Iranian Journal of Fisheries Sciences 21(6) 1508-1526 2022
DOI: 10.22092/ijfs.2023.128417

Research Article

Environmental sustainability assessment of Nile tilapia (*Oreochromis niloticus*) breeding in biofloc system

Kazemi C.[1]; Bemani A.[1*]; Alizadeh M.[2]; Siyahati G.[1]; Ardakani T.[1]

Received: May 2022 Accepted: October 2022

Abstract

Sustainable development aquaculture industry entails the expansion of environmentally friendly systems. The aim of this study was to evaluate the environmental aspects of biofloc technology system (BFT) in Nile Tilapia (*Oreochromis niloticus*) culture. This research was performed on two systems including, Biofloc and flow-through system (FTS). Each system had three replications and carried-out for six months and sampled at intervals of 15 days. The density in each 30m^3 pond was 1500 juveniles of Tilapia (25 g b/w each). The studied parameters include fish weight, oxygen, temperature, ammonium, nitrite, pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) of effluent, feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), survival rate, and cost of culture. The results indicated that temperature, oxygen, nitrite, and ammonium were significant different (p<0.05) and lowest level of NO3(0.004mg/L), NH4 (0.002 mg/L) observed in BFT. There were significantly different between growth indices and all physiochemical factors except for pH, during the time. Effluent factors include BOD, COD and TDS in BFT were significantly lower than FTS (p<0.05). The quality of the effluent in BFT contained only 5%of the food with the effluent and 25% of the food reused in the food chain, while in flow-through, 25-30% of food discharged along with FTS. The water exchange rate in BFT was 1.5m^3/day(5%/day) and in flow-through was 30m^3/day (0.3 L/s). The cost of feeding in BFT was 25% lower than FTS system. Therefore, the results revealed that BFT, in addition to water quality improvement, leads to less feed consumption, higher quality production, and less environmental pollution.

Keywords: Sustainable Aquaculture; BFT; Flow through, Environmental Aspects, Pollution, Resource.

1-Department of Environmental Sciences and Engineering, Faculty of Agriculture and Natural Resources, Ardakan University, Ardakan, Iran.
2-Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension Organization, Tehran, Iran.
[*]Corresponding author's Email: a.bemani@ardakan.ac.ir

Introduction

The development of the aquaculture industry has led to environmental pollution in recent years, and therefore it is absolutely necessary to pay attention to the management and the type of farming system that is environmentally friendly. Lack of water resources and environmental pollution caused by the discharge of various effluents (agricultural, industrial, urban, etc.) are important factors hindering the development of aquaculture industry. The use of new technologies such as intensive aquaculture in recirculating systems has played a significant role in increasing their production efficiency (El-Sayed, 2021; Mugwanya *et al*., 2021; Shourbela *et al*., 2021). By using these systems, the daily water exchange rate has been reduced to almost 1% of the volume of water in the breeding ponds (Twarowska *et al*., 1997; Santhana Kumar *et al*., 2018; Dauda, 2020; Khanjani *et al*., 2021). Today, attention to recirculating aquaculture systems is increasing due to greater biological security and environmental benefits (Shi *et al*., 2017; Legarda *et al*., 2019; Liu *et al*., 2021; Ogello *et al*. 2021). When water is recycled in the recirculating aquaculture breeding systems, few risks like pathogens and foreign species entry into the breeding system, and the issues related to wastewater discharge that causes environmental contamination is minimized (Ray *et al*., 2012). In recent years, the development of the aquaculture industry has led to environmental pollution, and therefore it is necessary to consider the management and the type of breeding system that is environmentally friendly. The waste generations in large volumes, the use of flour and fish oil as feedstock are other unstable components in aquaculture. One of the sustainable aquaculture development aims is the production of higher fish quantum without a significant increase in using natural resources of water and land (Avnimelech, 2007).

Another goal of sustainable aquaculture development is the development of environmentally friendly systems. Amongst the major issues that are widely affecting the aquaculture industry are the impact of farm effluents, high dependence on fish flour as well as fish oil as feed supply, and the disease outbreak (Gao *et al*., 2012). Ammonium is not only one of the end products of protein metabolism, but also the main excretion substance of aquatic animals in an aquaculture system. Nonetheless, ammonia and ammonium are both toxic for aquatic species, but non-ionized ammonia has higher toxicity concerning ammonium ions. It should be noted that the toxicity threshold is highly dependent on the intensity, size and type of species, particle size and organic matter resistance, the activity level of compounds, metals, nitrate, salinity, and pH.

Today, attention to Recirculating Aquaculture Systems (RAS) is increasing due to greater biological security and environmental benefits. When water is reused in recirculating aquaculture systems on a cyclical basis, there are some risks, such as the entry of pathogens and foreign species into the breeding system, and problems with wastewater discharge that cause pollution (Ray *et al*., 2012). Biofloc technology system (BFT) is a newly identified technique to solve the mentioned problems. BFT is characterized as ‘‘the utilize of aggregates of bacteria, algae or protozoa, held together in a matrix along with particulate organic matter to improve water quality, waste treatment and disease prevention in intensive aquaculture systems (Santhana Kumar *et al*, 2018; Khanjani *et al*., 2021). Therefore, the development of a sustainable aquaculture industry entails the expansion of systems that provide a cost/benefit ratio for community economic support and production sustainability (Avnimelech, 2009; Shi *et al*., 2017; Mugwanya *et al*., 2021).

This goal will be achieved through the development of more efficient feeding programs, the use of inexpensive production systems, and the utilization of high-efficiency production technologies that require less energy, and when investment costs are controlled and managed. In addition to the three mentioned, aquaculturists must meet market demands to produce high-quality, healthy, attractive products and socially acceptable products. The use of new and appropriate techniques and technologies such as BFT in the reproduction of fish and shrimp is of great importance that can pursue important goals of sustainable aquaculture.

BFT is an environmentally-friendly aquaculture system that reuses organic and recycles nutrients for production. A sustainable approach to such a system is based on the growth of microorganisms in the ship environment that has the least water exchange. This technology has important advantages such as minimizing water consumption and recycling of nutrients and organic matter, and in addition, reduces the entry of pathogens into the breeding system and leads to improved biological safety on the farm (Avnimelech, 2007). Moreover, large-scale production in aquaculture biofloc systems can have environmental benefits in marine and coastal ecosystems, and by replacing soybeans or fishmeal with biofloc compounds in aquatic nutrition, aquaculture wastewater and its environmental effects can be controlled. Using BFT, the levels of mycotoxins and anti-nutritional factors in the aquatic feed are limited and the use and need for feed, which is costly, is generally reduced (Suárez-Puerto *et al*., 2021). Reduction of Feed Conversion Ratio (FCR) and improvement of Growth Rate (GR) in shrimp and even fish have been reported using the biofloc breeding system (Wasielesky *et al*., 2006; Jamal *et al*., 2020, Aghabarari *et al*., 2021).

