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Production Performance of Catfish (*Clarias gariepinus* Burchell, 1822) Cultured With Added Probiotic *Bacillus* sp. on Biofloc Technology

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Abstract

Biofloc technology (BFT) is one of the most developed aquaculture technologies, which aims to improve the efficiency of feed use by providing nutrients for flocs to be used by fish as a supplementary feed. Also, BFT serves to improve water quality through the breaking down of fish waste materials assisted by heterotrophic bacteria. Bacteria used in this study were *Bacillus* sp. as probiotics in BFT. This study aimed to examine the production performance of catfish maintained with a biofloc system on an industrial scale, without experimental design. The average weight of catfish when stocking was 5.9 ± 0.0 g/fish with a density of 7000 fish in 9 unit circular ponds. This studies showed after 78 days of culture, bodyweight gain about 28.6 g/fish to 41.7 g/fish, highest specific growth rate was K2 (2.4 ± 0.2% BW/day), the highest survival rate K2 (98.87 ± 6.64% BW/day), highest grow rate K1 (41.7 ± 5.8 g) and the best FCR K9 (0.95 ± 0.11).

INTRODUCTION

Catfish production in Indonesia has continued to increase every year. According to DJPB (Aquaculture bureau) data (Direktorat Jenderal Perikanan Budidaya, 2016), the catfish production target in 2016 was 1,217,100 tons and in 2019 catfish production target is 1,770,600 tons with an average annual increase of 13.75%. Catfish is one of the most popular consumption fish in Indonesia. As an effort to fulfill the catfish production target, there needs to be a technological innovation in culture, one of which is by conducting intensive culture by increasing fish stocking density. On the other hand, an increased fish stocking density could cause the accumulation of uneaten feed, and organic and inorganic materials which become waste material and are toxic for cultured fish, causing stress to fish, which makes them susceptible to diseases such as bacterial, mycological, or viral diseases (Sukenda *et al*., 2016).

One breakthrough in the catfish aquaculture system to overcome several issues especially in high-stocking density culture is biofloc technology (BFT). This technology is based on the conversion of inorganic nitrogen, especially ammonia, by heterotrophic bacteria into microbial biomass which could then be consumed by the cultured organism, which is aimed to improve water quality and improve feed

utilization efficiency (Ekasari, 2009). Biofloc technology can be done by adding organic carbon to the maintenance medium to increase the C/N ratio and to stimulate the growth of heterotrophic bacteria (Crab *et al*., 2007). The nutritional quality of biofloc is influenced by, among others, the composition of the biofloc microorganisms (Ekasari *et al*., 2014).

The positive effects of biofloc system on African catfish production might be explained by three factors, that is, biofloc environment provides good and stable water quality, served as an additional food source for the fish and the environment and consumption improved the fish robustness against stress and disease (Fauji *et al*., 2018)

According to Bakar *et al*. (2015), the waste from biofloc technology-cultured catfish could also be utilized as bacterial biomass (flocs) which results in a high growth rate and could improve survival rate. Catfish culture activity usually faces the motile Aeromonas septicemia disease caused by *Aeromonas hydrophila* which could cause mortality. High density could exacerbate the virulence of the infection. The disease could cause up to 80% or even 100% mortality in a relatively short time.

These facts were supported by Lukistyowati and Kurniasih (2012) who stated that the disease outbreak caused by *A. hydrophila* could cause up to 80-100% mortality in the fish population in a short time (1-2 weeks). In a biofloc medium, the bacteria *Bacillus* sp. administered is expected to suppress the growth of this harmful bacteria. This was stated in the study by (Ulkhaq *et al*., 2014) where the administration of the probiotic *Bacillus* sp. in catfish dumbo culture was able to suppress the growth of *A. hydrophila* and could result in a 92.23% survival rate. The present study was aimed to evaluate the production performance of Clarias gariepinus catfish with the administration of probiotic *Bacillus* sp. in the biofloc technology. The purpose of the current study was to study analyze the production performance of catfish raised using a biofloc system with the addition of *Bacillus* sp. as decomposing bacteria.

