Chapter

Novel Biofloc Technology (BFT) for Ammonia Assimilation and Reuse in Aquaculture In Situ

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Abstract

Ammonia is one of the most harmful risks for success of fish and shrimp culture. There is no effective solution for harmlessness of ammonia in traditional aquaculture operations except exchanging water, which would bring negative effects on environment, or fixing expensive equipment. Biofloc technology (BFT) that appeared in recent years supplies a novel solution for this issue without exchanging huge water and fixing equipment. This technology could assimilate ammonia almost in real time with many other supplemental benefits. Because of the very high nutritional value for fish and shrimp, bioflocs, the by-product of BFT, could also be reused as a complemented food in situ or a gradient for feedstuff to replace expensive fishmeal or be processed to pellet diet to feed fish and shrimp directly. However, some aspects with regard to the effective use of biofloc as a food source for fish and shrimp, such as high lipid content, productivity, and palatability, need to be further researched in detail.

Keywords: ammonia, assimilation, reuse, biofloc technology, aquaculture

1. Introduction

The world population will exceed 9 billion people by the middle of the twenty-first century, indicating proportionate food should have to provide. Fisheries and aquaculture are the critical important sources against this challenge of food and nutrition [1]. Between 1961 and 2016, the average annual increase in global food fish consumption (3.2%) outpaced population growth (1.6%) and exceeded that of meat from all terrestrial animals combined (2.8%). Total fish production in 2016 reached 171 million tones, of which 88% was directly utilized for human consumption. In per capita terms, food fish consumption grew from 9.0 kg in 1961 to 20.2 kg in 2015, accounting for about 17% of their average per capita intake of animal protein consumed by the global population [1].

Since the late 1980s, the fishery production has been stable without obvious increase. But aquaculture has become more and more important, which production grew faster than other major food production sectors. The contribution of aquaculture to the global production of capture fisheries and aquaculture combined has risen continuously, reaching 46.8% in 2016 and representing 53% of fish production for food uses [1].

However, the development of aquaculture has faced challenges because of lack of land and water source and degradation of environment [1]. Therefore, turning of

aquaculture to intensive even high intensive model from extensive or semi-extensive model is a tendency all over the world. Intensive aquaculture utilizes limited land source to culture more fish and shrimp by excessively increasing aquatic animal density with little water exchange or even zero water exchange. However, one of the most harmful risks for success of fish and shrimp in intensive aquaculture system, especially in closed intensive culture system with little water exchange, is the accumulation of ammonia. Unfortunately, there is no effective solution for harmlessness of ammonia in practical operations except exchanging water or fixing some very expensive equipment for water treatment [2].

Biofloc technology (BFT) that appeared in recent years supplies a novel solution for this issue without exchanging huge water or fixing equipment [2]. BFT could assimilate ammonia almost in real time and reuse the by-product as a natural food source in situ in aquaculture water column. In this chapter, problems referred to ammonia in aquaculture (Part 2 and Part 3), principles of ammonia removal (Part 4), main operations of BFT (Part 5), applications of using biofloc produced as a by-product of BFT in aquaculture (Part 6 and Part 7), and some highlighting issues that should be paid attention to or need to be further researched (Part 8) are introduced in brief.

2. Toxicity of ammonia to fish or shrimp

Ammonia is one of the most harmful inorganic nitrogen compounds for fish or shrimp in aquaculture (another is nitrite), whose accumulation in pond water may deteriorate water quality, reduce growth, increase oxygen consumption, alter concentrations of hemolymph protein and free amino acid levels, and even cause high mortality [3]. For example, in water with pH 8.05 and temperature 23°C, the 96 h median lethal concentration (LC_{50}) value of ammonia on *Litopenaeus vannamei* (Pacific white-leg shrimp, the most important cultured crustacean species in the world [1]) juveniles is 35.4 mg/L at salinity of 25‰, indicating that the safety level of ammonia for rearing *L. vannamei* juveniles is only 3.55 mg/L [3]. A research conducted by the author of this chapter revealed that in a closed aquaculture system for *L. vannamei* without water exchange, the ammonia concentration accumulated up to a very high level of 10.81 mg/L, with an average of 6.35 mg/L, leading to a mortality rate as high as 70%.

There are two existent types for ammonia, ionic type (NH_4^+) and free type (NH_3), both of which in general named together as total ammonia nitrogen (TAN). In fact, the toxicity of TAN is mainly from the free NH_3; in water, the 96 h LC_{50} value to *L. vannamei* juveniles is 1.57 mg/L under conditions of pH 8.05, temperature 23°C, and salinity of 25‰, and the safety level is 0.16 mg/L [3]. Van Wyk et al. [4] even recommend a value of ≤0.03 ppm or mg/L for NH_3 when farming this shrimp in recirculating freshwater systems. There is an equilibrium between the two existences of ammonia in water [5]:

$$NH_3 + H^+ \leftrightharpoons NH_4^+ \tag{1}$$

This equilibrium indicates that NH_4^+ and NH_3 exist in water at the same time and their proportions are determined by the pH of the water body so that the toxicity of TAN is highly related to water pH. Actually, the relationship among water pH and the concentrations of NH_4^+ and NH_3 could be descripted with an equation [6] as follows:

$$\frac{[NH_3]}{[NH_3] + [NH_4^+]} = \frac{10^{pH}}{\exp\left(\frac{6344}{273+T}\right) + 10^{pH}} \tag{2}$$

