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STUDY REGARDING NITRIFICATION IN EXPERIMENTAL AQUAPONIC SYSTEM

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Abstract

The aim of this paper was to understand how nitrogen cycle evolving through early experimental aquaponic system due to growth of nitrification bacteria such as Nitrozomonas and Nitrobacter. In this respect, it was design an aquaculture system, with different composition of fish species: Carassius auratus (10 pieces) and Hypostomus plecostomus (2 pieces). After we've found that the nitrogen cycle was established, we've chosen the suitable plants for the hydroponic system. The fishes were fed by organic meal; it was used a feeding rate ratio for design calculations of (0,6 grams / day) and the feed input was kept relatively constant. The pH was controlled on a daily basis (pH was maintained constant by addition of calcium carbonate) and oxygen dissolved (that has been kept constant by using a proper pump). Nitrogen forms (ammonium - NH4, nitrite - NO2 and nitrate - NO3) were determined on a daily basis as well and in the same time with pH measurement, in order to make correlations between evolution of nitrogen concentrations and nitrification bacteria growths. After 30 days of experiment it was found that ammonium - NH4 concentrations are directly and significantly correlated with nitrite -NO2 concentrations (the Pearson correlation factor is r = 0.74). It means that Nitrosomonas bacteria formed an effective biofilm. After 10 days from initiation of aquaculture experiment we also observed that nitrate concentrations values are correlated with nitrites concentrations values (which demonstrates the Nitrobacter growing). The correlation coefficient is negative (r = - 0.75) because in the absence of plants, the nitrates were accumulated in water. In conclusion, according to experimental data, the ammonium- NH4 and nitrite -NO2 concentrations are stabilized after 30 days, reaching values which are lower than legislation in force imposing.

Key words*: Nitrosomonas, Nitobacter, nitrogen cycle, nitrite, nitrate, aquaponic*

INTRODUCTION

Aquaponics refers to any system that combines conventional aquaculture (raising aquatic animals such as snails, fish, crayfish or prawns in tanks) with hydroponics (cultivating plants in water) in a symbiotic environment. In normal aquaculture, excretions from the animals can accumulate in the water, increasing toxicity. In an aquaponic system, water from an aquaculture system is fed to a hydroponic system where the by-products are broken down by nitrifying bacteria into nitrites and then into nitrates, which are utilized by the plants as nutrients, so the water is recirculated back to the aquaculture system.

Aquaponics has ancient roots. Aztec cultivated agricultural islands known as chinampas in a system considered by some to be the first form of aquaponics for agricultural use (Boutwelluc, 2007 and Rogosa, 2013) where plants were raised on stationary (and sometime movable) islands in lake shallows and waste materials dredged from the chinampa canals and surrounding cities were used to manually irrigate the plants (Boutwelluc, 2007 and Rogosa, 2013). Also, South China, Thailand, and Indonesia who cultivated and farmed rice in paddy fields in combination with fish are cited as examples of early aquaponics systems (FAO, 2001). These aquaponic farming systems existed in many Far Eastern countries, in USA, and Canada.

In Romania, aquaponics exists in Snagov, Focsani, and Timisoara towns.

Parts of an aquaponic system

Aquaponics consists of two main parts, with the aquaculture part for raising aquatic animals and the hydroponics part for growing plants (Rakocy, 2016 and Diver, 2006). Aquatic effluents, resulting from uneaten feed or raising animals like fish, accumulate in water due to the closed-system recirculation of most aquaculture systems. The effluent-rich water becomes toxic to the aquatic animal in high concentrations but this contain nutrients essential for plant growth (Rakocy, 2016). Although consisting primarily of these two parts, aquaponics systems are usually grouped into several components or subsystems responsible for the effective removal of solid wastes, for adding bases to neutralize acids, or for maintaining water oxygenation (Rakocy, 2016). Typical components include:

- *Rearing tank*: the tanks for raising and feeding the fish;
- *Settling basin*: a unit for catching uneaten food and detached biofilms, and for settling out fine particulates;
- *Biofilter*: a place where the nitrification bacteria can grow and convert ammonia into nitrates, which are usable by the plants (Rakocy, 2006);
- *Hydroponics subsystem*: the portion of the system where plants are grown by absorbing excess nutrients from the water;
- *Sump*: the lowest point in the system where the water flows to and from which it is pumped back to the rearing tanks.