METHODOLOGY

Place and Time

This study was conducted in February to June 2018. Fish grow out were carry out in "Unit Pembesaran Kolam Bundar, Bos Lele Semplak Bogor", West Java. Analysis of the effects of pathogenic infections on fish and water contamination worked by Fish Health Laboratory and Fish Environment Laboratory, BDP FPIK IPB, by sending samples.

Research Material

The materials used in this study were 9 units of 3 m diameter pool (pond volume 9.2 m^3), 1 unit trickle filter, scale, hand net, and water tub. The materials used this study was catfish fingerling as much 63,000 fish (ABW 5.9 g/fish), 63 m^3 well water, *Bacillus* sp. as probiotic, commercial fish feed, and dolomite.

Research Design

This research is a pre-production trial of catfish culture on an industrial scale using the biofloc system. This study did not use a different treatment plan. In this study all units given the same treatment, as catfish culture in a circular ponds unit with the application of a biofloc system to reach market size.

Work Procedures

Media Preparation

This is not an experimental application but is already an industrial scale, this research is a continuation of previous (Yusuf *et al*., 2015). Ponds used 9 units of circular tarpaulin material with a metal wire mesh frame 7 mm. Dimensions of the pond were 1.10 m in height and 3 m in diameter. Water volume used in each pond of the nine ponds was 5.18 m3 with the LP 100 Resun aerator, 4 points airstone balls per unit, the water source was well water that had been

treated in a reservoir using a trickling filter and sedimentation. Ponds were placed indoors with a roof so that they were protected from rainwater and direct sunlight. Ponds are set up with coding K1, K2, K3, K4, K5, K6, K7, K8, and K9. In this experimental application, all units were gain the same treatment, that was 7000 fish/unit, using the same probiotic, at satiation feeding, and the same sources of water.

Pond Sterilization and Water Filling

Sterilization was conducted to kill any microorganisms in the ponds, with the expectation that the dominant microorganism to grow was *Bacillus* sp. Ponds were sterilized by washing using soap and rinsing with water then spraying a 60 ppm chlorine solution to the walls and base of the ponds. The other way is using 30 ppm chlorine that dissolved in water (Panigrahi *et al*., 2019). After that, the ponds left to dry for one day. Water was channeled into the ponds to a 60 cm depth and aerated until the smell of chlorine disappeared.

Probiotic Preparation

Firstly, sterilization of production equipment, then sterilization of raw materials such as pure molasses is heated into a production machine that has been set to a temperature of 100^{O}C for 10 minutes, put in a container, and cool at room temperature overnight. Run a contamination test trough molasses sample dilution, *Bacillus* sp. isolation, and coliform test. If the result was negative continued standard test feasibility of the probiotic bacteria by sample dilution of molasses, bacterial isolated, TPC (Total plate count) test, if it produces minimal 1 $\times$ 10^{6} CFU/ml *Bacillus* sp. (Yusuf *et al*., 2015) then probiotics are ready to use.

Floc Preparation in the Water in Each Pond

After the smell of chlorine was gone, the next step, development of the floc, was begun. Sea salt was added to ponds at a dose of 1 kg/m^3, then dolomitic lime $CaMg(CO_3)_2$ was added at a dose of 10 g/m^3. Finally, a probiotic that contained *Bacillus* sp. was added at a dose of 10 ml/m^3. Ponds were then aerated for 5-7 days for the floc to develop.

Catfish Fingerlings Distribution

Catfish fingerlings were distributed in the afternoon. Fingerlings were 7-8 cm long and stocking density was 7000 fish/unit. Before fingerlings were released, they were acclimatized for 15 minutes. After they were released on Day 0, the catfish fingerlings were not fed for 12 hours and had their behavior observed.

Production of Fermented Feed

To maintain bacterial homogeneity both in the pond and fish's digestive tract, a feed that containing *Bacillus* sp. was produced by adding bacteria to feed. The addition was done by mixing 5 ml of the probiotic that contained *Bacillus* sp. with 200 ml water and stirring until it dissolved. This probiotic solution was then mixed with feed at a dose of 5 ml/kg feed. The feed is stored in airtight containers. After 3 - 5 days, then fermented feed was ready, marked by the growth of mold.

