Improvement of water quality (Cu, Fe, Zn) in biofloc aquaponics systems by utilizing fish waste as a source of micronutrients

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Abstract. Biofloc Technology (BFT) has been used successfully in aquaculture and offers several advantages, one of which is improving the water quality. Fish and plant cultivation in the aquaponic system has been widely practiced in Indonesia, but very few BFTs are used in aquaponics, especially those related to micro-nutrient components. For this purpose, to improve water quality, we used BFT in a Nutrient Film Technique (NFT) Aquaponic Method. Cu (0.0193-0.3290 mg L^{-1}), Fe (0.4849-0.6133 mg L^{-1}), and Zn (0.1721-0.2589 mg L^{-1}) appeared to fluctuate from day-0 to 42. Even though there are parameters (Fe, Zn) that have exceeded the threshold, nitrifying bacteria, such as Biolacto bacteria, can develop well. The bacteria is capable of forming light brown flocks that do not show symptoms of heavy metal poisoning, so tilapia (*Oreochromis niloticus*) and Samhong mustard (*Brassica juncea* L.) may accelerate their growth. The fish weight growth rate for the aquaponics system using biofloc was 4.9438%, and the length growth rate for fish was 2.923%, while in the aquaponics system without biofloc, the fish growth weight was 3.85%, and the growth rate for fish length was 2.86%. Therefore, to improve water quality, the application of BFT in aquaponics is feasible, and more research is required to improve this technology.
Key Words: aquaponics, BFT, biolacto, NFT, water quality.

Introduction. Traditional aquaculture faces significant environmental problems, such as high water use, extensive land use, and nitrogen and phosphorus chemical-rich water production (Buhmann et al 2015; Hu et al 2015), primarily due to feed assimilation of cultivated species and poor feed management practices (Mariscal-Lagarda et al 2012). To meet high standards of productivity and minimum environmental impact, new strategies are needed. In modern aquaculture systems, profitability depends on the growth of fish and the density of fish stocks (Andrade et al 2015). The development of high-density systems, however, may contribute to the accumulation of toxic organic matter and inorganic nitrogen, and can interfere with sustainable cultivation development (Crab et al 2012).

Stress due to increased stocking density has detrimental effects on growth, feed utilization, antioxidant activity, immune system, well-being, and health of fish (Santos et al 2010; Suarez et al 2015; Naderi et al 2019). Stress can be reduced by improving immunity.

One of the ways to overcome the problems above is with biofloc-based aquaculture. The basic principle of biofloc technology (BFT) is the conversion of microbes from nitrogenous waste to microbial biomass (biofloc), which can be used *in situ* as a source of additional nutrients for fish. Microbial flocks continuously provide additional proteins (essential amino acids), polyunsaturated fatty acids, vitamins, and minerals (Ahmad et al 2019; Ebrahimi et al 2020). BFT was created to minimize waste disposal and improve biosecurity in aquaculture (Emerenciano et al 2011; Abbaszadeh et al

2019). In BFT, the balance between carbon and nitrogen stimulates the growth of microbial communities consisting of bacteria, microalgae, protozoa, and other invertebrates that play an important role in natural productivity, water quality, nutrient cycling, and serve as a complementary food source for livestock (Emerenciano et al 2011; Ahmad et al 2019; Ebrahimi et al 2020). However, high nutrient content, low to no water exchange, and a high stocking density can have negative effects (Crab et al 2012; Avnimelech 2015; Quinta et al 2015). BFT should have a continuous recirculation of water (Widanarni et al 2012; Krummenauer et al 2014) or recycling of nutrients for the processing of vegetables in an aquaponic environment (Buhmann et al 2015). The biofloc system has beneficial effects on growth, feed use, function of digestive enzymes, immune system, and stress and disease resistance (Fauji et al 2018; Bakhshi et al 2018; Liu et al 2018).