Live components

Animals. Freshwater fish are the most common aquatic animal raised using aquaponics, although freshwater crayfish and prawns are also sometimes used (Drive, 2006).

Plants. Green leafy vegetables with low to medium nutrient requirements are well adapted to aquaponic systems, including lettuce, basil, spinach, chinese cabbage, chives, herbs, and watercress (www.backyardaquaponics.com)(http://www.bioconlabs.com).

Bacteria. Nitrification, the aerobic conversion of ammonia into nitrates, is one of the most important functions in an aquaponics system as it reduces the toxicity of the water for fish, and allows the resulting nitrate compounds to be removed by the plants for nourishment (FAO, 2001). Ammonia is steadily released into the water through the excretion of the fishes as a product of their metabolism, but must be filtered out of the water since higher concentrations of ammonia (commonly between 0.5 and 1 ppm) can kill fish (Robert, 1997). Although plants can absorb ammonia from the water to some degree, but nitrates are assimilated more easily (Rakocy, 2016), thereby efficiently reducing the toxicity of the water for fish (FAO, 2001). Ammonia can be converted into other nitrogenous compounds by: *Nitrosomonas* bacteria that convert ammonia into nitrites, and *Nitrobacter* bacteria that convert nitrites into nitrates.

Nitrosomonas is a genus of rod-shaped chemoautotrophic bacteria (microbewiki.kenyon.edu). They are found in soil, freshwater, and on building surfaces, especially in areas that contains high levels of nitrogen compounds. Most species are motile with a flagellum located in the polar regions. They are important in the nitrogen cycle by increasing the availability of nitrogen to plants while limiting carbon dioxide fixation (http://microbewiki.kenyon.edu/index.php/Nitrosomonas). This organism oxidizes ammonia into nitrite as a metabolic process. *Nitrosomonas* use energy gained through the oxidation of ammonia to fix gaseous carbon dioxide into organic compounds. *Nitrosomonas* prefers an optimum pH of 6.0-9.0 and a temperature range of 20 to 30°C. This microbe is photophobic, and will generate a biofilm matrix or form clumps with other microbes to avoid light (http://microbewiki.kenyon.edu/index.php/Nitrosomonas)

Nitrobacter is a genus of mostly rod-shaped, gram-negative, and chemoautotrophic bacteria (http://www.bioconlabs.com). *Nitrobacter* plays an important role in the nitrogen cycle by oxidizing nitrite into nitrate in soil. Unlike plants, where electron transfer in photosynthesis provides the energy for carbon fixation, *Nitrobacter* uses energy from the oxidation of nitrite ions, NO_2^-, into nitrate ions, NO_3^-, to fulfill their energy needs. *Nitrobacter* have an optimum pH between 7.3 and 7.5, and will die in temperatures exceeding 49°C or below 0°C (http://www.bioconlabs.com). According to Grundmann, *Nitrobacter* seem to grow optimally at 38°C and at a pH of 7.9 (http://www.bioconlabs.com).

MATERIALS AND METHODS

Our aquaponic system consists of (Figure 1):

- rearing tank for raising and feeding the fishes (47x37x42 cm, V=73 cm^3);
- biofilter, a place where the nitrification bacteria can grow and convert ammonia into nitrates; biofiltration material with a high specific surface (600 m^2/m^3), is represented by expanded clay aggregates;
- a pump that flow back the water to the rearing tanks (model EHEIM compact 300, with following caracteristics: capacity 150-300 l/h, total head- 0.5 m, power - 5W).

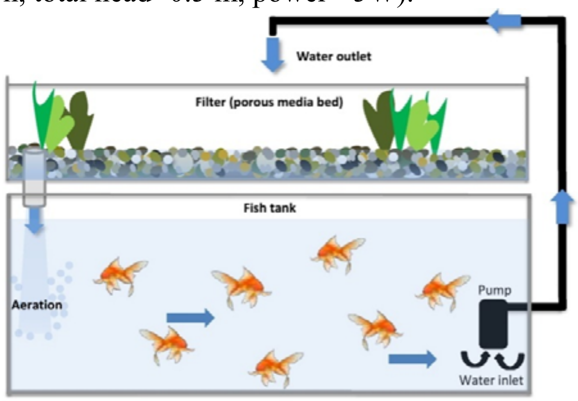

Figure 1. Aquaponic sistem

Live components:

- *Animals*. Freshwater fish (Figure 2) - *Carassius auratus* (10 pieces) and *Hypostomus plecostomus* (2 pieces);

Figure 2. The fish species from the aquarium.

