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Zooplankton growth in a Biofloc system with different carbon sources in a *Cyprinus carpio* culture

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Abstract
Yucca, moringa, macroalgae flours and coffee residuals were used to determine zooplankton growth in Biofloc system flocs. Four Rotoplas ® water tanks of 450 L capacity were filled with 300 L and 25 juveniles' stage of *Cyprinus carpio* were introduced with a mean length of 5 cm and mean weight of 2.10±0.44 g. Carps were fed with 5% of total weight of biomass with trout food pellets (45% of protein). The carbon source for each experimental container was supplied at a proportion of 0.001% of the total organism's weight. Each 15 days a sample of 250 mL of Biofloc of each tank were taken and microorganisms were counted in five groups: ciliates and flagellates, nematodes, annelids, and turbellaria form. All carbon sources allowed the establishment of Biofloc system. With moringa and coffee residuals, the nematodes appeared until the seventh sampling (37 org mL^{-1} and 24 org mL^{-1} respectively); with macroalgae and yucca, nematodes appeared in the fifth and third sample, reaching the quantity of 50 org mL^{-1} and 110 org mL^{-1} respectively. With this study, vegetal origin carbon sources can be used with good results.

Keywords: Biofloc, zooplankton, yucca, macroalgae, moringa, coffee residuals

1. Introduction

Biofloc system (BFT) has proved to be a widely used technique in fish and crustaceans culture, also maintains a good water quality in culture, adding an external carbon source together with food [1, 2]. Avimelech [3] mentioned that in Biofloc a conglomerate grows, called bioflocs, compound by bacteria, algae and protozoa that develops in the water column, along with detritus and dead organic particles. Ibekwe *et al*. [4] Emerenciano *et al*. [5] mentioned that bacterial growth community in Biofloc system, will allow a better decomposition of chemical contaminants and therefore a better water quality.

Microorganisms communities that live in Biofloc play an important role. Many studies have shown that using different carbon sources affect the Biofloc composition [6, 7]. Emerenciano *et al*. [5] mention that microorganisms populations that make up Biofloc play an important role in cultured fish and crustaceans nutrition, because they are a natural protein and lipids *in situ* source available during all day and represent a reduction in food costs between 40 to 50% [8]. Studies of Ekasari *et al*. [9, 10], Emerenciano *et al*. [11, 5] and Becerril *et al*. [12] have shown that Biofloc nutritional quality allows that fish and crustaceans in culture have good results, because it has been reported that Biofloc can contain between 25 to 50% of protein and 0.5 to 15% of lipids, nevertheless, protein and lipids values vary according to carbon source used to promote Biofloc and zooplanktonic community that develops in the system [5]. Main organisms that make up Biofloc are bacteria, microalgae, yeast, rotifers, protozoa (ciliates and flagellates), nematodes and crustaceans [13, 14, 15]. Zooplankton consumption that are in Biofloc flocs from fish and crustaceans in culture, have grate benefits like a higher growth rate, increase in feed conversion factor, and decrease in the balanced food consumption cost [8].

In the laboratories of Life Food Production and of Life Food Chemical Analysis of UAM-Xochimilco many studies have been made about microbial, phytoplankton and zooplankton composition that grow in Biofloc with different carbon sources, Monroy *et al*.[13], Monroy *et al* [14] and Becerril *et al* [12] made studies on the diversity of microorganisms in tilapia cultures with Biofloc and the nutritional importance for aquaculture and ecological function of microorganisms that make up Biofloc system; Becerril *et al* [16] made the study of the nutritional composition of produced bioflocs with molasses, coffe waste and rice bran like a carbon source; and Castro *et al*. [17] made the study of the composition of zooplancton and fitoplancton in a Biofloc system using molasses and molasses + rice flour as carbon sources. As well as the studies of new carbon sources of vegetal origin to produce Biofloc and determine the growth of zooplankton in Biofloc flocs, have become relevant and therefore it is the aim of this study.

2. Materials and Methods

2.1 Experimental design

The experiment took place in the Life Food Production laboratory at Universidad Autonoma Metropolitana, Unidad Xochimilco. Four Rotoplas® water tanks of 450 L of capacity were filled with 300 L of water (previously chlorinated) and maintained with continuous and vigorous aeration to help the formation of flocs. Environmental temperature was maintained 23°±2°C for all experiment. In each container were placed 25 juveniles of *Cyprinus carpio* of 5 cm length and average weight of 2.10±0.44 g. Carps were fed with particulate food pellets of 2 mm of diameter with 45% of protein content. Four carbon sources were used: coffee residuals, moringa, macroalgae and yucca flour. Experiment was maintained for six months (Fig. 1).