In this case, aquaponics may be an option that seeks to diversify the development of aquaculture (Buzby & Lin 2014). Aquaponics requires the integration of hydroponics with recirculating aquaculture systems, in which water is effectively recirculated for optimum plant nutrient assimilation (Deswati et al 2018; Deswati et al 2019; Deswati et al 2020a; Deswati et al 2020b). This is made possible by the symbiotic relationship between fish, bacteria, and plants (Liang & Chien 2013; Somerville et al 2014), in which bacteria naturally convert nutritional waste from fish farming into products that plants can absorb and are thus profitable, with a good plant growth and water quality (Espinosa Moya et al 2014). In aquaponics, a limited region, with product diversification, reasonable water usage, and practical complete use of feed, can provide high fish and plant productivity (Dediu et al 2012). The prospect of producing and offering food without pesticides or antibiotics in dry areas makes aquaponics a method that offers a high value-added, sustainable commodity. In an aquaponic environment, various and diverse plants can be cultivated (Love et al 2015). Furthermore, the development of leafy vegetables such as lettuce is the most promising because of its high market popularity, rapid growth, minimal nutritional requirements, and exceptional adaptation to aquaponic methods (Deswati et al 2018; Deswati et al 2019; Deswati et al 2020a; Deswati et al 2020b).

In the aquaponics system in Indonesia, recirculating aquaculture and hydroponics have been introduced and have become attractive models. Furthermore, aquaponics have been used to increase water quality, to minimize water loss and negative environmental impacts (Deswati et al 2019; Deswati et al 2020a; Deswati et al 2020b). In addition, the application of aquaponics can remove dissolved N and P at low concentrations. Therefore, aquaponics is an alternative technology to address the negative environmental impacts commonly associated with intensive fish farming and the activity of plants.

In aquaculture, BFT has been successfully used and provides many benefits, one of which is improving water quality. Fish and plant cultivation in aquaponics systems has been widely practiced in Indonesia, but very little BFT is used in aquaponics. We are attempting to perfect aquaponics in this research paper by pragmatically adopting biofloc technology, combining the new aquaculture systems with the new hydroponic systems. The presence of a rich microbiota and various micro and macronutrients, especially nitrates, usually results in a higher nitrate concentration in the BFT system compared to other systems. In the aquaponics system, the use of BFT is still debatable, and more research is needed.

Deswati et al (2018, 2019) conducted research on water quality using the field and drain (F&D-AP) method and nutrient film technique (NFT-AP) methods without employing biofloc. Furthermore, Deswati et al (2020a, 2020b, 2020c, 2020d) added biofloc to the F&D-AP method, increasing water quality. The aim of this paper is to discuss the use of biofloc in the NFT-AP method to improve water quality (Cu, Fe, Zn concentrations).

Material and Method. This study was conducted from January to June 2021 at Blasta Hydroponics House in Padang. Sample analysis was also performed at Andalas University's Applied Analytical Laboratory, which is part of the Department of Chemistry, Faculty of Mathematics and Natural Sciences.

Instrumentation and materials. Atomic absorption spectrophotometer (AAS) AA 240, analytical balance (Shimadzu), hot plate, fish tank (FT, 1 unit), mechanical filter tank (MFT, 1 unit), biofilter tank (BT, 1 unit), sum tank (ST, 1 unit), hydroponic subsystem (HS, 1 set), PVC pipe, water pump, water container, aerator, desiccator, and glassware were the instruments used in this research.

The materials used in this study were fish feed, Samhong mustard, planting media (rockwall), water from a tank with a density of 1000 ind. 0.8 m^{-3} (2-3 cm). Nile tilapia (*Oreochromis niloticus*) were used in this study. Biolacto bacteria, banana, eggs, pineapple, vitamin C, complex of vitamin B, yeast. Baker's yeast, yakult, molasses, granulated sugar, and chemicals were also used in the study.

Biofloc culture of bacteria. Biolacto bacteria (100 g), banana (150 g), pineapple (650 g), eggs (165 g), vitamin C (3 items), vitamin B complex (3 items), cassava fermentation (11.2 g), yeast (11 g), sugar granulate (1.25 kg), yakult (455 mL), molasses (75 mL) were used for the cultivation. The bacteria culture container used was a container with 19 L of water. A blender was used to mash some of the ingredients that have been prepared, including: pineapple, banana, vitamin C, vitamin B complex, and yeast tape. The egg yolk, sugar, refined ingredients and the Biolacto bacteria were added to the container. The aerator was installed and the system was closed so that no air could enter. The bacterial culture has been carried out for 2 weeks, and the active bacterial culture was characterized by mild fermented yeast odor.