Considering the generalities that were stated about the biofloc system and the main purpose of this study, which is to evaluate the environmental aspects of the biofloc system in Nile Tilapia breeding, the environmental effects are estimated by the scenario of the presence or absence of the biofloc system. The efficiency of this system is also determined in terms of environmental compatibility.

Materials and methods

This research is performed on two systems include Biofloc system (without water exchange) and Flow-through system (with water exchange) (factor a) in Nile Tilapia (*Oreochromis niloticus*) culture, which have two treatments and three replications that are sampled for 6 months at intervals of 15 days (factor b) in Inland Salt Water Fishery Research Station, Bafgh, Iran. In two systems of biofloc and flow-through, three 30m^3 ponds were studied in greenhouse environment wherein density in each pond was 1500 juveniles (25 g b/w each). The principal approach of bofloc system is to culture suitable microorganisms along with Nile Tilapia to produce a sustainable system, benefited by the minimum or zero water exchange. In Flow-through system water exchanged for maintaining appropriate water quality to produce Tilapia and relied on a water flow for collecting and removing metabolic wastes. The water exchange rate in biofloc system was 1.5 m^3 per day (i.e. 5% per day), whereas in the flow-through system was 30 m^3/day (i.e., 0.3 l/s). In biofloc system, by producing biofloc bacteria in breeding ponds and creating bacterial biomass, wastes are reabsorbed or removed (Crab *et al*., 2012, 2009; Hargreaves, 2013; Abdi Rad and Qaednia, 2015). This system was equipped with aeration system.

The studied parameters include fish weight, oxygen amount, temperature, ammonium, nitrite, acidity, BOD, COD, TDS of effluent, Feed Conversion Ratio (FCR). Specific Growth Rate (SGR), Protein Efficiency Ratio (PER), Survival rate, and cost of Nile Tilapia (*Oreochromis niloticus*) culture:

BOD measurement requires taking two samples at each site. One is tested immediately for dissolved oxygen, and the second is incubated in the dark at 20°C for 5 days and then tested for the amount of dissolved oxygen remaining. The difference in oxygen levels between the first test and the second test, in milligrams per liter (mg/L), is the amount of BOD. This represents the amount of oxygen consumed by microorganisms to break down the organic matter present in the sample bottle during the incubation period. Because of the 5-day incubation. COD was determined using Hach tubes and a method based upon the reduction of (orange) potassium dichromate to (green) chromium salts at high temperature, followed by absorbance measurement using a dedicated colorimeter. Total Dissolved Solids (TDS) is a measure of the combined inorganic and organic substances dissolved in water. It was measured by using TDS meter (AQUA LYTIC, AL450T-IR model). For the current state evaluation of biofloc system, the data related to energy flows and required materials are as inputs. Manufactured products, by-products, besides effluences, refuses, and wastes discharged into the environment are as output. They were collected for all the considered processes. Data collection was performed in two parts, including the use of organizational information data and field sampling. To collect the data required in this project, some samplings from input and output flows were fulfilled to determine the exact amount of effective elements through measurements. To determine the effects of the biofloc system, this system was compared with the water exchange system (100%), in terms of energy consumption, materials, product quality, and discharged effluents from the breeding system. In addition, their positive influences, as well as negative impacts, were compared. The effects of these scenarios and the interaction of these systems on the environment were assessed by SAS statistical software version 9.1 (Fig. 1).

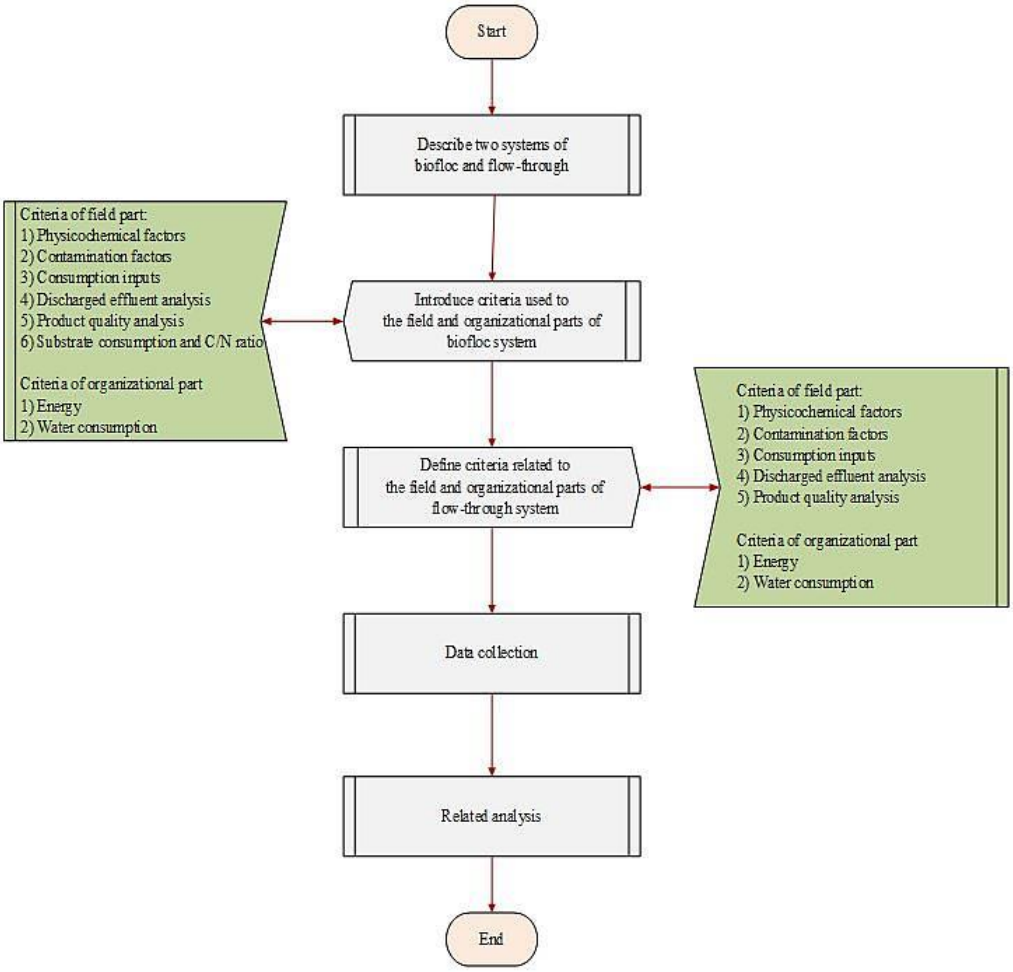

Figure 1: The flowchart of the research.

Criteria

The criteria used in the flow-through and biofloc systems based on two parts of field and organizational are given in Table 1. The framework of assessment criteria in this study is shown in Figure 2.

Table 1: Description of flow-through and biofloc systems based on two parts of field and organizational and criteria for the study.