- *Bacteria*. *Nitrosomonas* and *Nitrobacter* (their existence are demonstrated by our results);
- *Plants.* Our hydroponic system will include lettuce.

The fishes were feeding by organic meal and we always kept feed input relatively constant per body grams (0.6 grames per fish).

We ensure a good aeration, maintaining the oxygen dissolved at 10 mg/l. Since the nitrification process acidifies the water, calcium carbonate was added in the water to provide a buffer against acidification.

Control of growth and development nitrifying bacteria was set up by daily investigation of ammonia, nitrite and nitrate concentrations in water, pH and oxygen dissolved. We used a professional test kit for freshwater analysis (provide from JBL Company).

RESULTS AND DISCUSSIONS

Our results show that in operational 30 days of aquaculture system it develops a bacterial population (*Nitrosomonas*) capable to convert ammonium ions resulting from excretion process of the fishes, into nitrites. According to Table 1 and Figure 3 can be seen initiating the nitrogen cycle by *Nitosomonas* bacteria. Certain amounts of ammonia results in excretion processes are converted into nitrites, the coefficient correlation value being r = + 0.74, which defines direct and significant correlation. The nitrites formation is directly proportional to the concentration of ammonium ions.

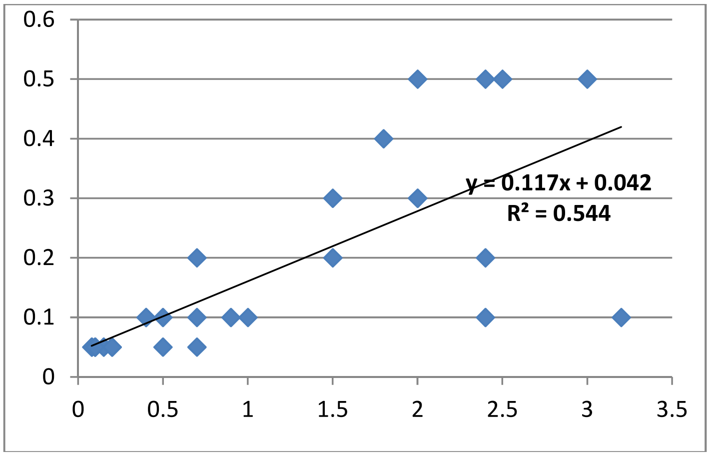

Figure 3. The correlation between concentrations of ammonium and nitrite in the aquaculture system, in the first 30 days of the experiment. Pearson correlation coefficient r = 0.74 defines a significant direct correlation

Table 1. The parameter values daily investigated: pH, ammonium, nitrite, nitrate, phosphat, and oxygen dissolved

Day	pH	NH_4	NO_2	NO_3	PO_4	OD
1	8	0.05	0.08	40	0.02	10
2	7.9	0.05	0.08	40	0.02	10
3	7.8	0.05	0.1	60	0.2	10
4	7.8	0.05	0.1	60	0.2	10
5	7.8	0.05	0.15	60	0.2	10
6	7.7	0.05	0.15	50	0.2	10
7	7.8	0.05	0.2	50	0.3	10
8	7.8	0.05	0.5	50	0.3	10
9	7.9	0.05	0.7	40	0.2	10
10	7.8	0.1	0.9	50	0.2	10
11	7.8	0.1	1	50	0.2	10
12	7.8	0.1	3.2	30	0.2	10
13	7.8	0.1	2.4	60	0.2	10
14	7.8	0.2	2.4	70	0.2	10
15	7.8	0.3	2	80	0.2	10
16	7.8	0.3	2	80	0.3	10
17	7.8	0.5	2.4	90	0.3	10
18	7.8	0.5	2.5	90	0.2	10
19	7.8	0.5	3	90	0.2	10
20	7.8	0.5	2	90	0.2	10
21	7.8	0.4	1.8	100	0.2	10
22	7.8	0.3	1.5	110	0.2	10
23	7.8	0.2	1.5	130	0.3	10
24	7.8	0.2	0.7	140	0.3	10
25	7.8	0.1	0.7	140	0.2	10
26	7.8	0.1	0.5	130	0.2	10
27	7.8	0.1	0.4	140	0.3	10
28	7.8	0.1	0.2	180	0.3	10
29	7.8	0.05	0.2	220	0.3	10
30	7.8	0.05	0.1	220	0.3	10

On the twelfth day of the experiment nitrite ions concentration recorded a growth peak, which demonstrate that *Nitrosomonas* species have grown up and developed. As a result, the concentration of ammonium ions starts to decrease. Simultaneously the concentration of nitrite are decreasing instead nitrate concentration are increasing. The concentration of nitrite ions starts to decrease linearly with decreasing of ammonium ions concentration, while the concentration of nitrate starts to increase. These facts mark the moment when *Nitrobacter* bacteria begin the activity of transforming nitrite to nitrate (Figure 4).