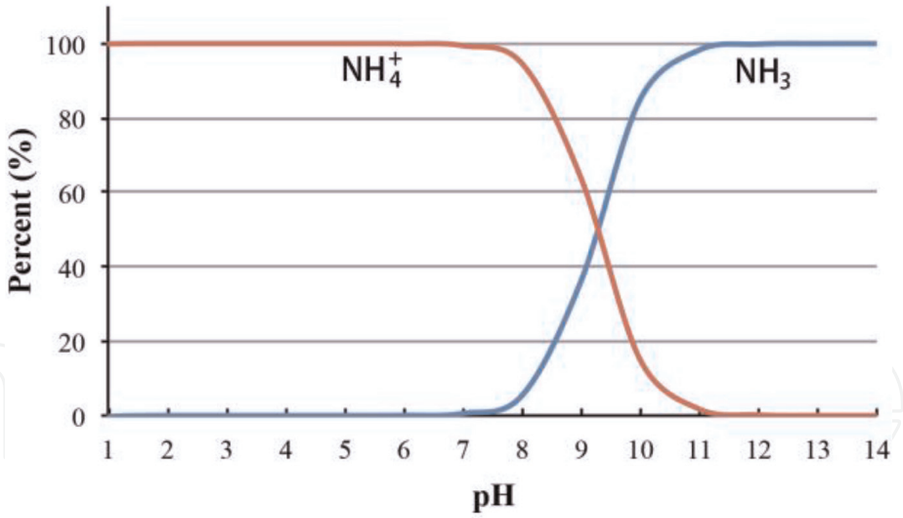

Figure 1.
Percent of NH_4^+ and NH_3 changing with pH under 25°C.

In this equation, T means the water temperature. Based on Eq. (1), the proportions of NH_4^+ and NH_3 along pH gradient under a certain temperature (such as 25°C) could be calculated and pictured, respectively (**Figure 1**). From **Figure 1**, it is well known that there is almost no NH_3 existing in water when pH is below 7.0; however, after that, the proportion of NH_3 will exponentially elevate with increasing of pH, as well as the toxicity of TAN.

3. Deriving of ammonia in aquaculture water body

Ammonia in aquaculture water body is mostly produced from artificial feeds fed to fish animals. Estimated about 78% of nitrogen existing in aquaculture water body comes from feedstuff [7]. Artificial formulated feed for aquaculture animals contains a very high content of protein; in general, the crude protein content in finfish feedstuff is 25–30% [8] and higher for crustacean animals, which is even up to 40–45% for shrimp species like white-leg shrimp [4]. However, the utilization efficiency of those feeds in water is very low. When feed is added to water, only 25% of protein nitrogen in feedstuff is assimilated to body growth of aquatic animals, and the rest of about an approximate 75% proportion will lose into the water body, via directly excreting as metabolic ammonia from gill, evacuating as urea and feces by cloaca system, or dissolving as other organic nitrogen compounds [9], which are further degraded as inorganic ammonia by microorganisms with hydrolysis enzymes.

4. The main routes for ammonia transformation in aquaculture

There are three routes for ammonia removal or transformation in aquaculture system: intake by photoautotrophic algae, nitrification and nitration of autotrophic nitrobacteria, and assimilation of heterotrophic bacteria [10].

4.1 Route 1: photoautotrophic intake by algae or phytoplankton

Actually, the intake route of ammonia by photoautotrophic algae is the process of well-known photosynthesis as follows [10]:

$$16NH_4^+ + 92CO_2 + 92H_2O + 14HCO_3^- + HPO_4^{2-} \rightarrow C_{106}H_{263}O_{110}N_{16}P + 106O_2 \quad (3)$$

Or when nitrate is as the nitrogen source

$$16NO_3^- + 124CO_2 + 140H_2O + HPO_4^{2-} \rightarrow C_{106}H_{263}O_{110}N_{16}P + 138O_2 + 18HCO_3^- \quad (4)$$

where $C_{106}H_{263}O_{110}N_{16}P$ represents the stoichiometric formula for algae.

In this process, the ionic ammonia of NH_4^+ is the first-order utilized inorganic nitrogen for synthesis of organic materials. However, a carbon to nitrogen to phosphorus ratio (C:N:P) of about 106:16:1 is also needed, indicating that to promote ammonia assimilation, exogenous additions of inorganic carbon and phosphorus sources are needed and that in general make the growth of algae, especially blue-green algae or cyanobacteria, to be very difficult to control and easily result in cyanobacteria blooming, a serious deterioration of water quality and a disaster for human daily life.

4.2 Route 2: autotrophic oxidation by nitrobacteria

Autotrophic nitrobacteria, the chemical autotrophic bacteria, can oxidize ammonia by using inorganic carbon sources without the need of phosphorus [10]:

$$\begin{aligned} NH_4^+ + 1.83O_2 + 1.97HCO_3^- &\rightarrow 0.0244C_5H_7O_2N \\ &+0.976NO_3^- + 2.9H_2O + 1.86CO_2 \end{aligned} \quad (5)$$

where $C_5H_7O_2N$ represents the chemical formula for microbial biomass.