Maintenance

Water quality management was conducted morning and afternoon before feeding. The feed (fermented feed) was given in the morning, afternoon, and evening. Probiotic was administered to the maintenance medium at a dose of 10 ml/m^3 every morning 15 - 30 minutes after feeding. Fish were not fed for half a day every 7 days, and a sampling of 10% of the stocking density per unit (weight increase) was conducted every 7 days.

Health and Feed Management

Fish health management was conducted based on biosecurity principles. If the pH was low, limewater was added to maintain the stability of the maintenance medium pH. Water was changed every week or if there was a fishy or sulfurous

odor detected. The volume of water changed was 30-50 % depending on the fish's condition. Feed was given *ad satiation* is the feeding method that gives feed as full as possible (Hastuti and Subandiyono, 2014), but if there was a loss of appetite, the feed was reduced or temporarily stopped. The behavior of fish and their physical conditions were observed, especially during feeding time. If any fish demonstrated any signs of illness, it was quickly placed in a separate pond.

Treatment for sick fish was treatment using high-concentration of salt, natural methods using medicinal plants such as the extract of guava leaves, banana pseudostem, betel leaves, and papaya leaves in the maintenance medium, and adding vitamins to feed. Culture equipment was not used shared (nets, water quality measuring tools that are immersed), especially between ponds where there were sick fish or where there had been sick fish to avoid disease cross-contamination.

Tested Parameters

Growth rate (GR)

Growth rate was calculated following equation Effendie (1997):

$GR = Wt - Wo$

GR = Growth rate (g)
Wt = Final mean weight (g)
Wo: Initial mean weight (g)

Specific growth rate

Specific growth rate fish was calculated using the equation as follows:

$$SGR = 100 \times \frac{(\ln Wt - \ln Wo)}{t}$$

SGR = Specific growth rate (%)
Wo = Initial mean weight (g)
Wt = Final mean weight (g)
t = Culture period (days)

Survival Rate (SR)

At the end of experiment fish were counted to determine the survival and mortality percentage according to following formula (Goddard, 1996):

$$SR = 100 \times \frac{Nt}{No}$$

SR = Survival Rate (%)
Nt = Number of fish at the end of experiment (fish)
No = Number of fish at the beginning of experiment (fish)

Feed conversion ratio (FCR)

$$FCR = \frac{\text{Total Feed}}{\text{Total Biomass}}$$

FCR = Feed conversion ratio
Total feed = Total amount of feed during culture period (kg)
Total Biomass = Total harvest biomass (kg)

Data Analysis

Production performance parameters include weight growth, growth rate (GR), specific growth rate (SGR), and survival rate (SR) referring to the Solanki *et al*. (2012), and the feed conversion ratio (FCR) refers to Yusuf *et al*. (2015). Growth was observed by sampling at weeks 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 of culture. Once the results were obtained, the data were analyzed using descriptive analysis and then presented in tables and figures using Ms. Excel.

RESULTS AND DISCUSSION

Production Performance

During the study, measurement of growth performance parameters which included absolute growth rate (GR), specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR) were measured. The average growth rate results are presented in Figure 1.

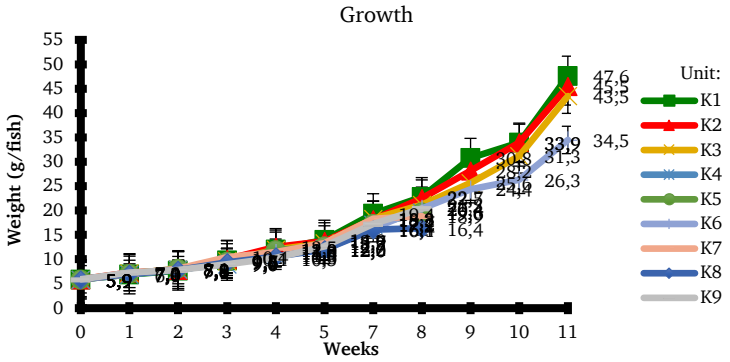

Figure 1. Average growth rate of catfish during culture of 11-week.

