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Evaluation of Various Sources of Bacteria on Biofloc Productivity

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Authors' contributions

This work was carried out in collaboration among all authors. Author Aisyah wrote the first draft of the manuscript. Authors AY and YA managed the outline of the manuscript and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Biofloc technology is a new alternative in addressing water quality problems in aquaculture which is adapted from conventional domestic waste processing techniques. The main principle applied in this technology is water quality management which is based on the ability of heterotrophic bacteria to utilize organic and inorganic N in water. In a balanced C and N condition in water, heterotrophic bacteria which will utilize N, both in organic and inorganic form, are present in water for the formation of biomass so that the concentration of N in water is reduced. Several types of bacteria that are often used in biofloc are *Bacillus sp., Bacillus subtilis*, *Pseudomonas sp., Bacillus lichenoformis, Bacillus pumilus*; *Lactobacillus* sp.; *Bacillus megaterium* . Microbes play key role in the biofloc systems. Microbes associated with floc after consumption help to improve digestion, reduces FCR reduces dietary protein level and heterotrophic bacteria, which together probiotic bacteria, inhibit the development of potential pathogen bacteria. With biofloc there are some improves in different aspects during culture like higher growth rates, increased survival, improved water quality, reduced amount of water used and decrease in diseases. Thus, role of microorganisms is important in biofloc system; therefore, it is necessary to carry out more studies related to identification of microbes that can be present in biofloc systems.

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1. INTRODUCTION

The high level of organic waste from artificial feed residue (pellets) and feces resulting from intensive catfish rearing will cause accumulation and deposition at the bottom of the rearing water medium, so a decomposition process is required. If the maintenance medium is not decomposed, the anaerobic bacteria will decompose it and form toxic gases such as sulfuric acid, nitrite, and ammonia and have a negative impact on the metabolism of the cultivated organism until death. To reduce waste organic matter and waste that will be disposed of into public waters, it is necessary to manage water quality so that the maintenance media remains in good condition. One of the efforts is a biological approach by utilizing bacterial activity to accelerate the decomposition process of organic waste [1]

Along with technological developments through a biological approach, biofloc technology has been applied to maintain the quality of cultivated waters. Biofloc technology is a technology using both heterotrophic and autotrophic bacteria that can intensively convert organic waste into a collection of microorganisms in the form of floc, which can then be used by fish as a food source [2,3]. In the floc there are several forming organisms such as bacteria, plankton, fungi, algae, and suspended particles which affect the structure and nutritional content of the biofloc, however the bacterial community is the most dominant microorganism in floc formation in the biofloc [4,5].

Biofloc technology is a new alternative in addressing water quality problems in aquaculture which is adapted from conventional domestic waste processing techniques [5,6]. The main principle applied in this technology is water quality management which is based on the ability of heterotrophic bacteria to utilize organic and inorganic N in water. In a balanced C and N condition in water, heterotrophic bacteria which will utilize N, both in organic and inorganic form, are present in water for the formation of biomass so that the concentration of N in water is reduced [5]. Theoretically, the utilization of N by heterotrophic bacteria in aquaculture systems is presented in the following chemical reaction [7]; NH_4^+ + = 1.18 $C_6H_{12}O_6$ + $HC0_3^-$ + 2.06O_2 -> $C_5H_7O_2N$ + 6.06 H_2O + 3.07 CO_2

In general, the following benefits can be generated from biofloc are prevention of the introduction of diseases to fish farms from incoming water, improvement of biosecurity, improvement of feed conversion, improvement of water quality improvement of water use efficiency, increasing land use efficiency, reduction of sensitivity to light fluctuations [8.9]

2. HISTORY OF BIOFLOC SYSTEM

At 1970's at French Research Institute for Exploration of the Sea, Oceanic Center of Pacific found biofloc system [10]. These types of systems with active microbial suspensions through continuous water circulation system was used for culturing various penaeid species including *Litopenaeus vannamei, L. stylirostris, Penaeus monodon* and *Fenneropenaeus merguiensis* [10,11,12]. After that they produced a world record of 20– 25 ton ha 1 year 1 with two crops, in a similar limited water exchange system leading to a better understanding of the benefits of biofloc to shrimps cultured *L. stylirostris* and *L. vannamei* in terms of increasing yield [13].

Back to 1980's by Steve Serfling and Dominick Mendola in solar aquafarms in California [14]. They developed an active microbial suspension system termed 'microbial soup' for farming of shrimp and fish [14]. Later, Balfour Hepher and his colleagues in Israel developed the concept of a 'heterotrophic food web', which was encouraged by constantly keeping uneaten feed and excreta suspended by paddle wheels installed in the ponds/tanks [14]. The high dissolved oxygen as well continuous mixing were suggested to aid in the ability of heterotrophic bacteria to partly convert the suspended organic material into microbial biomass, which flocculated and became available as additional nutrients to the fish [15].

