}NO_3^-$ concentration range between 20.5 and 26.9 mg/L with an average of 22.6 mg/L (*Fig. 4*).

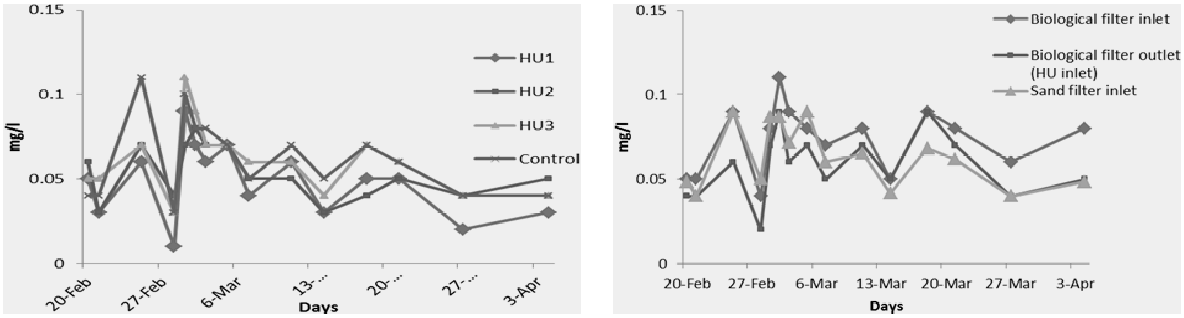

Fig. 3. Nitrite nitrogen evolution throughout the experimental period
(V1-HU1; V2-HU2; V3-HU3; V4–Control; biological filter inlet/outlet, mechanical filter inlet)

The $N\text{-}NO_3^-$ concentration registered for biological filter inlet (mechanical filter outlet) were between 20.28 and 25.07mg/L with an average of 21.53 mg/L and the ones for biological filter outlet (aquaponic module inlet) were between 20.64 and 26.87 mg/L, with an average of 22.56 mg/L. Also, the concentration from water samples taken from mechanical filter inlet ranged between 20.34 and 25 mg/L $N\text{-}NO_3^-$, with an average of 21.6 mg/L. By applying post-hoc Duncan test for multiple comparisons (ANOVA), it was found that differences between V1 outlet and V4 control variant outlet are statistically significant ($p<0.05$), also the differences between mechanical filter inlet and biological filter outlet concentration of $N\text{-}NO_3^-$, are statistically significant ($p<0.05$). Difference between biological filter outlet and V1, V2, V3 experimental variants outlet are statistically significant ($p<0.05$). Differences between the experimental variants (V1, V2, V3) are not statistically significant ($p>0.05$). Tukey and Duncan tests divided the values in three homogeneous subsets: V1 outlet; V2outlet + V3outlet + Mechanical filter inlet + Biological filter inlet; Biological filter outlet + Control variant outlet.

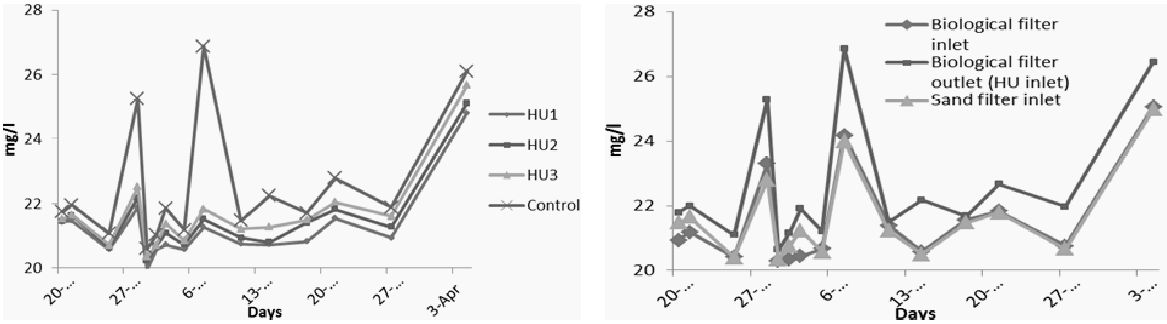

Fig. 4. Nitrate nitrogen evolution throughout the experimental period
(V1-HU1; V2-HU2; V3-HU3; V4–Control; biological filter inlet/outlet, mechanical filter inlet)