According to Figure 1. its showed that average catfish weight increased weekly. Fish's initial average weight at the beginning of the culture period was 5.9 ± 0.0 g/fish with a density of 7000 fish/pond in 9 units of circular ponds with biofloc technology system culture, resulting in different growth rates on different culture periods. The culture of fish in ponds K1, K2, K3, and K6 were chosen to be extended because their growth evaluation results were better than those fish in ponds K4, K5, K7, K8, and K9 after a disease outbreak. The daily growth rate K1, K2, K3 and K6 were 0.14 g/day, 0.13 g/day, 0.13 g/day, and 0.10 g/day this result relatively higher than previous study (Soedibya *et al.*, 2017), produce results 0.09 ± 0.02 g/day, 0.13±0.06 g/day 0.10±0.04 g/day and 0.10±0.03 g/day.

Therefore, fish in ponds K4, K5, K7, K8, and K9 were harvested early, as a result of mass mortality. This outbreak was caused by *Aeromonas hydrophila*. According to Lukistyowati and Kurniasih (2012), these bacteria cause disease outbreaks with high mortality rates (80-100%) in a short time (1-2 weeks). Virulence level of *A. hydrophila* which can cause mass death.

The highest final weight was demonstrated by ponds K9 (20.4 ± 5.1 g/fish), K4 and K5 (19.6 ± 4.9 g/fish), K7 (18.9 ± 4.9 g/fish), and K8 (16.4 ± 4.0 g/fish) with a 9 week culture period. Then, K1 (47.6 ± 13.4 g/fish), K2 (45.5 ± 12.6 g/fish), K3 (43.5 ± 11.8 g/fish), and K6 (34.5 ± 9.3 g/fish) with a culture period of 11 weeks. The growth rate is presented in Figure 2.

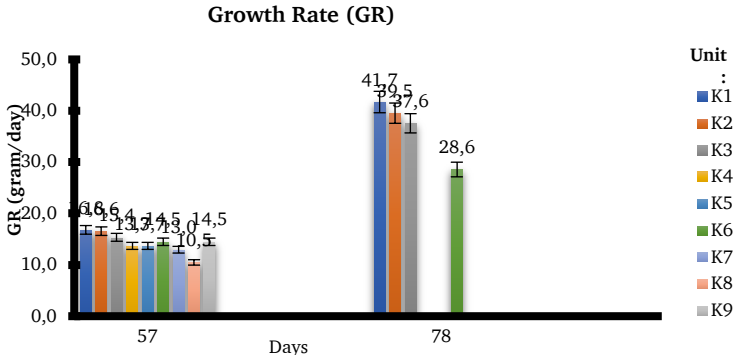

Figure 2. Absolute growth (growth rate) of catfish during 57-day and 78-day culture periods .

As presented in Fig. 2, the growth rate of catfish during the 57-day culture period, and the highest results were demonstrated by fish in ponds K1 (16.8 ± 1.9 g/day), K2 (16.6 ± 1.9 g/day), K3 (15.4 ± 1.9 g/day), K6 (14.5 ± 1.9 g/day), K9 (14.5 ± 1.9 g/day), K4 and K5 (13.7 ± 1.9 g/day), K7 (13.0 ± 1.9

g/day), K8 (10.5 ± 1.9 g/day), respectively. Then, extended culture period for 78 days resulted in the highest growth rate in K1 (41.7 ± 5.8 g/day), K2 (39.5 ± 5.8 g/day), K3 (37.6 ± 5.8 g/day), and K6 (28.6 ± 5.8 g/day), respectively. The results of this experiment showed that growth rate is smaller than results obtained by Putra *et al*. (2017) which can obtain growth in the range of 1.6 ± 0,28 g/day, whereas this study only gets daily growth 0.54 ± 0,27 g/day.