However, the growth rate of nitrobacteria is very low when compared to heterotrophic bacteria, which in turn leads to a low oxidized rate for ammonia. There are also no other efficient supplemental approaches to accelerate this process, which mainly relies on the natural development of nitrobacteria. Furthermore, an intermediate product of this process, nitrite or NO_2^-, another toxic inorganic nitrogen compound for aquaculture animals, would be produced. Nitrite is an unstable product with high oxidized ability comparable to oxygen and thus will oxidize Fe^{2+} in the center of hemoglobin to Fe^{3+}. As a result, oxygen could not combine to hemoglobin and transport to tissues, and thus animals will be asphyxiated, even though there is enough oxygen dissolved in water body [11]. Moreover, the oxidization of ammonia by nitrobacteria would cause numerous accumulation of nitrate (NO_3^-), another inorganic nitrogen compound which could be easily taken by phytoplankton, indicating a potential risk of algae blooming [10, 11]. Finally, the nitrification process could affect water quality, such as exhausting carbonate alkalinity (HCO_3^-) and resulting in reduction of water pH [10].

4.3 Route 3: assimilation by heterotrophic bacteria

Ammonia also could be assimilated by heterotrophic bacteria through a process different from those of photoautotrophic algae (route 1) and autotrophic nitrobacteria (route 2) [10]:

$$NH_4^+ + 1.18C_6H_{12}O_6 + HCO_3^- + 2.06O_2 \rightarrow C_5H_7O_2N + 6.06H_2O + 3.07CO_2 \quad (6)$$

where $C_5H_7O_2N$ represents the chemical formula for microbial biomass like route 2, or Eq. (5). Compared to route 2, sufficient dissolved oxygen is needed for the processing of bio-reaction of Eq. (6) as well, but about half of HCO_3^- will be exhausted. Differently, in Eq. (6) of route 3, carbohydrate ($C_6H_{12}O_6$) is needed, and about 40 times microbial biomass is produced.

5. Novel solution for ammonia assimilation and reuse in aquaculture based on route 3: biofloc technology (BFT)

Ammonia accumulation is the head issue faced in aquaculture, and there are several routes referring to ammonia clearance mentioned above. However, routes 1 and 2 are all not suitable to apply in aquaculture. For route 1, intake of phytoplankton or algae might produce a large number of algae exceeding the biological capacity of water body, and those planktons will be old and die quickly and release toxins harmful to aquatic animals. In regard to route 2, it is mainly applied for effluent treatment in sewage plant, which needs inferior procedures of wastewater, and thus is not suitable in aquaculture as well. Fortunately, according to the principles of route 3 displayed in Eq. (6), a novel technology, in generic nicknamed as biofloc technology (BFT), is developed for aquaculture in recent years, to be used as effectively and environmental-friendly for transforming of ammonia.

5.1 Principal operations of BFT

In accordance with Eq. (6), existing of carbohydrate will promote assimilation of ammonia, companied with synthesis of microbial biomass. However, the content of carbohydrate or C:N in aquaculture water body is lower than the need for bio-reaction of Eq. (6) in general. Although the C:N of bacterial cell composition is about 5:1 [12], it needs a C:N of 15:1 for blooming growth of heterotrophic bacteria to assimilate ammonia [13, 14]. In aquaculture water body, the carbohydrate is mainly from feedstuff added in [7], whose content is usually inadequate for blooming growth of heterotrophic bacteria. For example, taking white-leg shrimp feed usually used in China into consideration, the contents of ingredients, such as crude protein, lipid, fiber, ash, and moisture, are 40, 5.0, 5.0, 15, and 12%, respectively, indicating a calculated C:N of approximate 6:1 according to the relationship between contents of carbohydrate and feed ingredients [15, 16]:

$$\begin{aligned}\text{Carbon\%} = 0.80 \times \text{Lipid\%} + 0.53 \times \text{Protein\%} + 0.42 \\ \times \text{Carbohydrate\%} + 0.42 \times \text{Fiber\%}\end{aligned} \tag{7}$$

$$\text{Carbohydrate\%} = 100 - (\text{Protein} + \text{Lipid} + \text{Fiber} + \text{ash} + \text{moisture})\% \tag{8}$$

Therefore, additional exogenous organic carbon source containing carbohydrate ($C_6H_{12}O_6$) should be supplemented to prompt assimilation of ammonia by improving growth of heterotrophic bacteria, and this is one of the two principal operations for BFT [17].

The other principal operations for BFT are aeration and treatment of by-product. Known from Eq. (6), a huge number of dissolved oxygen is needed to assimilate ammonia by heterotrophic bacteria, and also massive bacteria biomass is produced as by-product, which needs to be treated.

5.2 Addition of carbohydrate

For assimilating 1 mole of ammonia, 1.18 mole of carbohydrate is exhausted according to Eq. (6), which indicates that when 1 g of NH_4^+ exists in water, about 12 g of $C_6H_{12}O_6$ should be added [10, 17]. This needs to supervise the ammonia concentration of water continuously, which is difficult to implement actually. Thus, a general manipulation is that carbon source is added only when ammonia concentration excesses 1 mg/L with the NH_4^+ to $C_6H_{12}O_6$ ratio (w:w) of 1:12 [10, 17]. Of course, the content of carbohydrate contained in material used as carbon source should be determined.

Another way for addition of carbohydrate to improve the bio-reaction of route 3 is adjustment of the C:N in water in real time. For this purpose, the contents of nitrogen in water are determined actually, and then materials rich in carbon or carbohydrate are added to adjust C:N. However, in fact, many times, the adjustment of C:N is not based on the actual carbon and nitrogen concentrations. Alternatively, only when feedstuff is fed, carbon source is considered to add, and the weight for addition is calculated according to the nitrogen content in feedstuff with a C:N of 15:1 [13].