Systems	Criteria of the organizational part	Criteria of the field part
Biofloc system	1) Energy; 2) Water consumption;	1) Physicochemical parameters including temperature, oxygen, acidity, nitrite, ammonium, and weight; 2) Contamination factors; 3) Consumption inputs; 4) Discharge effluent analysis; 5) Product quality analysis; 6) Substrate consumption and C/N ratio;
Flow-through system	1) Energy; 2) Water consumption;	1) Physicochemical parameters including temperature, oxygen, acidity, nitrite, ammonium, and weight; 2) Contamination factors; 3) Consumption inputs; 4) Discharge effluent analysis; 5) Product quality analysis;

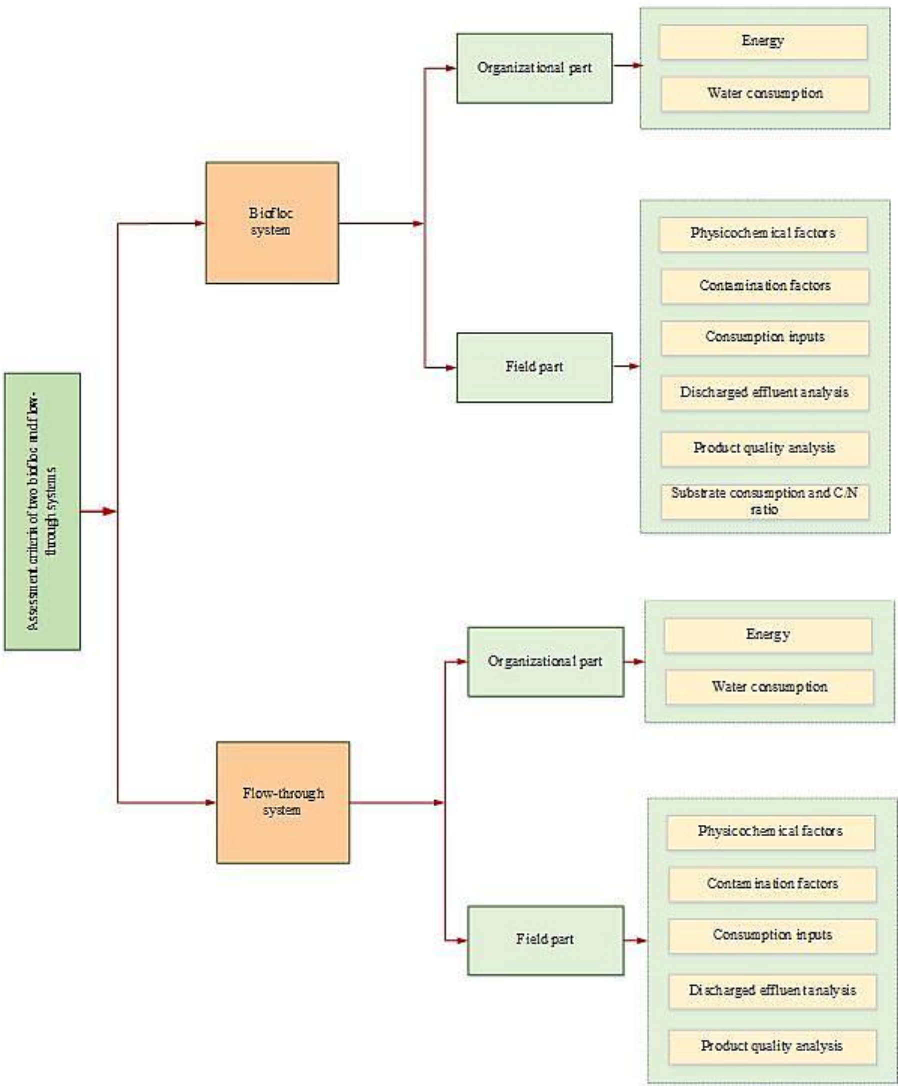

Figure 2: The framework of assessment criteria in this research.

Results

Table 2 shows the results of analysis of variance for water physicochemical parameters assessment in Nile tilapia breeding in flow-through and biofloc systems. The factors of temperature, oxygen, nitrite, and ammonium were significantly different (p<0.01) between two systems during the study period.

Table 2: Analysis of variance for water physicochemical parameters assessment in Nile tilapia breeding in flow-through and biofloc systems.

		Mean Square (MS)					
Source Of Variation (S.O.V.)	Degrees of Freedom (df)	Temperature	Oxygen	pH	Nitrite	Ammonium	Weight
Factor (a)	1	72.80**	2.80**	6.72**	0.03**	28.75**	40755.12**
Factor (b)	11	5.21**	0.16 ^{ns}	0.06*	0.00**	00.91**	323327.94**
Interaction (ab)	11	0.99**	0.23**	0.04 ^{ns}	0.00**	0.96**	1222.61 ^{ns}
Error	48	8.94	0.08	0.02	0.00	0.00	941.43
C.V.%		1.46	5.48	2.27	54.37	8.07	10.04

**, *, and ^{ns} indicate significance levels at 1%, 5%, and non-significant differences, respectively.
Coefficient of Variation (C.V.) signifies model efficiency: below 10 excellent and between 10-20 good.

According to Table 3, mean temperature (30.53°C) and weight (317.33) in the biofloc system is higher than in the flow-through system. The oxygen parameter in the flow-through (5.61 mg/L) was higher than the biofloc (5.21 mg/L) due to water exchange. In the biofloc system, pH (7.11), nitrite (0.006) and ammonium (0.034) is lower than the flow-through system.

Table 3: Mean comparison of water physicochemical parameters assessment in Nile tilapia breeding in flow-through and biofloc systems.

System	Temperature (°C)	Oxygen (mg/L)	pH	Nitrite (mg/L)	Ammonium (mg/L)	Weight of fish (gr)
Flow-through	28.52^{b}	5.61^{a}	7.72^{a}	0.052^{a}	1.29^{a}	296.25^{b}
Biofloc	30.53^{a}	5.21^{b}	7.11^{b}	0.00^{b}	0.034^{b}	317.33^{a}

^{ab} Means with common superscripts do not differ (p<0.05).

Results of mean comparison of interaction of systems and study period (Table 4) indicated that there was significant difference (p<0.05) between physiochemical factors in the biofloc and flow-through system during the time, except nitrite and ammonium which were not significantly differences (p<0.05) in biofloc system during the study period. Highest level of temperature and weight as well as lowest level of nitrite, ammonium, oxygen and pH observed in biofloc system during the time.

Table 4: Mean comparison of interaction of factor a and b.