It can be caused by a lack of carrying capacity. Factors that affect the carrying capacity include water quality, feed, and the size of the fish. Space and food supply are factors that also affect the growth of fish, where the fish will grow better if both factors can be met, and otherwise growth will slow if one or both are lacking. This result indicated that biofloc was able to increase growth (Soedibya *et al*., 2018).

The lower growth rate in this study is suspected of high density, increasing fish density may compromise animal welfare, which reduces survival and growth. Adverse effects of high density on fish production have been recorded in many aquaculture species mostly attributed to stress (van de Nieuwegiessen *et al*., 2009), it caused by the deterioration of water quality and competition for space and food. In addition to growth rate, can also be measured based on % daily growth i.e. with specific growth methods. The specific growth rate can be seen in Figure 3.

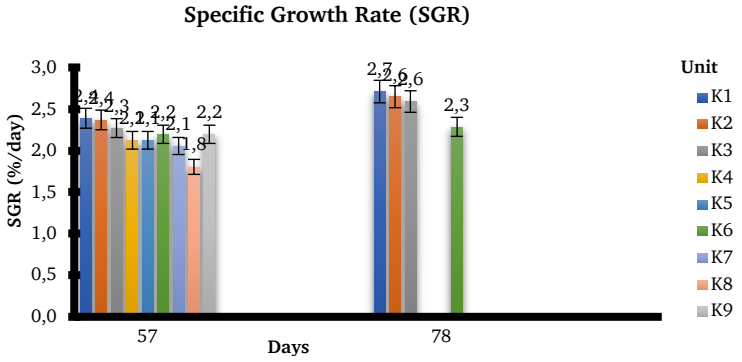

Figure 3. Specific growth rate of catfish during 57-day and 78-day culture period.

Specific growth rate (SGR) of catfish during the 57-day culture period and the highest results were demonstrated by fish in pond K1 and K2 (2.4 ± 0.2 %/day), K3 (2/3 ± 0.2 %/day), K6 and K9 (2.2 ± 0.2 %/day), K4, K5, K7 (2.1 ± 0.2 %/day), and K8 (1.8 ± 0.2 %/day), respectively, this result higher than previous studies where the gain on 50 days biofloc treatment were 0.87-1.15 %/day (Soedibya *et al*., 2017). According to Ekasari *et al*. (2016), the addition of *Bacillus* sp., cells into feed had a positive impact on the fish digestive system due to exogenous enzymes released by cells. Furthermore, for extended culture, which was 78 days, the results were K1 (2.7 ± 0.2 %/day), K2 and K3 (2.6 ± 0.2 %/day), and K6 (2.3 ± 0.2 %/day), respectively. This result relatively higher than Soedibya *et al*. (2018) which gets average results 1.04±0.12% /day.

Contrasts with Soedibya *et al*. (2018) who state that wide space enough to make the fish a lot of activities so that a lot of energy used for activity and metabolic processes than for growth, in this study showed that lower stocking density (1300 fish/m^3) produce relatively higher SGR. The survival rate parameter can be seen in Figure 4.

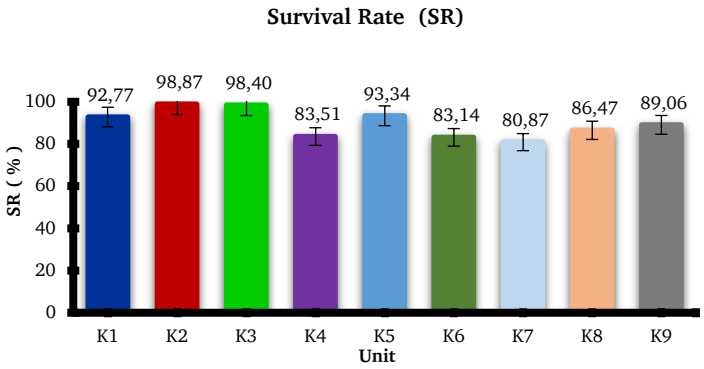

Figure 4. Survival rate of Catfish's during culture period.