Many materials could be used as carbon source for BFT system, such as acetate [18], glycerol [18], dextrose [19–21], cassava meal [22], cellulose [23], corn flour [24, 25], glucose [18], molasses [26–28], tapioca [29], wheat flour [28, 30], rice flour [16, 30], wheat bran [25, 31], rice bran [20, 29], starch [28, 32], poly-β-hydroxybutyrate (PHB) [33, 34], brewery residues [22], and sugar [32].

5.3 Aeration

The process of ammonia assimilation via heterotrophic microorganisms needs a huge number of oxygen, because of (1) oxygen consumption by respiration of blooming growth of bacteria and (2) oxidized fermentation of organic materials secreted by bacteria [17]. Thus, it is needed to usually equip a robust air blower to blow air into the water body to maintain a highly dissolved oxygen level in water [2], in general at least 5 mg/L [4]. In some cases, even pure oxygen is used for this purpose.

5.4 Treatment of by-product

A result induced by blooming growth of heterotrophic bacteria is substantial accumulation of suspended solids or bioflocs, one of the side effects of utilizing BFT. In a BFT system constructed by the author in the present article, bacteria secrete massive metabolic materials such as protein and polysaccharide, which could bond feeds, feces, debris, and other organic matters together, to become bioflocs and suspended in water under aeration condition (**Figures 2** and **3**). The author also found that sometimes the total suspended solid (TSS) content in BFT system would accumulate to above 800 mg/L (**Figure 3b**). That high level of TSS will be harmful to aquatic animals, which would lead to oxygen depletion, obstruction of fish or shrimp gills, and mortality due to asphyxiation [35]. Therefore, treatment of those accumulated TSS is an important operation for BFT [36].

There are three ways used for treating of TSS. The first one is in situ eaten by fish or shrimp as supplemental food [37, 38] which is also the most frequently used method. The second one is equipping a settling chamber to remove excessive solids [39]. And the last one is using separation systems for biofloc production and aquatic

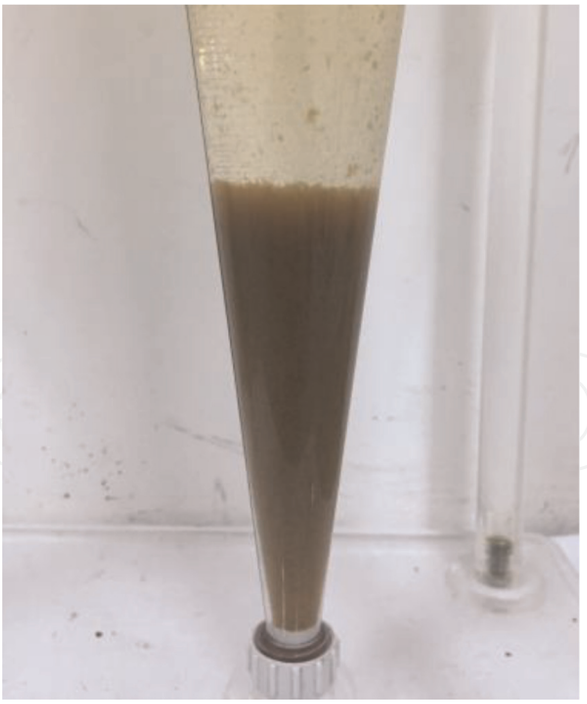

Figure 2.
Sedimentation of biofloc in an Imhoff cone.

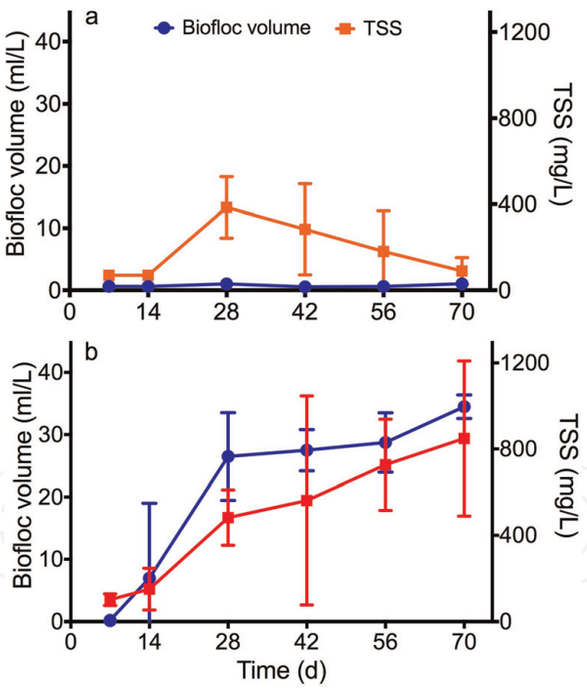

Figure 3.
Productivity characteristics of biofloc volume and TSS in closed traditional system (a) and BFT system (b) over time. Biofloc volume is defined as the volume of sinkable matter in 1 l water placed into an Imhoff cone in 15 min. TSS is represented as the mass (mg) of dry matter in 1 l water after filtering with a 0.45 μl membrane.

animal production, respectively, so that the increasing TSS produced in biofloc production system will not affect the growth of fish or shrimp raised in another system whose water quality should remain controlled by the former system. And for this purpose, four 10 m^3 composite tanks in general need to be fixed for water treatment of 12 tanks with a volume of 500 L per tank [40].