2.2 Food and carbon source

Carps were fed with 5% of the total weight of the population with particulate trout food (45% of protein) with 2 mm of diameter. The total of daily food was divided in two rations, one in the morning at 9:00 hrs and other at 16:00 hrs. Carbon source for each experimental recipient was supplied in a proportion of 0.0001% of total organisms weight. This was supplied once every day at 9:00 hrs.

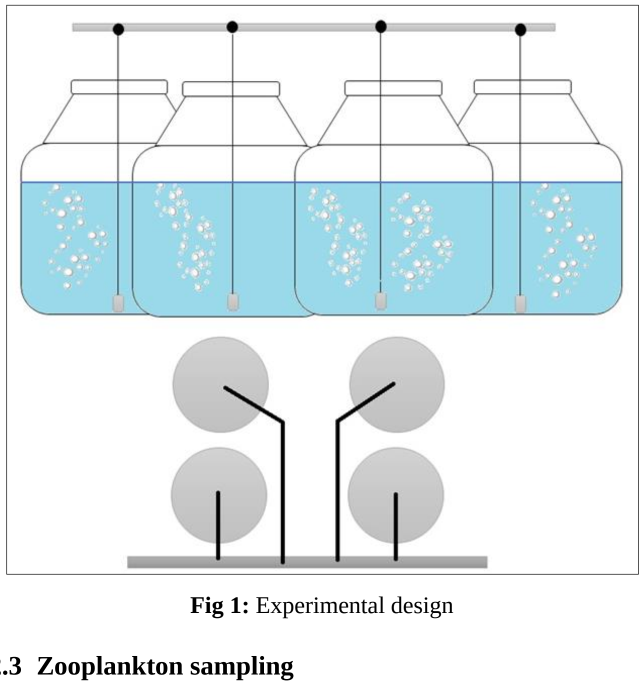

Fig 1: Experimental design

2.3 Zooplankton sampling

Each 15 days a sample of 250 mL of Biofloc from each tank was taken and it was left to be precipitated for 30 minutes, and then, fixed with formalin at 5%. Ten 1 mL samples were taken and with the aim of an optic microscope brand Leica model DM750 connected to an image program (Image® Pro Plus 7.0), microorganisms were counted.

Finally, the taxonomic identification of observed groups was made at genus level with aim of specialized literature of Aladro-Lubel [18].

3. Results

Founded microorganisms in the different carbon sources were divided in five groups: ciliates and flagellates, rotifers, nematodes, annelids and turbellaria form.

In Table 1 depicts the microorganisms that grew in the tank with macroalgae as carbon source. In the group of ciliates and flagellates nine genus were identified, from which *Colpidium* sp. and *Paramecium* sp. were present during all the treatment, reaching a total of 156 and 81 org mL^{-1} respectively. Ciliate *Coleps* sp. was the most abundant with 247 org mL^{-1} followed by flagellate *Paranema* sp. with 176 org mL^{-1}, appearing until the third and fourth sampling respectively. Within the group of rotifers four genera were founded, *Lecane* sp. and *Lepadella* sp. were the most abundant with 86 and 48 org mL^{-1}, even though they were not present in the sixth sampling. *Philodina* sp. and *Bdelloidea* sp. were only found at the beginning and end of the experiment. Nematodes appeared at the fifth sampling and remained until the end reaching a total of 50 org mL^{-1}.

Data of the zooplankton that grew in the treatment with coffee residuals is shown in Table 2. Regarding ciliates and flagellates, 12 genera were identified from which *Paranema* sp, *Coleps* sp. and *Paramecium* sp. reached highest values (253 org mL^{-1}, 212 org mL^{-1} and 180 org mL^{-1} respectively). Genera *Halteria* sp., *Euglena* sp. and *Aspidisca* sp. were founded until the sixth sampling. Within rotifera group *Philodina* sp. and *Lecane* sp. were present from the second sampling until the end of treatment. Genus *Philodina* sp. was the most abundant with 113 org mL^{-1}. Genera *Lepadella* sp. and *Mniobia* sp. appeared in the sixth sampling, but with very low values (7 org mL^{-1} and 6 org mL^{-1} respectively). Nematodes were present until the sixth sampling, reaching the 24 org mL^{-1}. It was observed that genera *Aleosoma* sp. and turbelarie were presented only in two samples each one with two organisms.