Treatments	Category	Temperature (°C)	Oxygen (mg/L)	pH	Nitrite (mg/L)	Ammonium (mg/L)	Weight (gr)
Flow-through	22.05.2019	27.30^{i}	5.43^{bcd}	7.83^{ab}	0.01^{cd}	0.26^{e}	24.33^{j}
	05.06.2019	27.50^{i}	5.53^{abcd}	7.56^{bc}	0.05^{ab}	0.24^{e}	24.00^{j}
	22.06.2019	29.16^{h}	5.56^{abcd}	7.76^{ab}	0.02^{cd}	0.55^{d}	53.67^{ij}
	06.07.2019	29.00^{h}	5.50^{abcd}	7.73^{abc}	0.02^{cd}	0.55^{d}	55.67^{ij}
	23.07.2019	30.20^{def}	5.43^{bcd}	7.76^{ab}	0.03^{bc}	0.89^{C}	153.33^{h}
	06.08.2019	30.00^{defg}	5.46^{abcd}	7.73^{abc}	0.03^{bc}	0.90^{C}	156.33^{h}
	23.08.2019	29.30^{gh}	5.53^{abcd}	7.73^{abc}	0.06^{ab}	1.80^{b}	315.00^{g}
	06.09.2019	29.53^{fgh}	5.63^{abc}	7.66^{abc}	0.06^{ab}	1.80^{b}	316.00^{g}
	23.09.2019	28.06^{i}	5.80^{ab}	7.83^{ab}	0.07^{a}	2.14^{a}	451.33^{e}
	07.10.2019	27.33^{i}	5.53^{abcd}	7.43^{cd}	0.07^{a}	2.10^{a}	493.33^{de}
	23.10.2019	27.36^{i}	5.90^{ab}	7.76^{ab}	0.07^{a}	2.13^{a}	593.33^{b}
	06.11.2019	27.50^{i}	6.03^{a}	7.90^{a}	0.07^{a}	2.14^{a}	600.67^{b}
Biofloc	22.05.2019	30.70^{de}	5.63^{abc}	7.10^{ef}	0.00^{d}	0.02^{f}	25.00^{j}
	05.06.2019	30.46^{dee}	5.80^{ab}	7.23^{de}	0.00^{d}	0.02^{f}	56.33^{ij}
	22.06.2019	31.50^{ab}	5.00^{de}	7.16^{def}	0.00^{d}	0.02^{f}	66.33^{ij}
	06.07.2019	31.83^{a}	5.10^{cde}	7.20^{de}	0.00^{d}	0.02^{f}	67.67^{ij}
	23.07.2019	31.03^{be}	5.16^{cde}	7.56^{ef}	0.00^{d}	0.02^{f}	185.00^{h}
	06.08.2019	31.90^{a}	5.2^{cd}	7.16^{def}	0.00^{d}	0.02^{f}	186.67^{h}
	23.08.2019	30.36^{dee}	5.2^{cd}	7.03^{ef}	0.00^{d}	0.02^{f}	372.33^{f}
	06.09.2019	30.46^{dee}	5.4^{bcd}	7.43^{de}	0.00^{d}	0.02^{f}	376.33^{f}
	23.09.2019	29.76^{efgh}	4.63^{e}	7.06^{ef}	0.00^{d}	0.02^{f}	529.00^{de}
	07.10.2019	29.70^{efgh}	5.00^{de}	6.86^{f}	0.00^{d}	0.02^{f}	559.00^{be}
	23.10.2019	29.26^{gh}	5.1^{cde}	6.96^{ef}	0.00^{d}	0.02^{f}	659.00^{a}
	06.11.2019	29.40^{fgh}	5.4^{bcd}	7.20^{de}	0.00^{d}	0.02^{f}	679.33^{a}

Means with similar letters in each column do not have significance difference at 5%, based on Duncan. test.

As observed in the Table 5, there was no significant difference (p<0.05) in pH levels during the study period (different time as factor b). As expected, nitrite and ammonium increased during the study period, which were controlled optimally at standard levels through feeding and aeration control.

Table 5: Mean comparison of water physicochemical parameters in different time as factor b.

	Treatments	Temperature (°C)	Oxygen (mg/L)	pH	Nitrite (mg/L)	Ammonium (mg/L)	Weight (gr)
1	22.05.2019	29.00^{e}	5.53^{abc}	7.46^{a}	0.01^{c}	0.14^{e}	24.67^{f}
2	05.06.2019	28.98^{e}	5.66^{ab}	7.40^{a}	0.03^{ab}	0.13^{e}	63.17^{f}
3	22.06.2019	30.33^{bcd}	5.28^{bc}	7.46^{a}	0.01^{bc}	0.32^{d}	60.00^{f}
4	06.07.2019	30.41^{bc}	5.30^{bc}	7.46^{a}	0.01^{bc}	0.32^{d}	61.67^{f}
5	23.07.2019	30.61^{ab}	5.30^{bc}	7.36^{a}	0.02^{abc}	0.46^{c}	169.17^{e}
6	06.08.2019	30.95^{a}	5.33^{abc}	7.45^{a}	0.02^{abc}	0.46^{c}	171,50^{e}
7	23.08.2019	29.83^{d}	5.36^{abc}	7.38^{a}	0.03^{a}	0.91^{b}	343.67^{d}
8	06.09.2019	30.00^{cd}	5.51^{abc}	7.55^{a}	0.03^{a}	0.91^{b}	346.17^{d}
9	23.09.2019	28.91^{e}	5.21^{c}	7.45^{a}	0.04^{a}	1.08^{a}	490.17^{c}
10	07.10.2019	28.51^{ef}	5.26^{bc}	7.15^{b}	0.04^{a}	1.06^{a}	526.17^{b}
11	23.10.2019	28.31^{f}	5.50^{abc}	7.36^{a}	0.04^{a}	1.07^{a}	626.17^{a}
12	06.11.2019	28.45^{ef}	5.71^{a}	7.55^{a}	0.04^{a}	1.08^{a}	640.00^{a}

Different superscripts indicate significant difference at p<0.05, where a > b > c.

The results of growth indices such as Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), and survival rate were compared, which are shown in Table 6. growth indices were significantly differences. SGR, PER and survival rate in biofloc system were higher than flow through system.

Table 6: Results of mean comparison of growth indices in Nile tilapia in flow-through and biofloc systems.

Treatments		Growth index			
		SGR	FCR	PER	Survival rate
Flow-through system	Mean	1.78 [ns]	1.33**	2.46**	95.76**
	Std. Deviation	0.04	0.01	0.15	0.37
	t	1.86	039.5	14.29	3.36
	df	4.00	4.00	3.99	4.00
	p value	0.13	0.00	0.00	0.00
Biofloc system	Mean	1.83 [ns]	0.93**	4.26**	96.40**
	Std. Deviation	0.02	0.01	0.15	0.32
	t	1.86	-29.5	14.29	3.36
	df	3.18	3.93	4.00	3.20
	p value	0.15	0.00	0.00	0.01

**. * and [ns] indicate significance levels at 1%, 5%, and non-significant differences, respectively.

Considering the results of analysis of varience of effluent factors (Table 7), there were not significantly differences between BOD, COD and TDS of the systems (p<0.01) during the study period.

Table 7: Analysis of variance of BOD, COD, and TDS of effluents obtained from Nile tilapia breeding in flow-through and biofloc systems.