6. Management of dissolved oxygen and ammonia in BFT system

6.1 Dissolved oxygen level

Although numerous amounts of oxygen will be consumed by respiration of a large number of flourishing heterotrophic bacteria, the author in this article supervised that the dissolved oxygen continuously sustained a high level in fact in a *L. vannamei* BFT system equipping air blower, such as root blower (**Figure 4**). In general, air blew into per minute with 1–5% of water volume is adequate to maintain a high dissolved oxygen level. For example, in a pond reserving 1000 m^3 water, the flow rate of root blower should be 10–50 m^3/min.

6.2 Ammonia assimilation efficiency

The speed of ammonia assimilation in BFT system is very fast; Avnimelech [13] reported that ammonia added to water body with a final concentration of 10 mg/L disappeared over a period of about 2 h post addition of glucose as carbon source. The author of the present chapter found that in the BFT system culture *L. vannamei*, a low ammonia level averaging 0.78 mg/L lasted during the whole period of culture in water body (**Figure 5b**). Moreover, nitrite and nitrate concentrations would also be limited at low levels in this BFT system (**Figure 5b**). Conversely, in the control system without water exchange, the average ammonia concentration was up to 6.35 mg/L, which in turn resulted in a very high level of nitrite of 11.78 mg/L subsequently (**Figure 5a**). Consequently, a very high shrimp mortality rate of 70% was found in this control water body, very far higher than that of the BFT system without mortality over the whole experimental period of 70 d (data not shown). Cardona et al. [41] reported that the survival rate of *Litopenaeus stylirostris* was 27.2% higher in the BFT system than that of the conventional system with huge water exchange, which was also contributed to the good water quality represented by those low ammonia and nitrite levels.

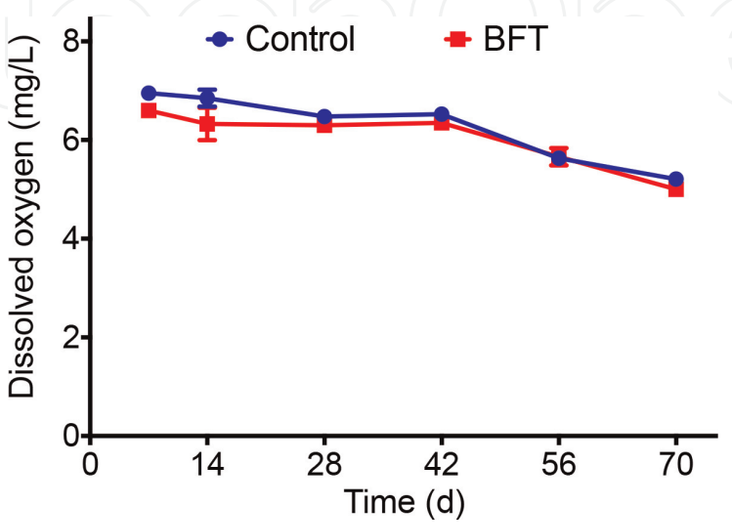

Figure 4.
Dissolved oxygen level in a BFT system over time.

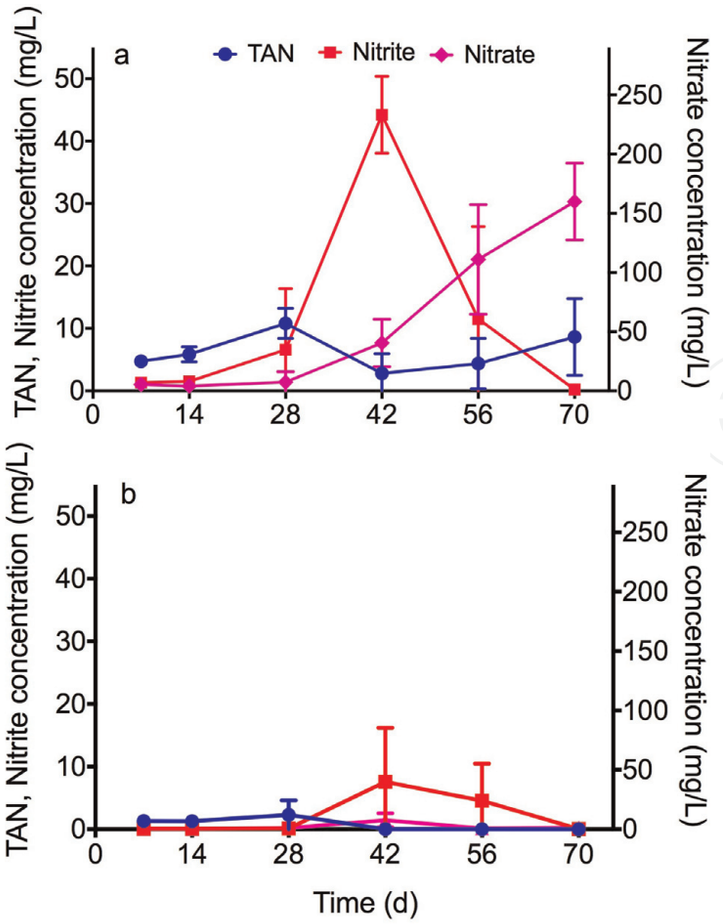

Figure 5.
TAN, nitrite, and nitrate concentrations in closed traditional system (a) and BFT system (b) at different time points.