In Table 3 depicts the results with the moringa treatment, it was identified 11 genera of ciliates and flagellates. Genera *Paramecium* sp., *Colpidium* sp. and *Paranema* sp. were observed from the beginning until the end of treatment, having a predominance of *Colpidium* sp. reaching levels of 182 org mL^{-1}. Genera like *Acineta* sp. and *Litonotus* sp. were observed only in the fourth sampling, being the minor counts (2 org mL^{-1} and 4 org mL^{-1} respectively). Regarding to rotifers, four genera were identified, from which genus *Lacane* sp. maintained during all the samplings, had values of 100 org mL^{-1}. *Philodina* sp. and *Lapadella* sp. were identified in the second and third sampling respectively, until the end of experiment, reaching 77 org mL^{-1} and 59 org mL^{-1} respectively. Appearance of nematodes and turbelaries were observed until the seventh and eight sampling with 37 org mL^{-1} and 2 org mL^{-1} respectively.

In Yucca treatment, the data is shown in Table 4. Regarding to ciliates and flagellates ten genera were identified, from which it was observed that *Paramecium* sp. and *Paranema* sp. were maintained form the first sampling until the end of experiment. Genus *Paranema* sp. obtained the highest count with 157 org mL^{-1}. Genus *Paramecium* sp. obtained 116 org mL^{-1} and *Colpidium* sp. with 106 org mL^{-1}. Genera *Stylonychia* sp., *Podophyra* sp. and *Acineta* sp. presented the lowest count of the experiment. Within the group of rotifers, five genera were collected, from which genus *Lacane* sp. was present during all the samples reaching a total of 76 org mL^{-1}. Genera *Philodina* sp. and *Lepadella* sp. were observed since the first sampling until the sixth (33 org mL^{-1} and 45 org mL^{-}

[1]). *Mniobia* sp. and *Bdelloidea* sp. were only observed in the fourth sampling with 4 org mL^{-1} and 6 org mL^{-1} respectively. Nematodes appeared in the third sampling and maintained until the end of experiment, reaching a total value of 110 org mL^{-1}.

4. Discussion

Ballester *et al*. [19] and Deng *et al*. [2] mention that microorganisms that grow in Biofloc play an important role in essential nutrients supply for organisms in culture. Previous studies prove that microbial flocs have essential aminoacids, lipids, vitamins and minerals, which can be consider as a food source for organisms in culture [20, 9, 21]. In open environmental cultures, it has been observed that with enough light, the production of phytoplankton is a rich source of food for the zooplanktonic communities and these produce nutrients for bacterial growth and formation of flocs in the Biofloc system. Ekasari *et al*. [9] (2014b), Emerenciano *et al*. [22] Becerril *et al*. [12] mention that the nutritional quality of Biofloc, can go between 25 to 50% protein and up to 15% of fat, depending on the carbon source that is used to produce flocs and that planktonic communities development and can be use as food for the cultured species. The use of other carbon sources, besides molasses, rice flour and coffee residuals, to produce bacterial flocs is important. Mexico has other vegetable products, like moringa, yucca and macroalgae's, that are already produced, and products exists in national market. There are no works that use yucca, moringa and macroalgae flour to produce Biofloc. Martínez-Córdova *et al*. [23] added *Yucca schidigera* to see the changes in the water quality and growth of shrimp, reporting that in the ponds where yucca flour was added the concentrations of ammoniacal nitrogen had the lowest values and with good shrimp growth. On the other hand, Pérez *et al*. [24] mentioned that the presence of ciliate *Paranema* sp. serves as an indicator of a good elimination of nitrogenous compounds in the system, which is related to this study, because the ciliate *Paranema* sp. was present during all experimental period. With yucca flour it was obtained the highest count of nematodes. This group is very important in Biofloc, because they have high contents of raw protein and essential fatty acids in their composition [25] and can be used as food for the species in culture, because Focken *et al*. [26] and Lourerio *et al*. [27] report the presence of nematodes in the stomach content of cultured fish in Biofloc system. Diverse studies have proved that microalgae are efficient to remove the dissolved nitrogenous compounds when are cultured together with shrimp and other fish [28, 29, 30]. In macroalgae species of genus *Gracilaria* sp., it has been documented ammonia removal levels between 0.34 and 3.60 mg in shrimp cultures [31]. Also, with macroalgae flour it was obtained ciliates that indicated a good transformation of nitrogenous compounds, because ciliate *Paranema* sp. was also present during study period. Unlike Yucca flour, in this treatment it was also obtained nematodes, but from the sixth sampling until the end.