Source Of Variation (S.O.V.)	Degrees of Freedom (df)	Mean Square (MS)		
		BOD	COD	TDS
Factor (a)	1	144.44**	144.41**	6.72**
Factor (b)	11	0.68 [ns]	0.62[ns]	0.06 [ns]
Interaction (ab)	11	0.05 [ns]	0.05[ns]	0.04 [ns]
Error	48	0.36	0.36	0.02
C.V.%		12.00	8.88	10.85

**. * and [ns] indicate significance levels at 1%, 5%, and non-significant differences, respectively.
Coefficient of Variation (C.V.) signifies model efficiency: below 10 excellent and between 10-20 good.

According to Table 8, the effluent factors include BOD, COD and TDS in the biofloc system was significantly lower than the flow-through system (p<0.05).

Table 8: Mean comparison of Factor a.

System	BOD (mg/L)	COD (mg/L)	TDS (mg/L)
Flow-through	6.45[b]	8.21[b]	496.29[b]
Biofloc	3.61[a]	5.38[a]	411.35[a]

ab Means with common superscripts do not differ (p<0.05).

According to Table 9, in each system separately, the means were not significantly different ($p<0.05$), especially in the biofloc system during the breeding period. It was observed that with increasing TDS during the breeding period, the amount of BOD and COD was also increased, which shows the relationship between the indices. The lowest value of BOD, COD, and TDS was observed in bioflocsystem.

Table 9: Mean comparison of interaction of two factors a and b.

Treatments row for two examined systems	Date	BOD (mg/L)	COD (mg/L)	TDS (mg/L)
Flow-through	22.05.2019	5.56^{b}	7.40^{b}	471.33^{abcde}
	05.06.2019	6.11^{ab}	7.87^{ab}	480.76^{abcde}
	22.06.2019	6.30^{ab}	8.06^{ab}	485.47^{abcde}
	06.07.2019	6.57^{ab}	8.33^{ab}	492.54^{abcd}
	23.07.2019	5.86^{ab}	7.62^{ab}	494.90^{abcd}
	06.08.2019	6.49^{ab}	8.25^{ab}	497.26^{abc}
	23.08.2019	6.76^{a}	8.52^{ab}	500.08^{abc}
	06.09.2019	6.38^{ab}	8.14^{ab}	501.50^{ab}
	23.09.2019	6.93^{a}	8.69^{a}	503.38^{ab}
	07.10.2019	6.90^{a}	8.66^{a}	504.80^{ab}
	23.10.2019	6.81^{a}	8.57^{a}	506.21^{ab}
	06.11.2019	6.70^{ab}	8.46^{ab}	517.29^{a}
Biofloc	22.05.2019	3.10^{c}	4.94^{c}	390.67^{e}
	05.06.2019	3.41^{c}	5.17^{c}	398.48^{de}
	22.06.2019	$3{,}56^{c}$	5.32^{c}	402.39^{cde}
	06.07.2019	3.60^{c}	5.56^{c}	408.25^{bcde}
	23.07.2019	3.67^{c}	5.63^{c}	410.20^{bcde}
	06.08.2019	3.72^{c}	5.68^{c}	412.15^{bcde}
	23.08.2019	3.78^{c}	5.68^{c}	414.50^{bcde}
	06.09.2019	3.82^{c}	5.70^{c}	415.67^{bcde}
	23.09.2019	3.86^{c}	5.72^{c}	417.22^{bcde}
	07.10.2019	3.88^{c}	5.79^{c}	418.40^{bcde}
	23.10.2019	3.94^{c}	5.85^{c}	419.57^{bcde}
	06.11.2019	3.98^{c}	5.88^{c}	428.76^{bcade}

means having similar letters in each column do not have a significant difference at 5%, based on the Duncan test.

As observed in the Table 10, there was no significant difference ($p<0.05$) in TDS level during the study period while BOD and COD levels were significantly differences during the study period.

Table 10: Mean comparison of Factor b.

Treatments	Treatments	BOD (mg/L)	COD (mg/L)	TDS (mg/L)
1	22.05.2019	4.33^{c}	6.17^{c}	431.00^{a}
2	05.06.2019	4.76^{abc}	6.52^{abc}	439.62^{a}
3	22.06.2019	4.93^{cab}	6.69^{abc}	443.93^{a}
4	06.07.2019	5.18^{ab}	6.94^{abc}	450.40^{a}
5	23.07.2019	4.56^{bc}	6.33^{bc}	452.55^{a}
6	06.08.2019	5.05^{abc}	6.82^{abc}	454.71^{a}
7	23.08.2019	5.29^{ab}	7.05^{ab}	457.29^{a}
8	06.09.2019	4.96^{abc}	6.72^{abc}	458.58^{a}

Table 10 continued:

9	23.09.2019	5.40[a]	7.16[a]	460.30[a]
10	07.10.2019	5.36[ab]	7.12[ab]	461.60[a]
11	23.10.2019	5.30[ab]	7.06[ab]	462.89[a]
12	06.11.2019	5.24[ab]	7.00[ab]	473.02[a]

Medians possessing similar letters in each column do not have a significant difference at 5%, based on the Duncan test.

Discussion

The expansion of aquaculture has faced many problems in commercial aquaculture due to the limitation of suitable lands as well as the high dependence on flour and fish oil as important constituents of aquaculture feed. Excessive use of water resources leads to water scarcity, saline infiltration, and other hydrological changes. In recent years, the development of the aquaculture industry has led to environmental pollution, and therefore it is necessary to consider the management and the type of breeding system that is environmentally friendly.

It must be noted that tilapia possesses characteristics that are compatible with this system, and in the present study it was demonstrated that water biochemical conditions as well as generative flocs quality, avails breeding feasibility of this species with biofloc system. Nevertheless, one of the most important factors that should be considered in biofloc systems like other intensive and super-intensive systems are oxygen, temperature, and nitrogenous compounds present in the water (Tierney and Ray, 2018; David *et al*. 2021; Khanjani *et al*. 2022a).

In the present research, Dissolved Oxygen (DO) and pH levels in biofloc treatments was lower in relation to flow-through treatments, which was probably due to the presence of heterotrophic consortia and increased respiration rate besides the addition of carbonaceous organic matter that causes reduced oxygen and increased carbon dioxide in biofloc tanks (Wasielesky *et al*., 2006; Jamal *et al*. 2020; de Lima Vieira *et al*. 2021). Studies have reported that the addition of carbonaceous organic matter to breeding reservoirs results in a temporary reduction of dissolved oxygen levels (De Schryver and Verstraete, 2009; Addo *et al*. 2021; Mohammadi *et al*. 2021). In 2001, a study on shrimp breeding systems without water exchange system reported that respiration of microorganisms presents in the system accounted for up to 60% of oxygen usage confirmed increased respiration rate in biofloc system. Similarly, Azim *et al*. (2008) carried out a study on variable oxygen levels in biofloc systems and reported that it fluctuated at 7, which does not have a significant difference with relation to the present study results whereby it fluctuated from 4.6 - 6. Since the size and density of flocs in biofloc system are temperature-dependent and excessive temperature increase causes excessive growth of flocs and this excess growth and density could lead to increased turbidity and reduced oxygen, so, the best temperature for biofloc system in tilapia breeding is 28-30°C (Ogello *et al*., 2021), wherein in the present study, the temperature was kept constant between 29 and 31°C. Nevertheless, an increased temperature of up to 32°C in tilapia has been reported in this system.