7. Approaches for reusing of biofloc as supplemental food

Treatment of suspended solids or bioflocs is one of the most important operations for using BFT. Usually, those solids or bioflocs are not removed just as a waste from water. In contrast, they are reused as a complemented food source for aquatic animals, especially omnivorous species such as shrimp and tilapia, in a system adopting BFT. During development of bioflocs, bacteria secrete protein and polysaccharide, which bond with feeds, feces, debris, and other organic matters together. Furthermore, the author of this article found that biofloc was also a nutritional resource that could attract zooplanktons to prey, such as protozoa, rotifers, nematodes, ciliates, and flagellates (**Figure 6**), which in turn provides live and fresh food rich in protein for fish and shrimp.

7.1 Three ways for food use of biofloc

There are three ways for biofloc used as food in aquaculture currently: (i) as a complemented food for fish or shrimp in situ [42–45], (ii) as a gradient for feedstuff to replace fishmeal [46–49], and (iii) as a normal feed to replace partial artificial feedstuff [50–55]. In brief, fish and shrimp consume biofloc rich in microbe, phytoplankton, and zooplankton as a food directly. Because animals take biofloc as a vice food source, thus in fact the biofloc hunted by fish and shrimp is only a few

parts of the whole. In other words, most of the bioflocs remain in the water body, which may be an obstacle for growth of fish and shrimp. For alleviating the negative effect of biofloc on animal growth, excessive parts should be collected from water body and could be taken as an alternative protein source for preparing feedstuff. Even more, biofloc is fed to fish or shrimp as feedstuff directly due to its whole and high nutritional value.

7.2 Factors influencing nutritional value of biofloc

Nutritional value of biofloc is important for its reuse. However, this value is affected by several factors. Because the development of biofloc is sponsored and prompted by accumulation of ammonia and addition of carbon source, it is suspected that feedstuff and carbon source [30], especially the last one, would impressively affect biofloc nutritional composition and value (**Table 1**). For example, protein content and oil content of feedstuff will affect those of in biofloc. With regard to carbon source, there are two main types of carbon sources: (i) simple structure carbon sources with easily dissolving ability in water, such as glucose, sucrose, and sodium acetate [17, 20], and (ii) complex compounds, like flour or bran of rice and wheat [56] and brewery residues, which are a by-product from beer production industry [22]. In general, complex carbon source is more difficult to dissolve and more powerful in improving biofloc nutritional value, which in turn improves the growth of fish or shrimp [17, 20]. This carbon source is not easy to degrade with big diameter so that animals in water could easily prey and eat them directly; thus, except for being used as carbohydrate, those materials also contain other nutritional materials essential for growth of fish and shrimp, such as proteins, oils, vitamins, and minerals, even carotenoids [57, 58].

7.3 Applications of biofloc as complemented food in aquaculture

BFT has been successfully used for culture of fish and shrimp, such as tilapia, carp, and *L. vannamei*. Results showed that BFT increased individual fish weight,

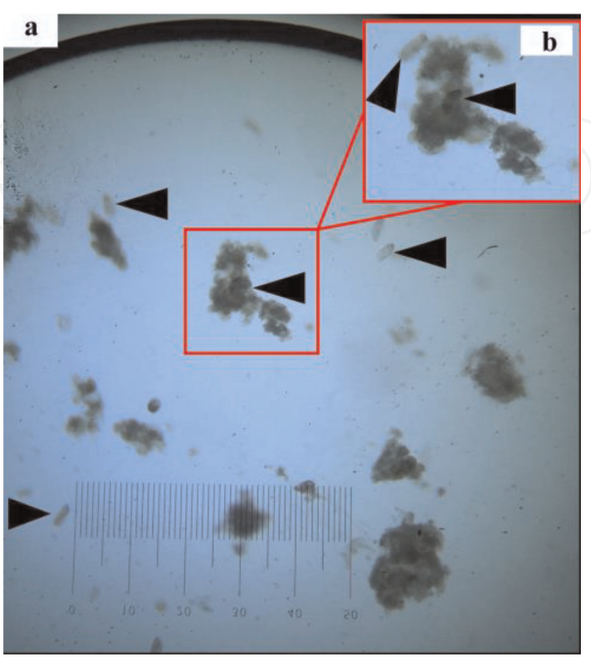

Figure 6.
Bioflocs observed with a light microscope. The minimal scale of the rule in the down part of the figure represents 25 μm. Arrows indicate free zooplanktons (a), and zooplanktons prey food from biofloc (b).