Regarding to moringa flour, there are some works that incorporate *Moringa oleifera* in balanced diets for red tilapia culture [32], with good results. Also, moringa is being used to see its potential in treatment of sewage water to improve water quality [33], finding that moringa seed powder (*M. oleifera*) is effective as natural flocculant and coagulant for the treatment of sewage water from the process of benefit of coffee and the chemical process of vegetable peeling. The use of this product to obtain flocs in a Biofloc system has not been studied, in this work it was founded that Biofloc system developed slowly, because until the seventh sampling nematodes were found (25 org mL^{-1}).

Unlike the reported results by Becerril *et al*. [16] regarding the use of coffee residuals as carbon source, it was observed the presence of nematodes in the sum of 24 organisms during the eight made samples. Becerril *et al* [16], mentions that systems based in microorganism's production are more stable that systems based in only phytoplankton production. Wang *et al*. [34], mention that systems based in microorganism play an important role in controlling the system water quality, because these participate in metabolize feces and other waste products from fish and crustaceans, as well as non-consumed food, transforming NH_3 and NO_2 in non-toxic nitrogenous compounds.

An important group in Biofloc are nematodes. Rey *et al*. [35], Monroy *et al*. [13] and Emerenciano *et al*. [5] mention that nematodes are one of the most important group in Biofloc systems and their abundance is determined by the presence of diverse ciliates (*e.g*., *Paramevium* sp. and *Colpidium* sp.) that serve as food. In this work with the use of vegetable flours, nematodes were found since the third sampling with yucca flour, so the fish can feed on these organisms and Lourerio *et al*. [27] suggest that are a rich source of *in situ* life food and available the 24 hrs for fish and crustaceans in culture.

Table 1: Identified zooplankton in macroalgae flour treatment.

Macroalgae **Ciliates and Flagellates**	**Sampling 1**	**Sampling 2**	**Sampling 3**	**Sampling 4**	**Sampling 5**	**Sampling 6**	**Sampling 7**	**Sampling 8**	**Sum**	**Sum per group**
Paramecium	12	18	9	8	6	-	12	16	81	
Stylonychia	2	4	2	1	-	-	-	-	9	
Colpidium	8	23	34	22	6	18	23	22	156	
Vorticella	6	8	4	1	4	-	-	-	23	
Halteria	4	12	4	1	-	-	-	-	21	
Coleps	-	-	24	34	45	54	48	42	247	
Amoeba	2	1	1	-	-	-	-	-	4	
Paranema	-	-	-	34	54	43	33	12	176	
Aspidisca	4	18	16	-	-	-	12	-	50	767
Rotifers										
Philodina	4	-	-	6	-	-	-	8	18	
Lecane	12	24	23	18	9	-	-	-	86	
Lepadella	8	-	18	14	8	-	-	-	48	
Bdelloidea	-	-	-	8	4	-	-	8	20	172
Nematodes	-	-	-	-	8	12	14	16	50	50
Turbelaries	-	-	-	2	2	-	-	-	4	4
Sum	62	108	135	149	146	127	142	124	993	