Nutrition application in the biofloc system. Biofloc nutrients, including: 50 g of dolomite lime, 250 g of salt, 75 mL of molasses and 80 mL of Biolacto bacteria per week were added to the FT per week.

Aquaponic systems series. There are 4 tanks in the NFT aquaponics system, namely FT (119 cm length, 100 cm width and 74 cm height), MFT (110 dm^3), BT (110 dm^3), ST (110 dm^3) and HS (rockwool as planting medium). FT (0.88 m^3 of water, 1000 tilapia fingerlings). The water from FT is flowed to the MFT, BT, ST, HS, and eventually back to the FT (Figure 1). This recirculation was constantly carried out and supported by a pump. The MFT was fitted with a plastic filter for the filtration of solid matter or water sediment. The MFT was filled with 110 dm^3 water.

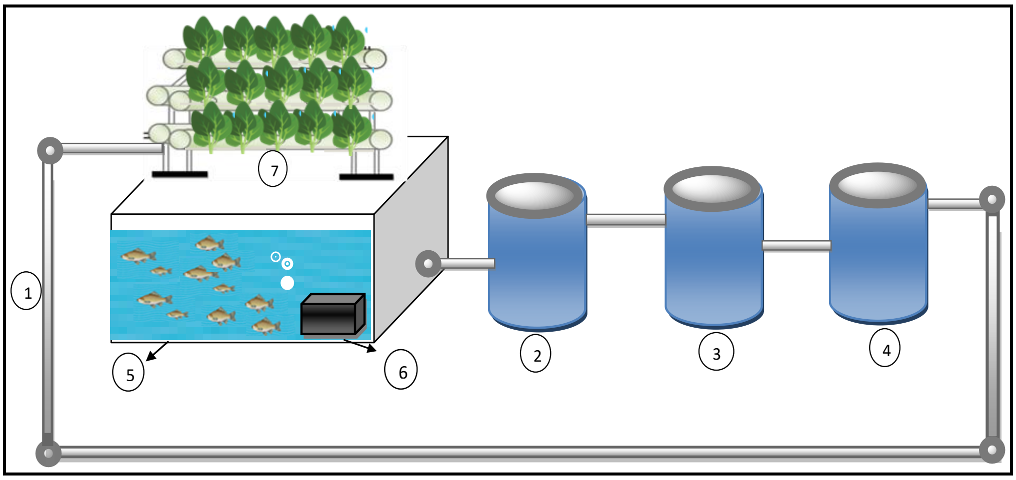

Figure 1. The circuit for the NFT aquaponic system; 1 - pipe; 2 - mechanical filter tank (MFT); 3 - biofliter tank (BT); 4 - sum tank (ST); 5 - fish tank (FT); 6 - aerator; 7 - hydroponic subsystem (HS).

In the BT, aerators, plastic filters, bio-balls, and bio-rings were used. Aerators maintained the oxygen required by microorganisms. The plastic filter helped to filter out residual solids or unfiltered sediments. Bioballs and biorings were installed at the bottom, to increase the surface for nitrifying bacteria. BT was filled with 110 dm^3 of water, with an addition of a 1:1 mixture of *Nitrosomonas* spp. and *Nitrobacter* spp.

The hydroponic plants used were Samhong mustard. It was sown for 2 weeks before being moved to HS. Rockwool, which had been cut into 1x1 cm pieces and inserted into a hollow pipe, was the growth medium used.

Analyzing the parameters of the aquaponic system. Before taking water samples from BT, HS, and FT, the aerator was turned off. Furthermore, Cu, Fe, and Zn concentrations in water, as well as Cu, Fe, and Zn concentrations in fish feed, were measured with an Atomic Absorption Spectrophotometer (AAS) at wavelengths of 324.7 nm, 248.3 nm, and 213.9 nm for Cu, Fe, and Zn, respectively. The results were compared with the water quality standards based on the Indonesian Government Regulation of the Republic of Indonesia Number 22 of 2021 class III.