Animals	Carbon sources	Feedstuff composition		C:N	Biofloc composition		Ref.
		CP	CL		CP	CL	
L. vannamei	Molasses and wheat bran	42.5	–	20:1	28.7–43.1	2.11–3.62	[59]
	Sucrose	35–40	7–9	–	24.01	3.31	[60]
	Molasses	38	9	15:1	27.43	0.86	[28]
	Starch				23.1	1.14	
	Wheat flour				30.73	2.18	
	Mixture of molasses, starch, and wheat flour with equal weight ratio				25.46	1.24	
Tilapia	Wheat flour	24	6.23	–	37.93	3.16	[61]
		35	6.24		38.41	3.23	
	Feed	22	12.3	11.6:1	50.6	2.6	[62]
		35	119	8.4:1	53.5	1.9	
	Poly-β-hydroxybutyric	30	4	–	34.06	6.58	[34]
	Glucose				38.53	6.06	
Farfantepenaeus paulensis	Molasses and wheat bran	40	13.1	20:1	30.4	0.47	[37]
		35	–	–	18.4	0.3	[63]
Labeo rohita	Wheat flour	29.6–35.4	4.2–16.5	10:1	35.4	1.1	[52]
Penaeus monodon	Wheat flour	40	–	10:1	24.3	3.53	[53]
Catfish	Glycerol	43	6	10:1	44.27	5.84	[64]
				15:1	38.65	7.35	
				20:1	32.64	10.78	
Green cucumber	Glucose	20.37	2.45	15:1	32.29	4.19	[65]
	Sucrose				28.04	4.30	
	Starch				21.67	3.83	
White cucumber	Glucose				27.27	4.25	
	Sucrose				27.48	3.89	
	Starch				21.23	3.76	

Note: CP, crude protein; CL, crude lipid.

Table 1.
Compositions of biofloc produced from different feedstuff and carbon sources.

weight gain, and protein efficiency ratio of tilapia by 12.54, 9.46, and 22.2%; decreased feed conversion rate (FCR) by 17.5% [66]; elevated total weight gain and specific growth rate increase by 128% and 112% [67], respectively; and also significantly increased the final weight, weight gain, and length of *L. vannamei* [37, 63]. And those results for enhancing growth of fish and shrimp are mainly contributed to biofloc. Research from Tierney and Ray [2] revealed that the shrimp in the BFT treatment received an estimated 87% of their carbon and 66% of their nitrogen from the pelleted feed source, whereas the rest of 13% and 34% came from the biofloc, respectively.

When bioflocs were eaten directly by fish or shrimp, the protein utilization efficiency of feed elevated by 29% [68], and the FCR decreased by about 18% for tilapia [66, 67], and also decreased for *Penaeus monodon* [69]. It is found that the protease, lipase, and amylase activities in the intestine, liver, and stomach of tilapia [66, 67] and *L. vannamei* were all significantly raised [25], which improves the digestion and intake of feed. A large number of heterotrophic bacteria are located in biofloc, most of which will secret enzymes like protease, lipase, and amylase, indicating that preying on biofloc would directly increase the content of those enzymes in digestive organs of fish and shrimp. Those bacteria also would secret digestive enzymes in vivo after entering, colonizing and propagating in the intestine [25]. In a BFT system for culturing *L. vannamei*, the feedstuff containing 35% of protein could be altered with feedstuff whose protein content decreased to 25%, because most nutrition materials lacking in low protein content feed could be compensated from biofloc. In another way, about 25% feedstuff could be saved when culturing *L. vannamei* with BFT because biofloc was hunted as a food in situ [54]. It is even reported that no feed should be added in some BFT systems for culturing *L. vannamei* and *Macrobrachium rosenbergii* [18, 37].

Biofloc is also used as an alternative protein source for fishmeal sometimes. The protein content in biofloc is evidenced to be very high, in general 25–40% [34, 63, 66, 70], in a case even up to 50% [62]. The essential amino acids were also rich in biofloc, and its composition was also highly in agreement with that in the fish body [71], indicating that it is valuable for growth of fish and shrimp. Dantas et al. [46] and Kuhn et al. [48] replaced 30% of fishmeal or soy meal with biofloc to manufacture feedstuff for feeding of *L. vannamei*, and results showed that the growth of shrimp is not affected. Kuhn et al. [47] thought that the whole soy meal could be replaced with biofloc and 67% for fishmeal. However, only appropriate replacement of fishmeal with biofloc could prompt the growth of shrimp obviously, approximately 15.16–16.5% [49].

In some cases, biofloc was collected and dried to make pellets and then fed to fish or shrimp like artificial or formulated feedstuff. Carps, *Labeo rohita*, and *L. vannamei* was cultured successfully with biofloc pellets according to a replacement ratio of 25, 50, and 50% to formulated feedstuff, respectively, without any negative effects on their growth [50–52]. However, when feedstuff was replaced 35% with biofloc, the growth of *L. vannamei* appears to be the fastest [72]. In fact, a replacement ratio lower than 10% could improve the growth of animals [55]. For example, when the replacement ratio was 4 and 8%, the individual mean body weight of *P. monodon* increased by 16.9 and 13.9%, respectively [53].

8. Prospects

Meeting future demand for fish is very important for global food security. However, barriers to growth have to be explicitly recognized to the environmental and economic pillars of sustainability [73]. Fortunately, BFT could fulfill those requests for sustainable development of aquaculture.

8.1 Environmental advantages of BFT for sustainable development of aquaculture under framework of the FAO

Except availability of land and water, environmental impact is another possible main constraint to aquaculture growth. Thus, aquaculture systems that reduce eutrophication risks and other environmental costs while providing income and extended social benefits should be developed [73]. For this purpose, the FAO thinks that herbivorous and omnivorous species should be promoted and integrated

aquaculture including multitrophic aquaculture is also an alternative, in which by-products (wastes) from one species are recycled to become inputs (fertilizers, food, and energy) for another [73].

