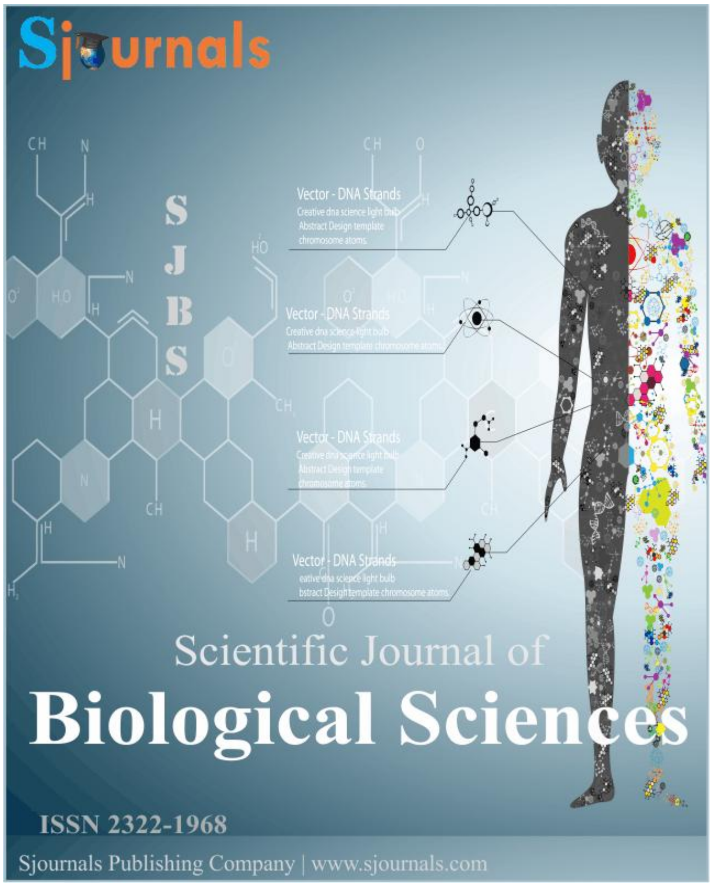
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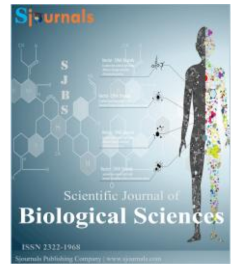

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Original article

Ecological succession of plankton in a biofloc system with molasses as carbon source

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A B S T R A C T

The objective of this research was to establish the ecological succession of the planktonic groups developed in a biofloc system for the cultivation of tilapia. For this purpose, 20 fish of 7±2 cm long and an average weight of 8.6 g were introduced in 200 liters' cylinders. Every day they were fed commercial food with 40% protein. The diet was calculated considering 10% of the body mass and molasses was added to promote the development of heterotrophic bacteria maintaining a C/N ratio of 15:1. Diversity and abundance of the organisms associated with the bioflocs was analyzed during 10 weeks by direct observation under an optical microscope (Olympus CBX50) connected to an image processing software. At the same time, physicochemical parameters (pH, nitrite, nitrates and ammonium) were measured using a HANNA Aquaculture Photometer auto analyzer (HI83203). Results showed a marked succession of planktonic organisms as the system matured, with the occurrence of facilitation, tolerance and inhibition mechanisms among the groups. Chlorophytes and cyanobacteria were among the facilitator species responsible for the transformation of nitrogen compounds into assimilable forms available for the development of other organisms with more complex structures, so that -throughout the experiment- ciliates and other protozoa appeared quickly, but with variations both in diversity and abundance among the groups. Later on, rotifers, one of the most conspicuous groups, increased represented by the genera *Lepadella*, *Phillodina*, *Lecane* and *Habrotrocha*. The last group to appear was that of nematodes (sixth week), which indicates that the system was already mature by providing sufficient carbohydrates for the formation of the collagen structures of this group.