Figure 4 shows the survival rate of catfish during the culture period, and the highest results in the first harvest were demonstrated by fish in ponds K5 (93.34 ± 6.64 %), K9 (89.06 ± 6.64 %), K8 (86.47 ± 6.64 %), K4 (83.51 ± 6.64 %), and K7 (80.87 ± 6.64 %), respectively. Meanwhile, for the second harvest, highest results were demonstrated by fish in ponds K2 (98.87 ± 6.64 %), K3 (98.40 ± 6.64 %), K1 (92.77 ± 6.64 %), and K6 (83.51 ± 6.64 %), respectively. Administration of *Bacillus* sp. probiotics at a dose of 104 cfu/mL can effectively suppress the growth of *Aeromonas hydrophila* and prevent MAS disease by increasing immune response and survival of African catfish (Ulkhaq *et al*., 2014).

The percentage survival of *Clarias gariepinus* cultured was generally high (80.87% to 98.87%). High percentage survival is attributed to air-breathing and relatively high tolerance of *C. gariepinus* to poor water quality conditions. This results in line with Shoko *et al*. (2016) that get results 95.42% to 98.83% survival rate.

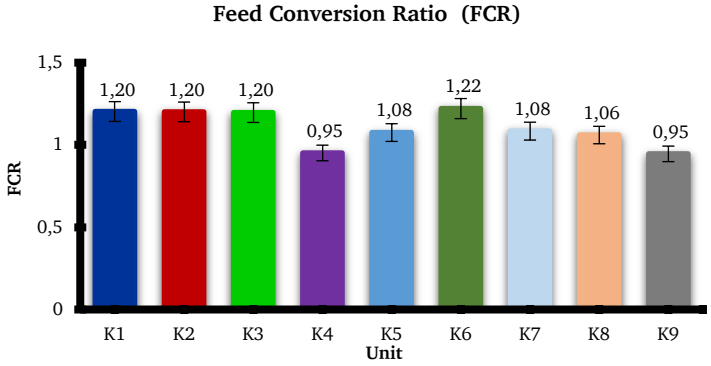

Figure 5. Feed conversion ratio of catfish during culture period.

Figure 5 showed the best feed conversion ratio for catfish during the course of the study, ranked for the first harvest as follows: fish in ponds K4 and K9 (0.95 ± 0.11), K8 (1.06 ± 0.11), K5 and K7 (1.08 ± 0.11). Meanwhile, for the second harvest, they were fish in ponds K1, K2, K3 (1.20 ± 0.11), and K6 (1.22 ± 0.11). This result relatively similar compared with the prior study, where the Feed conversion ratio of catfish grown in biofloc culture 0.91-1.42 (Yusuf *et al*., 2015).

Besides accelerate growth, biofloc is also had an important role as an alternative natural feed. This is because the biofloc contains a crude protein that reached 48–53% (Hastuti and Subandiyono, 2014) and therefore the Feed Conversion Ratio (FCR). According to Putra *et al*. (2017) produce an efficiency of 0.9 (below 1.00). This study results in relatively the same, where the FCR is in range 0.9-1.22. This is because besides fed on the commercial diet, the fish was also fed on floc that contains planktons. This

value is better than fish fed on a commercial diet without the application of biofloc (Jimoh *et al*.,2014). According to Azim *et al*. (2007), the nutritional quality of biofloc was appropriate at least for herbivorous and omnivorous fish species. In this field, the African catfish is categorized as omnivorous feeding habits (Rad *et al*., 2004).

Water reservoir capacity of 20 m^3 can fill 4 ponds in one charge, to fill entire ponds require 46.2 m^3 or 2,33 times from reservoir capacity. Water in the reservoir had a hardness value of 33.90 mg/L, whereas the optimum range is 50-200 mg/L at a pH of 6.5-9.0. Hardness is a measurement of the amount of calcium (Ca) and magnesium (Mg) salts that play an important role in maintaining fish's environmental quality, supporting calcium supply needed for the construction of bones, and reducing osmotic performance needed to replace blood electrolytes which are constantly lost through urine in large amounts. Iron (Fe) content in the reservoir water was 0.011 mg/L, while the optimum range is 0.00-0.05 mg/L. (Ogbonna and Chinomso, 2010). A high Fe content could reduce the quality of *Bacillus sp*. bacteria abundance which is why the Fe content in the water must be kept at a minimum.