During the mid-1990's, biofloc is developed what is today [16]. This system was developed to be a minimal to zero water exchange system and the addition of a carbon substrate that is low in nitrogen, were added to prevent the accumulation of nitrogen [14]. The idea of adding carbon substrate, where it was observed that bacteria feeding on carbonaceous substrate that are poor in nitrogen did take up nitrogen from water to produce cell proteins [12].

The high turbidity caused by the bioflocs appeared to go against the principle that the clearer pond water the better [17]. The eventual acceptance of biofloc was mainly due to reasons including; increased scarcity of freshwater, enacting stricter regulations on the amount of wastewater discharge by some developed countries [18] and severe outbreaks of viral shrimp diseases, which was fast spreading among the neighbouring farms that have connecting water use.

3. FACTORS AFFECTING THE BIOFLOC SYSTEM

The formation of biofloc occurs through the stirring of organic matter by aeration to dissolve it in the water column to stimulate the development of aerobic heterotrophic bacteria (sufficient oxygen conditions) attach to organic particles, decompose organic matter (take up C-organic), then absorb minerals such as ammonia, phosphate and other nutrients. in water.So that the beneficial bacteria will reproduce properly. These bacteria will form a consortium and floc formation occurs. The result is that the water quality is better and organic matter is recycled into flocks that can be eaten by fish [19,20]

Heterotrophic bacteria in pond water will thrive if the pond water is added with a source of (C) carbohydrates which can be directly utilized, for example sucrose, molasses and tapioca flour. These bacteria will use inorganic N, especially ammonia in water and synthesized into bacterial protein and single cell protein which can be used as a source of feed for reared shrimp or fish [21].

3.1 Biofloc Forming Bacteria

Biofloc formation by bacteria, especially heterotrophic bacteria, generally aims to increase nutrient utilization. avoiding environmental stress and predation [5,22]. The bacterial floc is composed of a mixture of various types of micro-organisms (floc-forming bacteria, filamentous bacteria, fungi), suspended particles, various colloids and organic polymers, various cations and dead cells [4,5,23] with sizes varying in the range of 100 - 1000 μm [5,24]. Apart from bacterial flocks, various other types of organisms are also found in biofloc such as protozoa, rotifers and oligochaeta [24,25]. The composition of the organisms in the flock will affect the structure of the biofloc and the nutrient content of the biofloc [26, 27].

In biofloc which was dominated by bacteria and green microalgae contained higher protein (38 and 42% protein) than biofloc which was dominated by diatom (26%) [27]. Abiotic environmental conditions also affect the formation of biofloc such as C / N ratio, pH, temperature and stirring speed [5,6,28]. Meanwhile, the mechanism of floc formation by bacterial communities is a complex process which is a combination of various physical, chemical and biological phenomena such as physical and chemical surface interactions of bacteria, and quorum sensing as biological control.

In the biofloc system, bacteria play a dominant role as heterotrophic organisms that produce polyhydroxy alkanoates which are useful in forming biofloc bonds. The growth of heterotrophic bacteria is influenced by the presence of dissolved organic carbon in water. Organic carbon elements will bind inorganic nitrogen which can be used for the growth of heterotrophic bacterial cells. Ammonia immobilization by heterotrophic bacteria is 40 times faster than by nitrifying bacteria. In the heterotrophic process, heterotrophic bacteria convert ammonia directly into bacterial biomass [29].

Bacteria capable of forming biofloc include *Zooglea ramigera, Escherichia intermedia, Paracolobacterium aerogenoids, Bacillus subtilis, Bacillus cereus, Flavobacterium, Pseudomonas alcaligenes, Sphaerotillus natans*, Tetrad and *Tricoda*. The characteristic feature of biofloc forming bacteria is its ability to synthesize polyhydroxy alkanoate (PHA) compounds, especially specific ones such as poly β-hydroxy butyrate. This compound is needed as a polymer material for the formation of polymer bonds between the substances forming the biofloc. *Bacillus* sp. and *Pseudomonas* sp. is a bacterial genera that can utilize carbon components and also has the ability to oxidize substrates containing C chains. *Bacillus* sp. bacteria. can produce a wide range of enzymes and is the most effective way to break down proteins.

3.2 Carbon Sources

Several sources of carbohydrates can be used as a source of carbon (C) for the formation of biofloc such as tapioca flour, cassava flour, sugar, and molasses. Molasses is a by-product that comes from making sugar cane (*Saccharum officinarum L*). United Molases defines molasses as an "end product" for the manufacture of sugar that no longer contains sugar that can be crystallized by conventional means. Molasses itself is a thick liquid and is obtained from the separation of sugar crystals. Molasses can no longer be formed into sucrose but it still contains high levels of sugar, amino acids and minerals. The sugar content in molasses is 75% and dry matter is 62% [30].