1. Introduction

Increasing demand for farmed fish for human consumption, demands to optimize available space for cultivation in order to make biomass production more efficient. However, this has resulted in problems associated with the modification of water quality, causing eutrophication from nutrient discharges of organic and inorganic components such as ammonium, phosphorus, organic matter, dissolved organic carbon and suspended solids (Collazos and Arias, 2015); overall, a condition of stress and poor conditions in the system that causes infectious diseases in farmed organisms. For this reason, a new technique called the *biofloc* system has been implemented, which uses this organic matter for the creation of closed systems that are not eutrophic and free of pathogenic bacteria. The system generates bioflocs incorporating an external carbon source; bioflocs are rich in bacteria, microalgae, rotifers, ciliates, nematodes and copepods that become a natural source of food available for growing organisms 24 hours a day (Avnimelech and Kochba, 2009; Monroy et al., 2013). It is important to consider that planktonic communities in aquatic environments respond quickly to changes in their immediate environment. These changes can be subtle and become evident either by prompting or inactivating certain metabolic pathways, or from the changes they can cause in the community's composition (Abreu et al., 2007) that lead to a climax community after successional changes that are dependent on nutrients available in the environment (Cabello et al., 2013; Crab et al., 2009). Despite the benefits observed with the use of Biofloc, information on the succession that occurs in planktonic organisms is scarce. Due to the above, the objective of this research was to describe these changes in the composition and abundance of zooplankton in a biofloc culture system for tilapia using molasses as a carbon source.

2. Materials and methods

This research was carried out in the Live Food Chemical Analysis Laboratory of the Universidad Autónoma Metropolitana -UAM-X.

2.1. Experimental design

To carry out the experiment, a Biofloc culture system was undertaken using the "macrocosm" type (Emerenciano et al., 2011). This involved six cylinders with a capacity of 200 liters, with an air diffuser in the center to guarantee continuous movement and resuspension of particles; 20 tilapias of an approximate length of 7±2 cm and an average weight of 8.6 g were introduced in the cylinders. Commercial food was supplied daily (Alimentos del Pedregal®, Toluca, State of Mexico, Mexico) with 40% protein content and 0.6-0.8 mm particles. The diet was calculated considering 10% of body mass and the amount of food was adjusted every 15 days. To promote the production of bioflocs in the cylinders, molasses was added as a carbon source maintaining a C/N=15:1 ratio, taking into account calculations on demand recommended by Avnimelech (2012).

2.2. Assessment of water quality and sedimentable solids

The evaluation of water quality parameters was done once a week. Water samples were taken from the bioflocs production cylinders, to measure water temperature (°C) and pH with a Hanna pHep 4 meter (HI 98127). Levels of Ammonium (NH_{4+}), nitrite (NO_{2-}) and nitrate (NO_{3-}) were also analyzed by spectrophotometry with a

HANNA Aquaculture Photometer auto analyzer (HI83203) according to HANNA standard methods (HANNA Company, 2003).

2.3. Diversity of microfauna associated with biofloc

For the observation and microscopic quantification of the organisms associated with the bioflocs, three samples of 10 mL of the culture water were taken, observed and accounted for directly with optical microscopy. The taxonomic identification of the groups in the samples was done with the help of specialized literature (Aladro-Lubel, 2009; Luna, 2006; Samanez et al., 2014).

2.4. Data analysis

From all the information obtained, a database was built in Excel (2013), descriptive statistics analysis was carried out, and frequency and abundance of the observed groups was determined.

3. Results and discussion

3.1. Physicochemical parameters

Fig. 1 shows that nitrite, nitrate, ammonium and pH had minimal variations and were kept within the optimal ranges for the growth of tilapia and the adequate development of the plant community. Therefore, we can ascertain that the recycling of nitrogen compounds in biofloc systems is efficient.