Results and Discussion. Fish feed contains key micro elements such as Cu, Fe, and Zn, which play an important role in fish growth. Fish and plants require certain metals in modest amounts, but high metal concentrations in water can poison fish and plants. The concentrations of heavy metals in fish feed were 12.9651, 103.1295, 40.6344 mg kg^{-1}, for Cu, Fe and Zn, respectively.

Cu concentration. The Cu concentration at day-0 to 42 continued to decrease (Figure 2), from 0.7225 to 0.0185 mg L^{-1}. From day-0 to 14 it decreased considerably, whereas the fluctuation was slight from day-14 to 42. However, Cu concentrations were still high on day-0 and 7, above the standard of quality, namely >0.02 mg L^{-1}, being one of the causes of the ineffectiveness of both FT and BT in the aquaponics system.

Copper is a micronutrient needed by plants in small quantities. In the aquaponics system, one of the sources of Cu originates from fish feed (12.9651 mg kg^{-1}) and it is usually still below the quality level (<100 mg kg^{-1}) (Alloway 2013). Fish are fed daily based on their percentage of body weight. Unconsumed food and fish waste collect at the bottom of the FT, potentially affecting water quality. The BFT, which is designed to increase the quality of water, also tends to function optimally. According to Somerville et al (2014), a high concentration of Cu in water can intoxicate fish and plants (Alloway 2013).

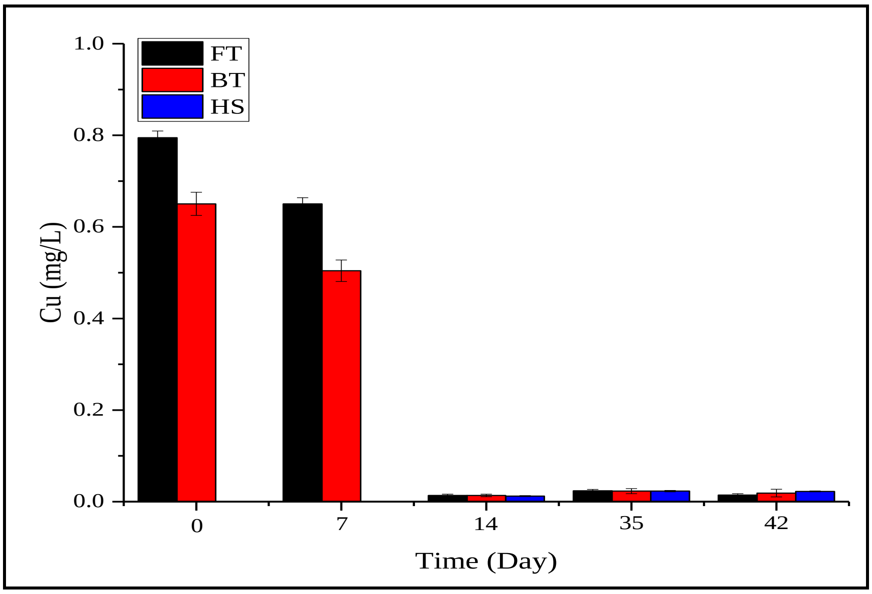

Figure 2. Cu (mg L^{-1}) concentration at various periods (days); FT - fish tank; BT - biofilter tank; HS - hydroponic subsystem.

In addition, Cu concentration decreases and reaches the quality level from day-14 to 42, and it is ideal for use in the aquaponic method for fish and plant cultivation. Since there is a bio-corral in the BFT, namely porous limestone, which acts as an adsorbent that can absorb Cu levels in water, the Cu concentration in BFT decreased. Limestone can be efficient in absorbing metals (Aziz et al 2001). The Cu concentration was also low after passing through the HS, and it is thought that the Cu bonded to plant roots is not taken back by water during the water recirculation process.

The obtained Cu concentrations indicate that BFT is suitable for use in aquaponic systems. High water concentrations of Cu can interfere with fish and plant growth. The chronic effects of Cu in fish can lead to renal cell changes, decreased growth and decreased immune response (Mega 2013). Cu concentrations of 2.5-3 mg L^{-1} in waters are strong enough to kill fish; at concentrations higher than 0.1 mg L^{-1}, Cu can harm plants (Widyaningrum et al 2007). High concentrations of Cu in water can lead to the accumulation in fish and plants, which are not suitable for consumption (Kamran et al 2013). The concentration of Cu obtained in this study is still acceptable for fish and plant growth.