From this point of view, in practical aquaculture operations, BFT utilizes by-products from agriculture industries, such as cassava meal [22], molasses [26–28], tapioca [29], wheat bran [25, 31], rice bran [20, 29], and brewery residues [22], as fertilizers for assimilating organic and inorganic materials. And in turn, its own by-product, biofloc, becomes complemented food for aquatic suspension or deposit feeders, like herbivorous fish. Some omnivorous aquatic animals, such as shrimp and tilapia, were all very suitable to be cultured with BFT [44, 62]. Due to the characteristics of in situ treatment of water quality and supplying of organic biofloc food, aquaculture systems that adopted BFT only need a few water exchange, even no water exchange, and decrease artificial feedstuff inputs, indicating reduced eutrophication risks of environment and the use of wild fish for aquaculture feeds to reserve balance of ecosystem.

8.2 Practical application of BFT for economical benefits

Rego et al. [74] analyzed the financial viability of inserting the BFT system (625 m^2 each pond) and maintaining the conventional culture system (2.86 ha each pond) for the marine shrimp *L. vannamei* in a farm located in the state of Pernambuco, northeastern Brazil. The total production costs of BFT were eight times higher than the conventional system. However, operating profit and profitability index were US$ 51,871.54 per hectare per year and 30.22% for BFT, and US$ 21,523.83 and 59.79% for the conventional system, respectively. In an investment analysis, indicators were favorable for both systems, with greater expressiveness of the net present value (NPV) for the BFT (US$ 142,004.42) and internal rate of return (IRR) 4.5 times higher for conventional system (131.86%). When the cash flows were designed for 10 years to the discount rates of 10, 13, and 16%, the BFT system showed greater sensitivity to changes in rates, reducing significantly the NPV when interest rates increased. Risk was only observed in the BFT system, with up to 15% of probability when subjected to the discount rate of 16%. Both shrimp production systems represent a significant investment alternative for the rural sector in northeastern Brazil, because even from the perspective of risk management, the IRR has 90% probability ranging from 7.66 to 59.40% for the BFT and from 67.96 to 201.03% for the conventional systems [75].

8.3 Further improvements for reuse of biofloc

Undoubtedly, BFT is a novel solution for transformation of ammonia in aquaculture. However, how to effectively reuse or deposit biofloc, the by-product of assimilation of ammonia in BFT system in situ, as a supplemental food for aquatic animals, needs more researches in detail.

The consumed efficiency of biofloc by fish or shrimp in situ is not adequate high, resulting in gradual accumulation of TSS in BFT system because of huge numerous organic materials produced by blooming growth of heterotrophic bacteria. Thus, the causes contributed to this low efficiency should be researched. Furthermore, the strategies for improving the utilization efficiency of biofloc should be assessed as well, such as improving accumulation of lipid of biofloc, which will increase the nutritional value. Usually, the total lipid content in biofloc is too low to be sufficient for demand of fish and shrimp (**Table 1**). Previous studies found that the lipid contents of biofloc were 0.5–0.6% [63, 70], 1.03% [66], or 4.0% [62], respectively,

which were all lower than the demands for lipid of aquatic animals [8]. For example, the recommended total lipid level in the diet for shrimp is in general higher than 6.5% [4]. Although external equipment could be used to settle the excessive part of biofloc, how to treat this deposit containing high content organic matter and bacteria, part of which may be pathogens, was also a problem [39].

The efficiency for producing biofloc also needs to be elevated, if biofloc is used as a gradient of formulated feedstuff for replacement of fishmeal or soybean meal or used to feed to aquatic animals directly as a food with whole nutritional gradients usually contained in artificial feedstuff. The productivity of biofloc recent is not adequate for those uses in practical operations.

Moreover, the improvement of biofloc palatability should be researched, which is important to the utilization of biofloc either eaten in situ or used as a food source [76]. Attractants or feeding promoting agents, like garlicin, betaine (trimethylglycine), trimethylamine oxide (TMAO), and s,s-dimethyl-β-propionic acid thetine (DMPT), could be taken into consideration as additives during development of biofloc in situ or preparing process for biofloc pellets. Thus, the effects of those agents on biofloc attraction to fish and shrimp should be studied in detail, respectively.

9. Conclusions

Biofloc technology (BFT) supplies a novel solution for this issue without huge water exchange, even zero water exchange. In general, ammonia would be removed quickly within several hours in a BFT system. Moreover, because of the very high nutritional value for fish and shrimp, bioflocs, the by-product of BFT, could also be reused as a complemented food in situ or a gradient for feedstuff to replace expensive fishmeal, and biofloc also could be processed to formulate diet to feed fish and shrimp directly. However, some aspects with regard to the effective use of biofloc as a food source for fish and shrimp, such as high lipid content, productivity, and palatability, need to be further researched in detail.

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Conflict of interest

The author declares no conflict of interest.

Notes/thanks/other declarations

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Appendices and nomenclature

BFT	biofloc technology
C:N (P)	carbon to nitrogen (to phosphorus) ratio
CL	crude lipid
CP	crude protein
DMPT	s,s-dimethyl-β-propionic acid thetine
FAO	Food and Agriculture Organization
FCR	feed conversion rate
IRR	internal rate of return
LC_{50}	median lethal concentration
NPV	net present value
NRC	National Research Council
PHB	poly-β-hydroxybutyric
ppm	part(s) per million
TAN	total ammonia nitrogen
TMAO	trimethylamine oxide
TSS	total suspended solids

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