Water treatment capacity. Díaz *et al.* (2012) mentioned that the nitrification performance of a biofilter is usually reported in literature as surface specific TAN removal or volumetric TAN removal rate. Nitrification rates in granular media are much more closely

related to volume of media than surface area provided by the media. In our case, volumetric TAN removal (VTR) was calculated and it ranged between 0.16 and 2.16 g/m^2/day, with an average of 0.79 g/m^2/day. The maximum values were registered after 10, 20, 28 and 37 days from the beginning of the experiment (*Fig. 5c*). Also, regarding TAN removal rate, we can say that it registered an upward trend in the second half of experimental period (*Fig. 5b*). In case of V1, the variation range is between 0 and 4.49 mg/L/day, with an average of 1.24 mg/L/day, for V2 the variation range is between 0 and 3.68 mg/L/day with an average of 1.09 mg/L/day and for V3 outlet we had a variation range between 0 and 2.4 mg/L/day, with an average of 0.71 mg/L (*Fig. 5b*). At the control variant, the range was between 0 and 0.21 mg/L/day with an average of 0.04 mg/L/day (*Fig. 5b*). The difference between V1 and V3 and also between V1, V2, V3 and the control variant are statistically significant ($p<0.05$). Differences between V1 and V2 are not statistically significant ($p>0.05$). Tukey and Duncan tests divided the values in three homogeneous subsets: V1+V2; V3; V4 control variant. Regarding nitrate removal rate, it has a relative alternating evolution, influenced by the nutritional requirements of plants in certain moments (*Fig. 5a*). An interesting thing is observed in case of control variant evolution where positive values are recorded in the first 19days of experiment and after that a negative evolution occurs, most probably because of a nitrifying bacteria bio-film appearance on the interior part of integrated system inlet pipes (*Fig. 5a*). In case of V1, the variation range of nitrate removal is between 5.78 and 32.87 mg/L/day, with an average of 16.4 mg/L/day, for V2 the variation range is between 4.33 and 27.82 mg/L/day with an average of 12.5 mg/L/day and for V3 outlet we had a variation range between 2.9 and 19.14 mg/L/day, with an average of 8.23 mg/L (*Fig. 5b*). At the control variant, the range was between -0.6 and 1.92 mg/L/day with an average of 0.24 mg/L/day (*Fig. 5b*). The difference between V1 and V3 and also between V1, V2, V3 and the control variant are statistically significant ($p<0.05$). Differences between V1 and V2 are not statistically significant ($p>0.05$). Tukey and Duncan tests divided the values in three homogeneous subsets: V1+V2; V3; V4 control variant.

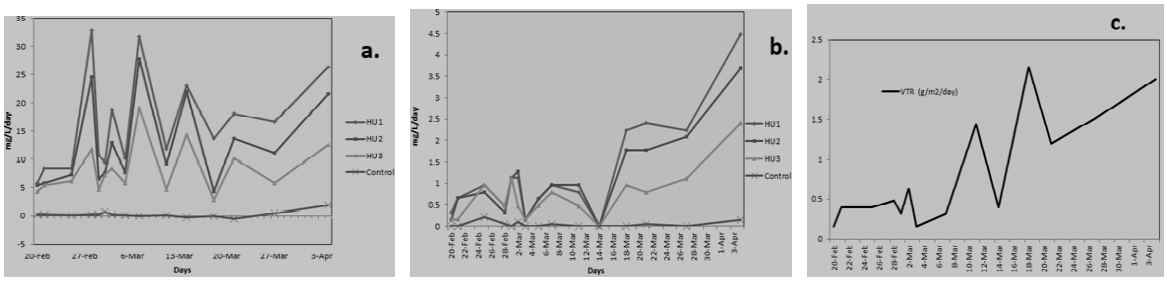

Fig. 5. *5a*. Nitrate removal rate; *5b.* TAN removal rate;
*5c.*Volumetric TAN removal of biological filter. (V1-HU1; V2-HU2; V3-HU3; V4–Control)