Fe concentration. Based on Figure 3, the Fe concentration from day-0 to 42 fluctuated between 0.4849 and 0.6133 mg L^{-1} and exceeded the acceptable normal limit of 0.3 mg L^{-1}. Fe is a micronutrient required by plants in small quantities. Fish feed (103.1377 mg kg^{-1}) is one of the sources of Fe used in the aquaponics system and is above the quality level (< 100 mg/kg) (Hasmalina et al 2017).

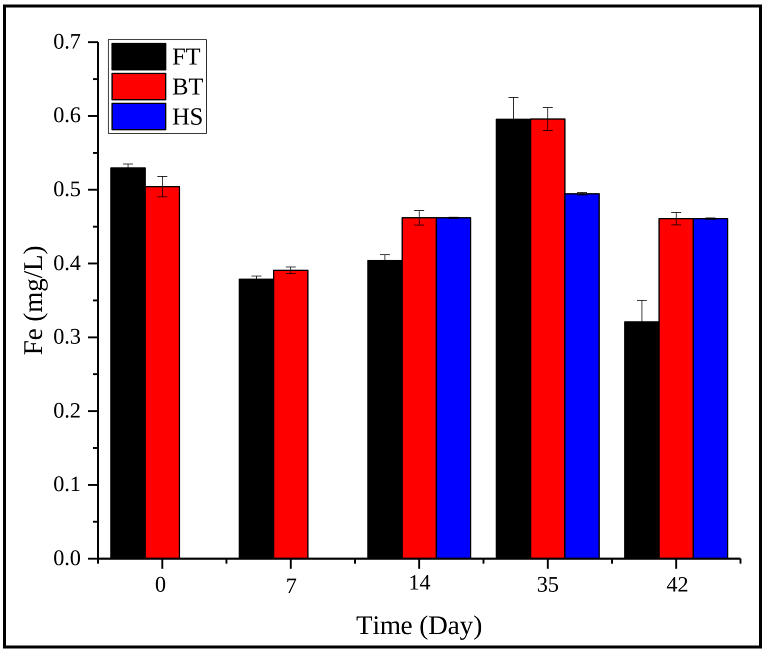

Figure 3. Fe (mg L^{-1}) concentration at various periods (days); FT - fish tank; BT - biofilter tank; HS - hydroponic subsystem.

The Fe value tends to decrease from day-0 to 7, presumably because Fe is required for fish growth, so that some of the Fe has been absorbed by the fish, as well as by hydroponic plants. Furthermore, the decrease in Fe concentration may be due to absorption or metal filtration processes in calcareous material contained in BT (Mega 2013).

The Fe concentration appeared to increase from day-7 to 35, possibly due to an increase in the amount of feed provided daily according to the growth of the fish, which also caused an increase in the remaining feed and feces of the fish, being one of the Fe sources that entered the system. After passing the HS, the Fe concentration was the same or decreased considerably, since the plant acts as a biofilter that can absorb Fe in water, thereby reducing the Fe concentration in water.

The Fe concentration decreased again from day-35 to 42, possibly because the bacteria were already employed, but not yet optimally improving the quality of the water. Biolacto bacteria breaks down the fish feed waste and feces in the FT into nutrients required by plants. In BT, the concentration of Fe decreased, because of the adsorbent of Fe, being believed that the biofilter was running properly.

Zn concentration. Zn is a micronutrient required by plants in small quantities. Fish feed (40.6384 mg kg^{-1}) is one of the sources of Zn used in the aquaponics system and is still below the quality level (< 100 mg kg^{-1}) (Hasmalina et al 2017).

The concentration of Zn fluctuated between day-0 and 42 (Figure 4), from 0.1721 to 0.2589 mg L^{-1}). The concentration of Zn is above the standard value (0.05 mg L^{-1}). The high concentration of Zn is due to the accumulation of residues from fish feed and organic waste. The Zn concentration in the aquaponics system using BFT, however, provides plants with the adequate concentration. In water, the concentration of Zn was high, which means that the water was polluted with Zn and likely affected fish and plants. In addition, a high concentration can have a negative effect on fish, causing structural damage, affecting fish growth and survival (Afshan et al 2014); the continued accumulation of Zn in fish can be caused by high concentrations of Zn in the water.