Table 2: Identified zooplankton in coffee residuals treatment

Coffee										
Ciliates and Flagellates	**Sampling 1**	**Sampling 2**	**Sampling 3**	**Sampling 4**	**Sampling 5**	**Sampling 6**	**Sampling 7**	**Sampling 8**	**Sum**	**Sum per group**
Paramecium	5	17	34	45	31	25	15	8	180	
Colpidium	-	-	-	5	15	18	8	10	56	
Vorticella	-	2	15	24	12	8	5	-	66	
Halteria	-	-	-	-	-	2	3	-	5	
Euglena	-	-	-	-	-	23	32	-	55	
Epystilis	3	12	8	5	-	-	-	-	28	
Coleps	-	-	5	8	48	72	56	23	212	
Anisonema	5	8	13	24	20	-	-	-	70	
Paranema	-	34	56	78	46	32	5	2	253	
Podophyra	-	-	2	1	-	-	-	-	3	
Acineta	-	2	8	4	-	-	-	-	14	
Aspidisca	-	-	-	-	-	12	23	4	39	981
Rotifers										
Philodina	-	8	23	45	21	12	2	2	113	
Lecane	-	2	12	18	8	4	1	2	47	
Lepadella	-	-	-	-	-	4	2	1	7	
Mniobia	-	-	-	-	-	-	4	2	6	173
Nematodes	-	-	-	-	-	4	8	12	24	24
Annelids										
Aelosoma	-	-	-	-	-	-	1	1	2	2
Turbelaries	-	-	-	-	-	-	1	1	2	2
Sum	13	85	176	257	201	216	166	68	1182	

Table 3: Identified zooplankton in moringa flour treatment

Moringa										
Ciliates and Flagellates	**Sampling 1**	**Sampling 2**	**Sampling 3**	**Sampling 4**	**Sampling 5**	**Sampling 6**	**Sampling 7**	**Sampling 8**	**Sum**	**Sum per group**
Paramecium	4	23	34	22	12	8	6	4	113	
Stylonychia	4	-	4	2	6	5	2	-	23	
Colpidium	8	34	40	23	34	23	12	8	182	
Vorticella	2	8	12	18	8	4	-	-	52	
Halteria	-	-	-	-	2	1	-	-	3	
Coleps	-	4	23	28	32	22	8	6	123	
Anisonema	-	-	-	-	2	4	-	-	6	
Paranema	18	32	28	34	22	12	8	4	158	
Podophyra	-	1	8	6	-	-	-	-	15	
Acineta	-	-	-	2	-	-	-	-	2	
Litonotus	-	-	-	4	-	-	-	-	4	681
Rotifers										
Philodina	-	14	9	23	18	8	4	1	77	
Keratella	-	-	-	2	4	1	-	-	7	
Lecane	12	8	14	23	17	12	8	6	100	
Lepadella	-	-	5	15	23	8	6	2	59	243
Nematodes	-	-	-	-	-	-	12	25	37	37
Turbelaries	-	-	-	-	-	-	-	2	2	2
Sum	48	124	177	202	180	108	66	58	963	

Table 4: Identified zooplankton in yucca flour treatment

YUCCA										
Ciliates and Flagellates	**Sampling 1**	**Sampling 2**	**Sampling 3**	**Sampling 4**	**Sampling 5**	**Sampling 6**	**Sampling 7**	**Sampling 8**	**Sum**	**Sum per group**
Paramecium	6	12	23	34	18	9	8	6	116	
Stylonychia	4	-	-	-	-	-	-	-	4	
Colpidium	0	8	34	23	12	6	12	11	106	
Vorticella	2	8	6	4	8	4	-	-	32	
Halteria	4	-	-	-	8	3	2	-	17	
Epystilis	0	12	6	4	2	-	-	-	24	
Coleps	0	8	12	18	34	12	-	-	84	
Paranema	8	8	10	16	43	32	18	22	157	
Podophyra	2	1	-	-	-	-	-	-	3	
Acineta	4	-	-	-	-	-	-	-	4	547
Rotifers										
Philodina	4	8	7	6	4	4	-	-	33	
Lecane	8	4	14	16	8	6	12	8	76	
Lepadella	4	9	12	14	4	2	-	-	45	
Mniobia	-	-	-	4	-	-	-	-	4	
Bdelloidea	-	-	-	6	-	-	-	-	6	164
Nematodes	-	-	8	12	23	18	23	26	110	110
Turbelaries	-	4	-	-	2	1	-	-	7	7
Sum	46	82	132	157	166	97	75	73	828	

5. Conclusions

Testing other carbon sources like Yucca, moringa and macroalgae flours, for flocs formation in Biofloc system, is becoming relevant; since we can count on alternate carbon sources that allow the growth of microorganisms which can control nitrogenous components in Biofloc system and avoid continuous water change and provide a food source available the 24 hours for fish and crustaceans in culture.

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