In biofloc system, conversion of toxic nitrogenous compounds is much more efficient because this process takes place via heterotrophic bacteria that are mainly in association with the Bacillus and Pseudomonas genus (Tierney and Ray, 2018; Abakari *et al*., 2021; Addo *et al*. 2021).Heterotrophic bacteria remove organic carbonaceous and waste nitrogenous matter from the water and use it to produce microbial protein, thereby reducing inorganic nitrogen concentration in the water (Jiménez-Ojeda *et al*. 2018; Tierney and Ray, 2018; Liu *et al*. 2022). Widanarni *et al*. (2012) in another study on biofloc systems evaluated the relationship between nitrite, nitrate, Total Ammonia Nitrogen (TAN), and suspended solids rate. They reported that increased suspended solids rate reduced the concentration of toxic nitrogenous substances.

In the present study, nitrite in treatments exhibited a significant difference. However, it should be noted that in treatment biofloc, wherein water exchange rate is low, due to control of carbon source and stimulation of heterotrophic bacteria present in the system, had a better performance in keeping nitrite content low. Gaona *et al*. (2016) revealed that with an increase in suspended solids concentration in experimental treatments, ammonia level did not show a significant difference, which is similar to present study findings where with increasing biofloc percent in the breeding system, ammonia level did not exhibit a significant difference ($p<0.05$). In this study, the highest levels of ammonia (2.14 mg/L) and nitrite (0.07 mg/L) were obtained in flow-through treatment and the lowest levels of ammonia (0.020 mg/L) and nitrite (0.004 mg/L) were reported in biofloc treatment respectively. Krummenauer *et al*. (2014) investigated the effect of water enriched with different biofloc ratios on water quality parameters. They demonstrated that the total ammonia nitrogen concentrations were 0.52, 0.09, 0.08, 0.11 and 0.04 mg/L, nitrite concentrations were 10.11, 1.26, 1.85, 1.56 and 0.54 mg/L in the treatments 0, 25, 50, 75 and 100% replaced with water containing flocs respectively, which is in concurrence with the present study results. The presence of heterotrophic and nitrifying bacteria leads to the removal of ammonia and nitrite at different stages of the breeding period, which eventually leads to nitrate production. The addition of molasses to biofloc treatments stimulated the activity of heterotrophic and nitrifying bacteria, which in turn decreased ammonia and nitrite levels and increased nitrate levels in biofloc treatments in relation to clear-water treatments, which are in concurrence with the results of Gaona *et al*. (2011). Addition of molasses to biofloc reservoirs caused stimulation and development of flocs, which was associated with an increase in the number of heterotrophic bacteria and production of flocs, which was in concurrence with the results of other researchers (Burford *et al*., 2004; Asaduzzaman *et al*., 2008; Avnimelech, 2009; Shi *et al*., 2017; Mugwanya *et al*., 2021).

The researchers reported that using a biofloc system to breed shrimp and great sturgeon has innumerable benefits such as improved Growth Rate (GR) and reduced Feed Conversion Ratio (FCR), which is in concurrence with the present study results (Aghabarari *et al*., 2021; Goswami *et al*., 2022). In this paper, the growth performance in biofloc and flow-through treatments showed a significant difference. In general, the growth performance of tilapia in treatments without water exchange was better than flow-through treatments, which were confirmed by other researchers' results. Improved growth performance and Specific Growth Rate (SGR) (Ballester *et al*., 2007), in various studies, improved growth coefficient and increased weight (Wasielesky *et al*., 2006; Xu and Pan, 2012), reduced Feed Conversion Ratio (FCR), reduced feed cost (Burford *et al*., 2004), improved feed intake a utilization efficiency performance (Xu and Pan, 2012) and high survival coefficient (Mishra *et al*., 2008) in biofloc treatment has been reported in relation to flow-through treatment.

Burford *et al*. (2004) reported that Vannamei shrimp could consume over 29% of biofloc present in the aqua as a food. In the present experiment, in biofloc system, 25% of biofloc circulated in the food chain which has a better performance in reduction of feeding costs in relation to the flow-through system. For example, to produce 3 tons of fish in biofloc system, 2.8 tons of food is utilized (25% of utilized food is protein) and 150 kg (0.15 tons or 5%) is converted to wastes, while, for example, to produce 3 tons of fish in a flow-through system, 4 tons of food is utilized (30% of it is protein), of which 1.2 tons (30%) is converted to wastes. In other words, in biofloc system, only 5% of food is discharged with the effluent and in a flow-through system, 25-30% of food is discharged with the effluent.

In the present study, biofloc system had 30% lesser cost in relation to the flow-through system. Nevertheless, considering the cost of molasses (5%) in biofloc system, in fine, it can be said that biofloc system had a 25% lower feeding cost. In biofloc system, only 5% of feed is discharged and in a flow-through system, 25-30% of feed is discharged along with the effluent. This in turn indicates a difference in the quality of discharged effluent in both systems. Much lower levels of TDS at biofloc system output showed that water-soluble matter load, which to an extent is accounted as an indicator of water contamination, decreased as a result of algae treatment performance. The maximum permissible TDS index value in aquaculture effluent is proposed as 500 mg/L (Coldebella *et al*., 2018). The measured TDS values at biofloc system output were at the desired range, whilst their values in flow-through reservoirs exceeded the permissible level that indicated a significant influence of biofloc system on the quality of discharged effluent. High TDS in the flow-through system itself is a factor depicting high BOD and COD of an effluent, which demonstrated that effluent quality of the flow-through system was lower than biofloc system.

In the last decade, our country is struggling with many issues and problems. One of these challenges is the water shortage crisis and environmental issues caused by the discharge of sewage into the environment. In the present research, in biofloc system, the water exchange rate was 1.5 m^3/day (i.e. 5% per day), whereas, in the flow-through system, the water exchange rate was 30 m^3/day (i.e. 0.3 l/s). To produce 3000 kg fish in biofloc system, daily 15 m^3 of water is required and for production of same fish quantum in a flow-through system, daily 90 m^3 of water is needed.

In this study, in both systems, 1500 i.e. 25g fish were considered, wherein for production of one-ton fish, in a period of approximately six months, the weight of each fish should reach 700 grams (i.e. 1500×700=1,050,000 g). However, considering table 5, the biofloc system enters its growth stage faster than flow through.

Power consumption conditions were similar in both systems and because of the central aeration system, each pump consumed one-kilowatt electricity. However, in biofloc system, due to water circulation, electricity consumption was higher in relation to the flow-through system.

The experimental results of this study showed significant advantages in using the aquaculture system of biofloc. In biofloc system, the unconsumed foods and feces are converted into microbial protein and reused. In general, considering the benefits of this system including limited water exchange during breeding and water quality improvement for the next cycle, recycling of excreted nutrients through bioflocs, limited use of natural resources, improved quality of effluent, minimize the effluent of aquaculture system, reducing environmental effects, control of toxic inorganic nitrogen and conversion to microbial protein, consumption of microbial protein by fish, the necessity of using this new technique in the aquaculture of the country and especially in the cultivation of tilapia in a dense greenhouse is felt.