As nitrates are converted into nitrites they accumulate in water and become bioavailable for the plants.

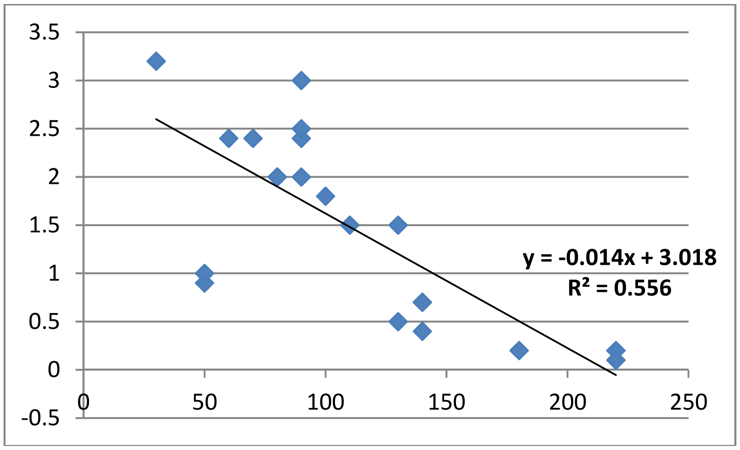

Figure 4. Correlation between concentrations of nitrite and nitrate in experimental aquaculture system, starting with the twelfth day. Pearson correlation coefficient r = - 0.75 defines a significant inverse correlation

Given that our aquaponic does not yet mounted hydroponic system, we cannot predict the future evolution of nitrates. However, conclusive data on the development of nitrifying bacteria biofilm (*Nitrosomonas* and *Nitrobacter*) supports the view that nitrates are successfully assimilated by the lettuce plants that will be cultivated on clay aggregates.

CONCLUSIONS

Bacteria are a crucial and pivotal aspect of aquaponics. The nitrifying bacteria convert the fish waste, which enters the system mainly as ammonia, into nitrate, which is fertilizer for the plants.

This is a two-step process, and two separate groups of nitrifying bacteria are involved. The first step is converting ammonia to nitrite, which is done by the ammonia-oxidizing bacteria. These bacteria are often referred to the genus *Nitrosomonas*. The second step is converting nitrite to nitrate is done by the nitrite-oxidizing bacteria. These are commonly referred to the genus *Nitrobacter*.

Nitrifying bacteria are relatively slow to reproduce and establish colonies, requiring days and sometimes weeks, and therefore the patience is one of the most important management parameters when establishing a new aquaponic system.

To develop extensive colonies of nitrifying bacteria is necessarily to use a biofiltration material with a high specific surface (optimal between 300 m²/m³ and 600 m²/m³), like volcanic gravel or expanded clay.

The water pH must be kept range of 7–8.2, not lower. Generally, nitrifying bacteria work better at higher pH, the *Nitrosomonas* group preferring a pH of 7.2–7.8, and the *Nitrobacter* group preferring a pH of 7.2–8.2. (http://www.bioconlabs.com)(http://microbewiki.kenyon.edu/index.php/Nitrosomonas).

Nitrifying bacteria are photosensitive until they fully establish a colony, and sunlight can cause considerable harm to the biofilter. It's necessarily to keep aquarium shaded from direct sunlight.

Bacterial function must be monitored by testing for ammonia, nitrite and nitrate. The test provides information on the health of the bacterial colony. Ammonia and nitrite should always be 0–1 mg/litre in a functioning and balanced aquaponic unit (http://www.fao.org/3/a-i4021e/i4021e05.pdf). According to our experimental data, the ammonium- NH4 and nitrite -NO2 concentrations are stabilized after 30 days, reaching values lower than legislation imposed (0.05mg/l, respectively 0.1 mg/l).

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