If Zn accumulates in the gills of fish, it can cause hypoxia, leading to its death. The danger of Zn is serious because it is not restricted to the environment and cannot be biologically destroyed (Afshan et al 2014; Gusain et al 2018). Zn accumulation can also happen in plants if the concentration in the water is too high. According to Alloway (2013), the signs of plant poisoning caused by Zn can be chlorosis, which occurs in the strands of young leaves, and leaf color change to yellow or white (Alloway 2013).

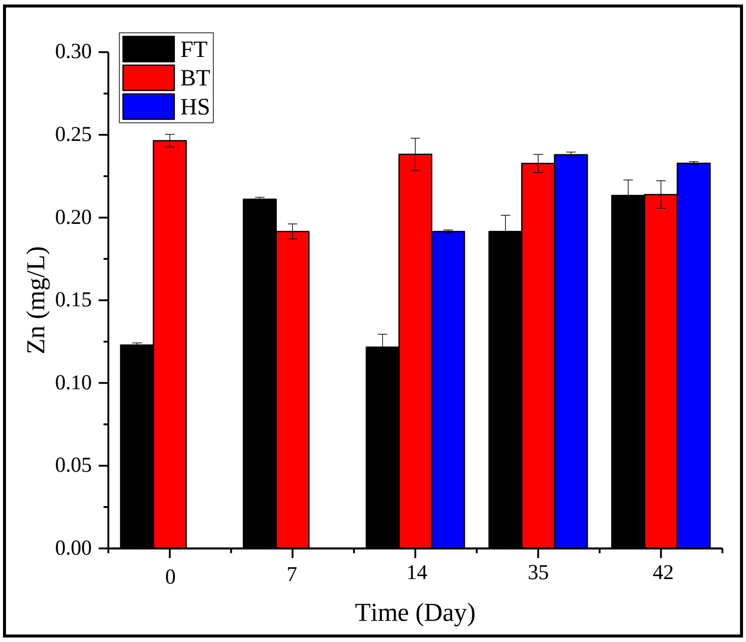

Figure 4. Zn (mg L^{-1}) concentration at various periods (days); FT - fish tank; BT - biofilter tank; HS - hydroponic subsystem.

Based on the aforementioned investigation of Cu, Fe, and Zn concentrations, the presence of these metals has no negative impact on bacterial development since bacteria have a high metal affinity and can absorb heavy metals. Bacteria may flourish and are typically immune to heavy metals in heavy metal-contaminated settings because they create biosurfactant/emulsion compounds that can absorb various heavy metals such as Cd, Cr, Pb, Cu, and Zn (Ratna 2005). Biolacto bacteria are gram-positive, with a high adsorption capability (Patel et al 2017). This is owing to the high presence of peptidoglycan and teichoic acid in their cell walls. These bacteria contain an important resistance mechanism for preventing cell damage and attaching metal ions to the cell surface. Lactic acid bacteria are also vital for attaching metal ions to peptidoglycan hydroxyl groups and protein functional groups.

One indicator that bacteria can still thrive effectively and that biofloc has been successfully generated is the light brown watercolor in FT, which boosted fish and plant growth and exhibits no signs of heavy metal toxicity. The biofloc formation process is interrupted and even fails to form when the pool water is light brown and in the shape of clumps that travel with the current (Aiyushirota 2009), or when the color of the water in the pond is too heavy, green, or reddish-brown.

Conclusions. Researchers and practitioners in Indonesia have carried many integrated studies, experiments and actual farming regarding fish and plant cultivation in aquaponic systems, but only a few have used BFT. In order to improve Cu, Fe and Zn concentrations, we have used BFT in the Nutrient Film Technique - Aquaponic Method. Although the threshold has been exceeded by Fe and Zn, nitrifying bacteria can grow well, as can Biolacto bacteria, which are capable of producing flocks that are light brown in color and do not exhibit heavy metal poisoning symptoms. The better concentrations of Cu, Fe and Zn will eventually accelerate fish and plant growth. In order to improve water quality, the application of BFT in aquaponics is therefore feasible, and more research is required to improve this technology.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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