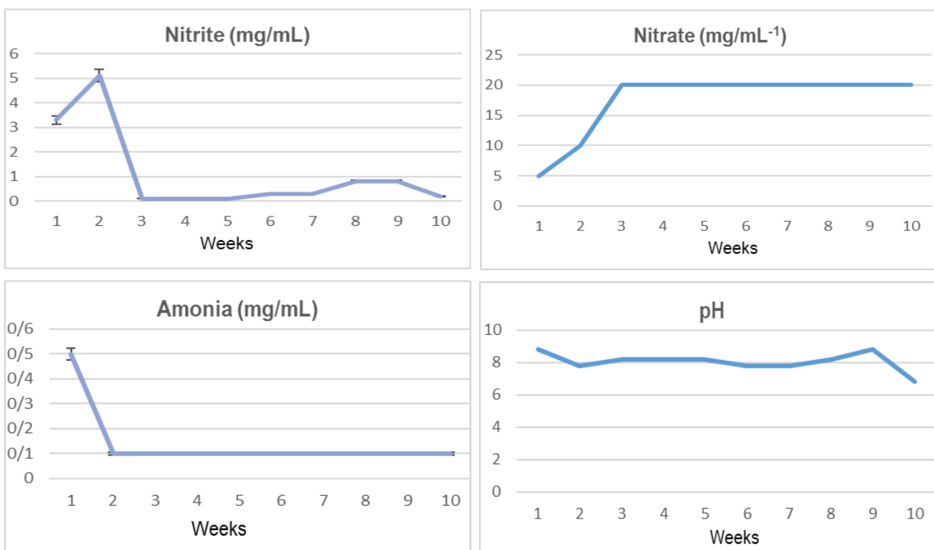

Fig. 1. Concentration of nitrite, nitrate, ammonium and pH in the biofloc system during 10 weeks of the experiment.

3.2. Abundance and diversity of zooplankton

3.2.1. Microalgae

Colonization of microalgae in the system began since the first week; the first two groups to appear were chlorophytes and filamentous algae and both groups remained throughout the experiment. Chlorophytes were the most abundant, with a total of 3022 organisms, followed by filamentous algae and diatoms (Fig. 2).

3.2.2. Amoebas

Fig. 3 shows the results of the abundance of amoeboid groups, where it can be observed that the genus *Arcella* is the most abundance throughout the experimental period, while the abundance *Acanthocystis* and *Actinophrys* was lower than 50 org/mL^{-1}.

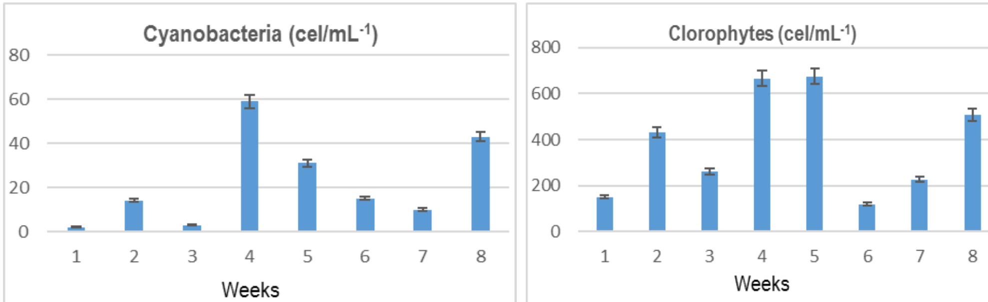

Fig. 2. Groups and total amounts of microalgae found in Biofloc systems during the experiment.

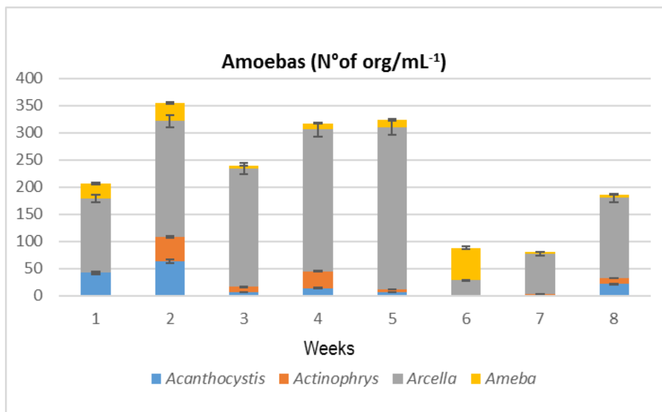

Fig. 3. Abundance of amoebas in the biofloc system.