3.3 Source of Nitrogen

Nitrogen in water is usually found in the form of ammonia (NH3), ammonium ($NH4^{+}$), nitrite (NO2-) and nitrate (NO3-) and several other organic nitrogen compounds. These nitrogen compounds are greatly influenced by the oxygen content in water, when the oxygen content is low, nitrogen changes to ammonia (NH3) and when the oxygen content is high, nitrogen changes to nitrate (NO3).

Broadly speaking, the conversion of N in water and sediment is grouped into three types, namely photoautotrophic conversion by algae and aquatic plants, chemoautotrophic by oxidation by nitrifying bacteria and immobilized through heterotrophic by heterotrophic bacteria [7].

Nitrogen in aquaculture systems mainly comes from artificial feed which usually contains protein in the range 13 - 60% (2 - 10% N) depending on the needs and stage of the organism being cultured. Protein in feed will be digested, but only 20-30% of the total nitrogen in the feed is used as fish biomass, the remaining nitrogen in the feed is in the form of metabolic waste in the form of urine and feces and uneaten feed.

Protein catabolism in the body of aquatic organisms produces ammonia as a final product and is excreted in the form of non-ionized ammonia (NH_3) through the gills. At the same time, the bacteria mineralize the organic nitrogen in the inedible feed and feces to become ammonia. As a result of these two processes, the application of high protein feed in the culture system will result in the accumulation of ammonia both as a result of excretion from the cultured organism and as a result of bacterial mineralization.

The presence of non-ionized ammonia (NH_3) in the culture medium is avoided because it is toxic to aquatic organisms even at low concentrations. The ammonia concentration in the culture medium must be lower than 0.8 mg / L to avoid the toxic effects of ammonia on aquatic organisms.

3.4 Availability of Aeration

The high bacterial density in water will lead to higher oxygen demand so that aeration for oxygen supply in the application of biofloc technology is indispensable. In addition to playing a role in supplying oxygen, aeration also functions to stir water so that biofloc suspended in the water column does not settle. Precipitation of biofloc at the bottom of the container must be avoided in addition to preventing anaerobic conditions at the bottom of the container due to the accumulation of biofloc, as well as to ensure that biofloc can still be consumed by cultured organisms.

4. SOURCE OF BACTERIA FROM THE BIOFLOC SYSTEM

Several types of bacteria that are often used in biofloc are *Bacillus sp., Bacillus subtilis*, *Pseudomonas sp., Bacillus lichenoformis, Bacillus pumilus* [31]; *Bacillus megaterium* [32,33]. Of the several types of bacteria, *B. megaterium* is a heterotrophic bacterium that is rarely applied but has a good role in improving water quality in the application of biofloc technology [32]. Besides being able to improve water quality, biofloc technology is expected to increase feed efficiency which affects the weight gain of fish.

5. MECHANISM OF BACTERIA IN THE BIOFLOC SYSTEM

The maintenance of water quality, mainly by the control of bacterial community over autotrophic microorganisms, is achieved using a high carbon-nitrogen (C:N) ratio. High carbon-to-nitrogen ratio is required to guarantee optimum heterotrophic bacteria growth and to produce new bacterial cells [42]. The heterotrophic bacteria are responsible for capturing nitrogenous compounds released by the fish and use them in their growth, thus eliminating ammonium and nitrite toxicity [38]. Thus, the microbial protein generated within the system is utilized by cultured species as a part of diet and nitrogen content in the system is assimilated in the cultured species.