Acknowledgement

The paper is from a postgraduate student thesis, Thereby the authors of this research highly appreciate the support of Faculty of Agriculture and Natural Resources, Ardakan University.

References

Abakari, G., Luo, G. and Kombat, E.O., 2021. Dynamics of nitrogenous compounds and their control in biofloc technology (BFT) systems: A review. *Aquaculture and Fisheries*, 6(**5**), 441-447.

Abdi Rad, Z. and Qaednia, B., 2015. Shrimp breeding with biofloc system. *Shrimp Crustaceans*, Q. 1, 12–15 (in Persian).

Addo, F.G., Zhang, S., Manirakiza, B., Ohore, O.E. and Shudong, Y., 2021. The impacts of straw substrate on biofloc formation, bacterial community and nutrient removal in shrimp ponds. *Bioresource Technology*, 326, 124727. https://doi.org/10.1016/j.biortech.2021.124727

Aghabarari, M., Abdali, S. and Yousefi Jourdehi, A., 2021. The effect of Biofloc system on water quality, growth and hematological indices of Juvenile great sturgeon (Huso huso). *Iranian Journal* of *Fisheries Sciences*, 20, 1467–1482. https://doi.org/10.22092/ijfs.2021.125140

Asaduzzaman, M., Wahab, M.A., Verdegem, M.C.J., Huque, S., Salam, M.A. and Azim, M.E., 2008. C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn Macrobrachium rosenbergii production in ponds. *Aquaculture*, 280, 117–123. https://doi.org/10.1016/j.aquaculture.2008.04.019

Avnimelech, Y., 2007. Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. *Aquaculture*, 264, 140–147. https://doi.org/10.1016/j.aquaculture.2006.11.025

Avnimelech, Y., 2009. Biofloc technology – A practical guide book. The World Aquaculture Society. Bat. Rouge, Louisiana, United States 176, 227–235.

Azim, M.E., Little, D.C. and Bron, J.E., 2008. Microbial protein production in activated suspension tanks manipulating C:N ratio in feed and the implications for fish culture. *Bioresource Technology*, 99, 3590–3599. https://doi.org/10.1016/j.biortech.2007.07.063

Ballester, E.L.C., Wasielesky, W., Cavalli, R.O. and Abreu, P.C., 2007. Nursery of the pink shrimp Farfantepenaeus paulensis in cages with artificial substrates: Biofilm composition and shrimp performance. *Aquaculture*, 269, 355–362. https://doi.org/10.1016/j.aquaculture.2007.04.003

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H. and Pearson, D.C., 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. *Aquaculture*, 232, 525–537. https://doi.org/10.1016/S0044-8486(03)00541-6

Coldebella, A., Gentelini, A.L., Piana, P.A., Coldebella, P.F., Boscolo, W.R. and Feiden, A., 2018. Effluents from fish farming ponds: A view from the perspective of its main components. Sustain. 10, 1–16. https://doi.org/10.3390/su10010003

Crab, R., Kochva, M., Verstraete, W. and Avnimelech, Y., 2009. Bio-flocs technology application in over-wintering of tilapia. *Aquacultural Engineering*, 40, 105–112.

https://doi.org/10.1016/j.aquaeng.2008.12.004

Crab, R., Defoirdt, T., Bossier, P. and Verstraete, W., 2012. Biofloc technology in aquaculture: Beneficial effects and future challenges. *Aquaculture*, 356–357, 351–356. https://doi.org/10.1016/j.aquaculture.2012.04.046

Dauda, A.B., 2020. Biofloc technology: a review on the microbial interactions, operational parameters and implications to disease and health management of cultured aquatic animals. *Reviews in Aquaculture*, 12, 1193–1210. https://doi.org/10.1111/raq.12379

David, L.H., Pinho, S.M., Keesman, K.J. and Garcia, F., 2021. Assessing the sustainability of tilapia farming in biofloc-based culture using emergy synthesis. *Ecological Indicators*, 131, 108186. https://doi.org/10.1016/j.ecolind.2021.108186

de Lima Vieira, J., dos Santos Nunes, L., Rodrigues de Menezes, F.G., Vieira de Mendonça, K. and Viana de Sousa, O., 2021. An integrated approach to analyzing the effect of biofloc and probiotic technologies on sustainability and food safety in shrimp farming systems. *Journal of Cleaner Production*, 318, 128618. https://doi.org/10.1016/j.jclepro.2021.128618

De Schryver, P. and Verstraete, W., 2009. Nitrogen removal from aquaculture pond water by heterotrophic nitrogen assimilation in lab-scale sequencing batch reactors. *Bioresource Technology*, 100, 1162–1167. https://doi.org/10.1016/j.biortech.2008.08.043

El-Sayed, A.F.M., 2021. Use of biofloc technology in shrimp aquaculture: a comprehensive review, with emphasis on the last decade. *Reviews in Aquaculture*, 13(**1**), 676-705. https://doi.org/10.1111/raq.12494

El-Sayed, A.F.M., 2021. Use of biofloc technology in shrimp aquaculture: a comprehensive review, with emphasis on the last decade. *Reviews in Aquaculture*, *13*(1), pp.676-705.

Gao, L., Shan, H.W., Zhang, T.W., Bao, W.Y. and Ma, S., 2012. Effects of carbohydrate addition on Litopenaeus vannamei intensive culture in a zero-water exchange system. *Aquaculture*, 342–343, 89–96. https://doi.org/10.1016/j.aquaculture.2012.02.022

Gaona, C.A.P., Poersch, L.H., Krummenauer, D., Foes, G.K. and Wasielesky, W.J., 2011. The Effect of Solids Removal on Water Quality, Growth and Survival of *Litopenaeus vannamei* in a biofloc technology culture system. *International Journal* of *Recirculating Aquaculture*, 12, 54–73. https://doi.org/10.21061/ijra.v12i1.1354

Gaona, C.A.P., Serra, F. da P., Furtado, P.S., Poersch, L.H. and Wasielesky, W., 2016. Biofloc management with different flow rates for solids removal in the *Litopenaeus vannamei* BFT culture system.

Aquaculture International, 24, 1263–1275. https://doi.org/10.1007/s10499-016-9983-2.

Goswami, N., Sufian, S.A., Khandakar, M.S., Shihab, K.Z.H. and Zishan, M.S.R., 2022. Design and development of smart system for biofloc fish farming in Bangladesh, in: *2022 7th International Conference on Communication and Electronics Systems (ICCES)*. pp. 1424–1432. https://doi.org/10.1109/ICCES54183.2022.9835915

Hargreaves, J.A., 2013. Biofloc production systems for aquaculture southern regional aquaculture center. SRAC Publ. 1–12.