3.2.3. Ciliates

The diversity and abundance of ciliates found in the system is represented in Fig. 4. The genus *Aspidisca* was the most abundant with 686 organisms during the first two weeks, but the population decreased while the *Vorticellas* group increased.

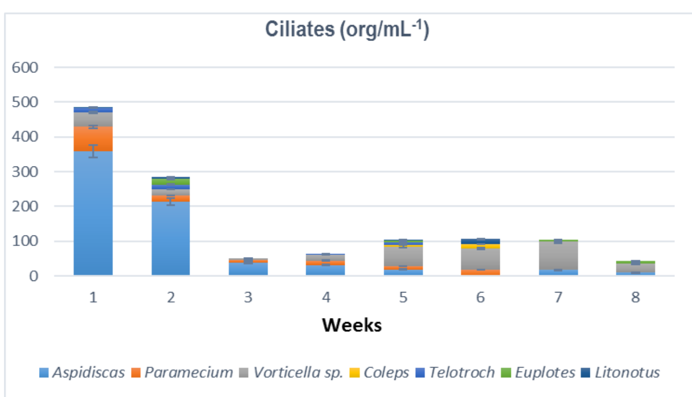

Fig. 4. Ciliate groups and total number of organisms in these groups found in the biofloc system during the experiment.

3.2.4. Rotifers

Fig. 5 shows the abundance and diversity of rotifers identified in the biofloc, where it is observed that although rotifers appeared from the beginning, diversity and abundance of the observed groups increased after the fourth week; most abundant were genera *Lepadella*, *Lecane* and *Philodina* with average values of 70, 40 and 38 organisms/mL^{-1} respectively.

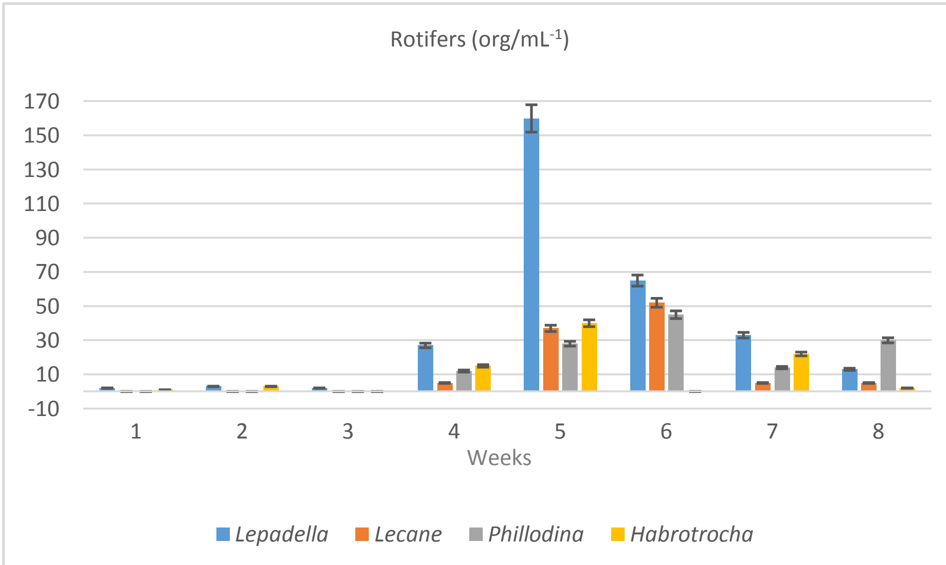

Fig. 5. Groups and total number of rotifers found in the biofloc system during the experiment.

3.2.5. Nematodes

Nematodes were not identified until the sixth week of the experiment, with a total of 16 organisms, however, due to an erroneous manipulation of the system at that time (addition of clean water), the organisms were no longer observed after the eighth week.

Results of this investigation showed variations in the diversity and abundance of the planktonic groups that developed in a biofloc system for the culture of tilapia throughout ten weeks of experimentation. A succession of microbial communities was observed during the system's maturation process. The first week it was possible to identify groups such as cyanobacteria and microalgae; later, groups of ciliated and amoeboid protozoa appeared to finally give way to rotifers and -in the last weeks- nematodes were present. Bentzon et al. (2016), assure that microbial communities in aquatic environments respond quickly to changes in their immediate environment. These changes can be subtle and become evident either by prompting or inactivating certain metabolic pathways, or from the changes they can cause in the community's composition and functionality as happened in this research.