Table 1. Comparison of bacteria found in biofloc systems

No.	Source of C	Bacteria Found	Species cultivated on Biofloc	Result	Reference
1	Corn meal	*Bacillus sp.*	*Litopenaeus vannamei*	Improvement in water quality by reducing nitrogen content in the system and assimilating in the cultured species:-It was significantly higher than the 34.7% recycled into shrimp biomass in the control.	[34]
2	Tapioca starch	Rotifers: Lecane, Trichocerca, Polyarthra and Asplanchna. Oligochaeta: Tubifex	*Macrobrachium rosenbergii*	Phytoplankton & algal periphyton : as autotrophic organisms, forming the base of the aquatic food web Heterotrophic organisms: contributing as consumer or decomposer to the pond ecosystem.	[35]
3	Tapioca flour wheatflour	Vibrionaceae, Enterobacteriaceae Alteromonadaceae and Micrococcaceae	*Litopenaeus vannamei*	Improves water quality than control	[36]
4	Sugar beet molasses and sugar corn starch	The highest total number of bacteria and Lactobacillus spp. was observed in corn starch and then sugar beet mollases	*Cyrinus carpio*	Helps to improve digestion and growth of an animal.	[37]
5	Sucrose	Heterotrophic bacteria, filamentous cyanobacteria, dinoflagellates, flagellates, ciliates and rotifers.	*Marsupenaeus japonicus*	Boflocs treatment resulted in a higher shrimp yield, higher protein efficiency ratio, and lower feed conversion rate.	[31]
6	Wheat flour	Protozoa, Rotifera Oligochaeta Paramecium, Tetrahymena and Petalomonas	*Oreochromis niloticus*	Total numbers of organisms are increase in low protein fed tanks and increase high protein fed tank higher than control.	[38]
7	Wheat bran and molasses	Phytoplankton, periphyton, zooplankton, microbial floc and benthic macro invertebrates reported in freshwater prawn monoculture ponds.	*Farfantepenaeus paulensis*	Play important roles in the maintenance of water quality and in the provision of essential nutrients for shrimp.	[39]
8	Sugarcane molasses	Vibrionaceae, Enterobacteriaceae Alteromonadaceae and	*Litopenaeus vannamei*	Improves water quality than control	[36]

No.	Source of C	Bacteria Found	Species cultivated on Biofloc	Result	Reference
		Micrococcaceae			
9	Molasses (M) Molasses + rice powder (M+RP)	Abundant group: Tintinids, Ciliates, Copepods, Spirulina and Nematodes.	*Oreochromis niloticus*	Growth of heterotrophic bacteria, which together probiotic bacteria, inhibit the development of potential pathogen bacteria in aquaculture	[40]
10	Glucose (Glu) - Starch (Sta) Glycerol (Gly)	At glycerol dominant in Proteobacteria and Bacteroidetes. While at glucose and starch Cyanobacteria.	-	Proteobacteria : symbiotic bacteria in aquaculture : removes organic matter.	[41]

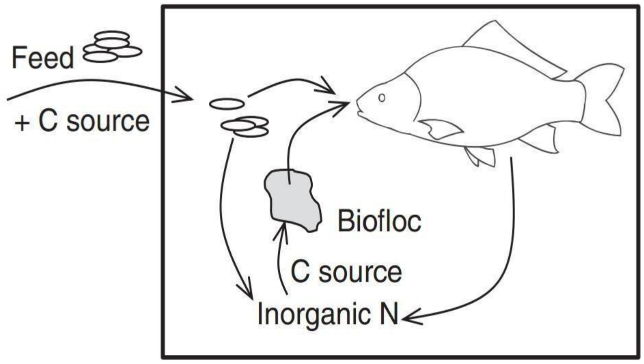

Fig. 1. Illustration of Biofloc System [46]

The mean N content of the shrimp biomass harvested was higher than the shrimp harvested from without biofloc system [34]. In biofloc, diverse planktonic groups develop in a natural way, such as rotifers, protozoans (ciliates and flagellates), crustaceans and nematodes which play an important role in nutrient recycle, maintenance of water quality and in nutrition of cultured animals [43]. Zooplantkton present in the biofloc help to increase growth rate, improves feed conversion ratio [44]. Phytoplankton uses nutrients released from water for growth and produces dissolved oxygen during respiration. The diversity of microbes associated with biofloc depends on the carbon source and the cultured species [45].

The effects of bioflocs on growth, feed digestibility and utilization, and immune response of tilapia have been investigated by a number of researchers. Long [47] assessed the effects of BT on the growth, digestive enzymes activity, haematology, and immune response of GIFT tilapia, Glucose was added as a carbon source to the biofloc system to keep a carbon/nitrogen (C/N) ratio of 15/1.

Biofloc shares some similarities with probiotics, since they both contain live microbes. While probiotics consist of live microbial cells, bioflocs are complex aggregates of living, dead cells, colloids, cations and organic polymers [48]. Some of the active compounds in bioflocs include bromophenols, carotenoids, chlorophylls, poly-beta-hydroxybutyrate and phytosterols [27; 48; 49] and some of these are well known to have anti-bacterial properties [50].

6. CONCLUSION

Microbes play key role in the biofloc systems. Microbes associated with floc after consumption help to improve digestion, reduces FCR reduces dietary protein level and heterotrophic bacteria, which together probiotic bacteria, inhibit the development of potential pathogen bacteria. With biofloc there are some improves in different aspects during culture like higher growth rates, increased survival, improved water quality, reduced amount of water used and decrease in diseases. Thus, role of microorganisms is important in biofloc system; therefore, it is necessary to do more studies related to identification of microbes that can be present in biofloc systems.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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