Nitrogen recovery by faeces, fish and plants biomass. The nitrogen percentage of aquaponic cultured spinach was determined by biochemical analyzes. The results were compared with the one of marketable spinach (*Fig. 6.a*). For nitrogen content of spinach leaf, an average content of 3.91 g% dry weight for both V1 and V2 experimental variants was recorded, lower than V3 spinach leaf nitrogen content of 4.6 g% dry weight. The differences between first two variants and the third variant are statistically significant ($p<0.05$). The initial average nitrogen content of spinach shoot was 3.05 g% dry weight and the average nitrogen content obtained from market spinach leaf was 4.49 g% dry weight. The differences between spinach from first two variants and market spinach nitrogen leaf content were statistically significant ($p<0.05$). Tukey and Duncan tests divided the values in two

homogeneous subsets: V1+V2, V3+market spinach. The values were similar to Roe *et al.* (2013) 0.42 g% dry weight for field culture spinach. The spinach nitrogen content from roots registered the main average values: 6.5 g% dry weight at V1, 7.27 g% dry weight at V2 and 9.69% dry weight at V3. The differences between first two variants (V1, V2) and the third variant (V3) are statistically significant ($p<0.05$). Also Tukey and Duncan tests divided the values in two homogeneous subsets: V1 + V2; V3. The nitrogen biochemical content from rainbow trout meat was also made and the following values were registered: at the start of the experiment an average value of 2.38 g% fresh weight was registered and at the end of the experiment an average value of 2.85 g% fresh weight was registered for F1 fish group and 2.86 g% fresh weight for F2 group. The nitrogen retention was calculated and the following average values were obtained: 3.62 g/fish for F1 group and 4.47 g/fish at F2 group. The evolution of nitrogen from faeces, throughout the experimental period, registered an upward evolution in the first days of experiment, in case of F1 fish group and then a constant evolution. Also, in case of F2 group, the evolution had a slight upward trend till the third week of experimental period and then it has stabilized (*Fig. 6.b*).

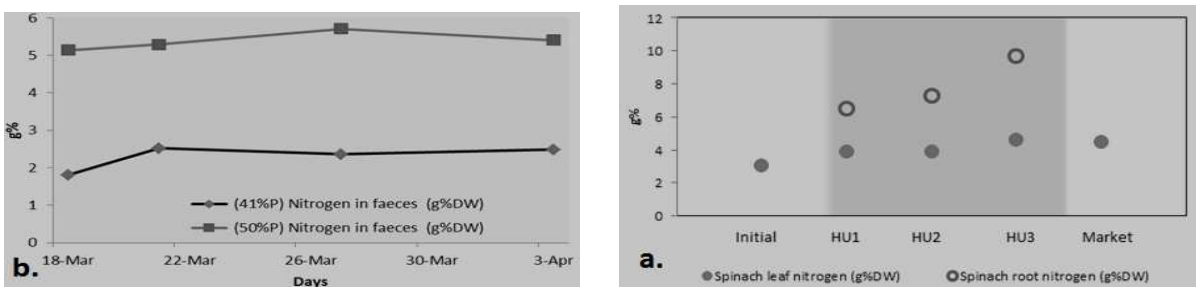

Fig. 6. Nitrogen recovery by 6.a. plants root and leaf; 6.b. faeces
(V1-HU1; V2-HU2; V3-HU3; V4 – Control)

The average value of nitrogen in dry faeces at F1 fish group registered a value of 2.28 g%dry weight and for F2 the value was 5.38 g% dry weight. The differences between the groups were statistically significant ($p<0.05$).

CONCLUSION

As a conclusion to this study it can be state that plant density applied in V1 case is the best from all three tested densities in terms of water chemical treatment. Also, by analyzing the nitrate removal rates, it must be pointed out that plants have different evolution periods in their lifetime and therefore different nutrient absorption rates. So, in an integrated aquaponic system is very important find a balance between plants absorption rates and administrated feed quantity.

The trickling biological filter nitrification performances were situated within normal range and had a good evolution, given also the statistically significant differences between ammonium nitrogen and nitrate nitrogen concentrations between its inlet and outlet. Another observation can be made regarding the nitrate removal rate from V4 control variant where negative values evolution occurred towards the end of experimental period. An explication can be the appearance of a heterotrophic bacteria bio-film on the interior part of integrated system inlet pipes, on the route between biological filter outlet and aquaponic modules inlet. The nitrogen content of spinach obtain in V3 experimental variant is similar with the one of market and field culture. Lower results were obtained for nitrogen content of spinach from V1 and V2 variants.

As recommendation for a future upgrade of the integrated system, it can be said that the performance of the mechanical filter must be improved, the number of aquaponic modules should grow and also the density of 59plants/m^2 should be applied for all hydroponic units for improving the water treatment performance. Also, for a better and more consistent absorption of nutrients, a higher light intensity can be also used.

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