Jamal, M.T., Broom, M., Al-Mur, B.A., AL Harbi, M., Ghandourah, M., Al Otaibi, A. and Haque, M.F., 2020. Biofloc technology: Emerging microbial biotechnology for the improvement of aquaculture productivity. *Polish Journal of Microbiology*, 69, 401–409. https://doi.org/10.33073/PJM-2020-049

Jiménez-Ojeda, Y.K., Collazos-Lasso, L.F. and Arias-Castellanos, J.A., 2018. Dynamics and use of nitrogen in biofloc technology - BFT. AACL Bioflux 11, 1107–1129.

Khanjani, M.H., Alizadeh, M. and Sharifinia, M., 2021. Effects of different carbon sources on water quality, biofloc quality, and growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings in a heterotrophic culture system. *Aquaculture International*, 29, 307–321. https://doi.org/10.1007/s10499-020-00627-9

Khanjani, M.H., Sharifinia, M. and Hajirezaee, S., 2022a. Recent progress towards the application of biofloc technology for tilapia farming. *Aquaculture*, 552, 738021. https://doi.org/10.1016/j.aquaculture.2022.738021

Krummenauer, D., Poersch, L., Romano, L.A., Lara, G.R., Encarnação, P. and Wasielesky, W., 2014. The Effect of Probiotics in a *Litopenaeus vannamei* biofloc culture system infected with *Vibrio parahaemolyticus*. *Journal of Applied Aquaculture*, 26, 370–379. https://doi.org/10.1080/10454438.2014.965575

Legarda, E.C., Poli, M.A., Martins, M.A., Pereira, S.A., Martins, M.L., Machado, C., de Lorenzo, M.A. and do Nascimento Vieira, F., 2019. Integrated recirculating aquaculture system for mullet and shrimp using biofloc technology. *Aquaculture*, 512, 734308. https://doi.org/10.1016/j.aquaculture.2019.734308

Liu, W., Du, X., Tan, H., Xie, J., Luo, G. and Sun, D., 2021. Performance of a recirculating aquaculture system using biofloc biofilters with convertible water-treatment efficiencies. *Science of the Total Environment*, 754, 141918. https://doi.org/10.1016/j.scitotenv.2020.141918

Liu, W., Xu, B., Tan, H., Zhu, S., Luo,

G., Shitu, A. and Wan, Y., 2022. Investigating the conversion from nitrifying to denitrifying water-treatment efficiencies of the biofloc biofilter in a recirculating aquaculture system. *Aquaculture*, 550, 737817. https://doi.org/10.1016/j.aquaculture.2021.737817.

Mishra, J.K., Samocha, T.M., Patnaik, S., Speed, M., Gandy, R.L. and Ali, A.M., 2008. Performance of an intensive nursery system for the Pacific white shrimp, *Litopenaeus vannamei*, under limited discharge condition. *Aquacultural Engineering*, 38, 2–15. https://doi.org/10.1016/j.aquaeng.2007.10.003

Mohammadi, G., Rafiee, G., Tavabe, K.R., Abdel-Latif, H.M.R. and Dawood, M.A.O., 2021. The enrichment of diet with beneficial bacteria (single- or multi- strain) in biofloc system enhanced the water quality, growth performance, immune responses, and disease resistance of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 539, 736640. https://doi.org/10.1016/j.aquaculture.2021.736640

Mugwanya, M., Dawood, M.A.O., Kimera, F. and Sewilam, H., 2021. Biofloc systems for sustainable production of economically important aquatic species: A review. Sustain. 13. https://doi.org/10.3390/su13137255

Ogello, E.O., Outa, N.O., Obiero, K.O., Kyule, D.N. and Munguti, J.M., 2021. The prospects of biofloc technology (BFT) for sustainable aquaculture development. *Scientific African*, 14, e01053. https://doi.org/10.1016/j.sciaf.2021.e01053

Ray, A.J., Seaborn, G., Vinatea, L., Browdy, C.L. and Leffler, J.W., 2012. Effects of biofloc reduction on microbial fynamics in minimal-exchange, superintensive shrimp, *Litopenaeus vannamei*, culture systems. *Journal of the World Aquaculture Society*, 43, 790–801. https://doi.org/10.1111/j.1749-7345.2012.00607.x

Santhana Kumar, V., Pandey, P.K., Anand, T., Bhuvaneswari, G.R., Dhinakaran, A. and Kumar, S., 2018. Biofloc improves water, effluent quality and growth parameters of *Penaeus vannamei* in an intensive culture system. *Journal of Environmental Management*, 215, 206–215. https://doi.org/10.1016/j.jenvman.2018.03.015

Shi, M.M., Ruan, Y.J., Li, J.P., Ye, Z.Y., Liu, G. and Zhu, S.M., 2017. Numerical study of dense solid-liquid flow in hydrodynamic vortex separator applied in recirculating biofloc technology system. *Aquacultural Engineering*, 79, 24–34. https://doi.org/10.1016/j.aquaeng.2017.08.002

Shourbela, R.M., Khatab, S.A., Hassan, M.M., Van Doan, H. and Dawood, M.A.O., 2021. The effect of stocking density and carbon sources on the oxidative status, and nonspecific immunity of nile tilapia (*Oreochromis niloticus*) reared under biofloc conditions. *Animals*, 11, 1–13. https://doi.org/10.3390/ani11010184

Suárez-Puerto, B., Delgadillo-Díaz, M., Sánchez-Solís, M.J. and Gullian-Klanian, M., 2021. Analysis of the cost-effectiveness and growth of Nile tilapia (*Oreochromis niloticus*) in biofloc and green water technologies during two seasons. *Aquaculture*, 538, 736534. https://doi.org/10.1016/j.aquaculture.2021.736534

.

Tierney, T.W. and Ray, A.J., 2018. Comparing biofloc, clear-water, and hybrid nursery systems (Part I): Shrimp (*Litopenaeus vannamei*) production, water quality, and stable isotope dynamics. *Aquacultural Engineering*, 82, 73–79. https://doi.org/10.1016/j.aquaeng.2018.06.002

Twarowska, J.G., Westerman, P.W. and Losordo, T.M., 1997. Water treatment and waste characterization evaluation of an intensive recirculating fish production system. *Aquacultural Engineering*, 16, 133–147. https://doi.org/10.1016/S0144-8609(96)01022-9

Wasielesky, W., Atwood, H., Stokes, A. and Browdy, C.L., 2006. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp Litopenaeus vannamei. *Aquaculture* 258, 396–403. https://doi.org/10.1016/j.aquaculture.2006.04.030

Widanarni, Ekasari, J. and Maryam, S., 2012. Evaluation of biofloc technology application on water quality and production performance of red tilapia *Oreochromis* sp. Cultured at different stocking densities. *HAYATI Journal of Biosciences*, 19, 73–80. https://doi.org/10.4308/hjb.19.2.73.

Xu, W.J. and Pan, L.Q., 2012. Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. *Aquaculture* 356–357, 147–152. https://doi.org/10.1016/j.aquaculture.2012.05.022.