Cyanobacteria (*Anabaena sp*) were found in the pioneer groups; these are frequent when nitrogenous compounds in water increase (Moss, 2001). Due to the fact that in the farming system there was no water exchange, the metabolic waste of fish increased thereby allowing the colonization of cyanobacteria, which, in turn, were responsible for transforming those compounds into assimilable forms for other organisms thus controlling the nitrogen compounds in the system. For this reason, cyanobacteria are considered facilitating species, as they are capable of modifying the environment so that other species can establish and grow (Walker, 2005). Also, by including carbohydrates, lipids and proteins available in the environment to their structures, they are a source of biomass that is available for the next trophic level (Becerril et al., 2017).

Another one of the colonizing groups were chlorophytes identified from the first week of observations until the end of the experiment. Although variations in abundance were observed over time, these variations were caused by the fluctuations of available nutrients such as nitrogen and phosphorus, as well as the rate of consumption of filtering zooplankton organisms present in water (Colina, 2016). Studies such as the one by López (2014) mentions that microalgae play an important role in the development of aquaculture, since they constitute the first live food for the early stages of development of almost all cultivated organisms, being highly nutritious and easy to eat due to their size.

In this research, a total of nine genus of ciliates could be identified, although not all were present throughout the experimental period, so that a variation was observed between the genera identified and the abundance per group throughout the culture. Within the dominant genera are: *Aspidiscas*, *Vorticella* and *Paramecium* that reached maximum abundances of 686, 244 and 138 organisms/mL^{-1} respectively; these values are higher than those reported by Ballester et al. (2010) in a post larva culture of *Farfantepenaeus paulensis* in a biofloc system, where ciliates numbers ranged from 39 to 169 individuals per mL^{-1}. In another study, Monroy et al. (2013) mention that ciliates are species that appear in the early stages of the biofloc, although there may be variations between the genera identified according to the carbon source, the species being cultivated and farming conditions; they report *Paramecium* and *Colpidium* as dominant genera.

Another interesting group is that of amoebas, which are always present in intermediate and mature phases as pointed out by Pérez-Uz et al. (2009) who mention that amoebas are important biological populations for the elimination of nitrogen in advanced systems. They indicate good nitrification rates in these systems, making them suitable candidates to take into account in the development of an efficiency index. In this study, naked and testate amoebae of the genus *Arcella* were the most abundant having up to 1378 org/mL^{-1}.

In Biofloc systems, rotifers are the most conspicuous group being *Lecane* and *Lepadella* genera the most reported (Betancourt et al., 2016; Castro et al., 2016). This group presents a variety of species with different habits and food preferences. Although they are considered filtering organisms, they have different mechanisms for the capture of food particles, so that their abundance and diversity is determined by the quality of available phytoplankton, organic matter and other compounds present in the water. They are intermediate species, and some are found in the climax stage of aquatic environments (Lamper and Somer, 2007).

In relation to nematodes, abundance was very low since they were only observed from the sixth to the ninth week and the maximum abundance reached was 12 organisms/mL^{-1}. It should be noted that this decrease in nematodes, coincided with the erroneous manipulation of the system when clean water was added in the sixth week, and although this happened only once, it could have affected the available nutrients for this group of organisms to remain more time in the system. From studies carried out with biofloc for both fish and shrimp farming, few report the presence of nematodes; apparently, they are mature stage organisms since they require a large amount of available nutrients for the formation of their collagen cuticles (De Lara et al., 2005).

Finally, it is important to note that the planktonic communities developed in biofloc culture systems to feed fish and crustaceans, constitute a dynamic heterotrophic community, fundamental in the transfer of matter and energy from the trophic web at higher levels into aquatic production systems. It is also recognized that "grazing" or rate of consumption, available nutrients and environmental variations, significantly affect the abundance, diversity and ecological succession that occurs in the planktonic populations that develop in production systems such as biofloc.

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