Total ammonia total (NH_3) content of healthy fishpond water was 1.371 mg/L and the sick fishpond was 0.323 mg/L, whereas the optimum is $NH_3 \leq$ 0.01 mg/L. Nitrite (NO_2) content of healthy fish pond water was 0.001 mg/L and sick fish pond was 0.268 mg/L, whereas optimum is $NH_2 \leq$ 0.1 mg/L. Visually, sick fish had bloated, yellowish-red bodies which are signs of ammonia poisoning in fish.

Ammonia and nitrite are toxic to fish because when the environment or pond water is too saturated with ammonia, fish are unable to release ammonia from their bodies in the form of urine or feces, causing fish to be poisoned by internal ammonia. Ammonia is generally toxic to fish (Shiwanand and Tripathi, 2013) when reaching out of 1.5 mg/L (Yusoff *et al*., 2011). The concentration of 2 mg/L nitrite causes a slow growth rate of fish and 4 mg/L causes acute death (Yusoff *et al*., 2011). The acute concentration of nitrite in yellow catfish (*Pelteobagrus fulvidraco*) sized 0.029 ± 0.049 g was 8.74 mg/L (Zhang *et al*., 2012), while nitrite content of 3.92 mg/L lowered daily growth rate below 2% in African catfish (*C. gariepinus*) (Roques *et al*., 2015).

Relatively low hardness in the water reservoir could be corrected by applying CaO or $CaCO_3$ at a dose of 10-160 ppm. The best dose for growth and survival of Indian carp *Labeo rohita* is 150 ppm (Rajkumar *et al*., 2018). While a high Fe content could be corrected by using filter substrates (zeolite, volcanic sand, activated charcoal, and bioballs) and aeration in the reservoir. The filtering system can be designed in a trickle filter system where the filter substrates are placed inside containers which are then stacked.

Water flowing from the well is passed through the containers containing filter substrates and into a reservoir tank. The volume of the reservoir was 60 m3 so the required filter system was 20 m3/filter container for each type of filter material used, as seen in Figure 6.

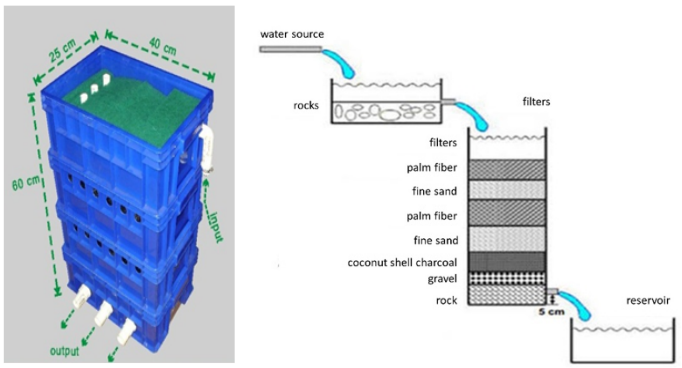

Figure 6. System of reservoir water filtering.

High ammonia and nitrite contents in healthy and sick fishponds could be corrected when *Bacillus sp*. bacteria which has an important role in the culture of fish using biofloc system work effectively, where the optimum abundance which is ≥ 1 x 10^6CFU/ml is reached. Additionally, a water replacement system where at least 10 cm is replaced daily needs to be done, and removal of sediments on the floor of the pond before every feeding to minimize the content of toxic organic materials in the maintenance medium is important.

CONCLUSION

Fish growth increased in size at every sampling but it was very slow due to a disease outbreak. In addition, the water source was contaminated with bacteria and had a high iron 0.011 mg/L, while the optimum range is 0.00-0.05 mg/L, it causing less than optimum growth of probiotic bacteria, so fish culture system with a high stocking density potentially led to stress, illness, inhibited growth, and even mass mortality.

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