Water Quality Improvement of Media Culture for Tilapia (*Oreochromis niloticus*) with Cleaner Production Method

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Abstract. The tilapia (*Oreochromis niloticus*), is known as a high adaptability and brackish water tolerance fish. This fish is also has a meat with high protein content, that ranges about 65 -75%. Generally the tilapia is cultured using a conventional system with high density. It is caused degradation of water quality of media culture, and finally increase mortality rate of fish cultured. The application of tilapia cultivation with cleaner production method by giving enzyme into the feed to upgrade the efficiency of feed utilization, presumed that could improve the water quality of cultivation media. It is due to the lower of feed and feces residues. Therefore the concentration of toxic compounds, such as ammonia, nitrite and sulfide, will be lower.

The experiments were conducted for 35 days with a completely factorial randomized design. The first factor was the dosage of enzyme in the feed, consisting of 4 dosages, and the second factor was the duration of the test fish maintenance (5 weeks). Water quality variables examined included ammonia, nitrite and sulfide. The results showed that enzyme dosage had no significantly impact on ammonia, nitrite and sulfide concentrations in the test media culture. However, the feeding with enzyme in low dosage, resulted less concentration of ammonia, nitrite and sulfide than it was without enzyme. The duration of fish cultured has significantly effect on the concentration of ammonia, nitrite and sulfide in the test media. While it is no significantly correlation between dosage and duration of maintenance.

Keywords: *Oreochromis niloticus*, cleaner production, ammonia, nitrite and sulfide

1. Introduction

Tilapia (*Oreochromis niloticus*) is a potentially of cultivated fish. Since this fish is very tolerant in any environmental condition. Tilapia is still survives, even if it is maintained at high density [1]. In addition this fish is has a high protein content, that is about 65 -75% meat [2]. The genus *Oreochromis* has a high adaptability and tolerance to water quality over a wide range, including in brackish waters [3].

Tilapia production increased year to year in Indonesia, since the year 2010 to 2014, according to Statistics of Production Directorate, General Directorate of Cultivation. Increasing of tilapia production among others due to fishery intensification, with a high density of dissemination per unit area of land. Such fish intensification, however, may resulted a big problem with water pollution in the cultivation media, due to liquid waste from the cultivation activities.

The Increase of awareness or partisanship of the world community towards the effort of environmental conservation, emphasizes the importance of applying cultivation patterns that can maintain environmental preservation, especially related to waste management [4]. The Increase of freshwater fish and marine fish demands, and the limitation availability of water and land for fish cultivation, causing the need for modernization and revision of cultivation technology [5].

There are several ways that can be done in order to control water pollution. One of them is a control by using cleaner production technology known as cleaner production, that is continuous application and integrated prevention strategy which are applied to various processes, products and services to get economic value, social, health, security and environment benefit [6].

The application of water pollution control by using cleaner production method so far is only limited in manufacturing industry, while the application over aquaculture industry is still very rare [5].
Actually, this method is also possible to be applied in the aquaculture industry. It is mainly in order to reduce the amount / volume and concentration of waste by using raw material and machine production which contained in fish and its environment.

The raw materials in the cultivation industry consist of seed and feed, while the machine production is media cultivation for fish. That is consists of water and other requirement, i.e. freshwater pond, brackish water pond or other ponds. In order to control pollution in media cultivation, among other it is used recirculation and bio filter systems.

The higher the price of raw materials to make feed, encourage the use of feed in low-protein rate (about 15%) which is given enzyme supplements to improve the digestibility of feed, so that more feed can be converted into fish meat biomass [7, 8]. As a result of the application of low-protein feed technology with enzyme supplementation, it is estimated that the volume of feces produced less, so as the concentration of toxic compounds derived from the process of feces decomposition and the rest of high protein feed will also be less.

This study is conducted to review the use of enzyme that has been used to improve feed efficiency, to suppress the production of liquid waste in the aquaculture system, as the implementation of cleaner production principles.

2. Materials and methods

The material used in this study was tilapia which was obtained from Siwarak Fish Hatchery, Semarang Regency. Tilapia used had a length of 2 ± 0.5 cm. During the experiment the tilapia was fed artificial feed / mill feed with a dose of 5% of body weight, with the frequency of giving 2 times a day namely in the morning and evening. Before given to tilapia, formerly the feed was mixed with enzyme with dosage 0.0125%, 0.025%, 0.050% and 0.075%. During the experiment fish was kept in an aquarium with capacity 20 liters, and was filled with 15 liters of water. Maximum maintenance density was 1 g biomass fish/ liter of media.

Water quality measurement was performed on physical parameters, included temperature, chemical parameters including: pH, dissolved oxygen, ammonia (NH₃-N), nitrite (NO₂-N) and Hydrogen Sulfide (H₂S) concentrations. Measurement of water quality was conducted twice a day, in the morning and afternoon, except for ammonia, nitrite and hydrogen sulfide, the measurement was taken once a week.

Table 1. Equipment and methods used in the measurement of water quality variables

| No | Variable            | Measuring instrument | Method          |
|----|---------------------|----------------------|-----------------|
|    | Temperature         | Digital Thermometer  | Conductivity meter |
| 3  | pH                  | pH meter digital     | Conductivity meter |
| 4  | Dissolved oxygen    | DO meter digital     |                 |
| 5  | Ammonia (NH₃-N)     | AAS                  | Spectrophotometry |
| 6  | Nitrite (NO₂-N)     | AAS                  | Spectrophotometry |
| 7  | Sulfide (H₂S)       | AAS                  | Spectrophotometry |

The experimental design used was a completely randomized design with factorial pattern. Factor I was the influence of enzyme dose in feed (consisting of 4 treatments level and 1 control, each treatment was repeated 3 times) against the quality of fish cultivation media. Factor II was the influence of fish culture duration towards the quality of fish cultivation media. The fishes test were treated for 35 days and they were weighted every 7 days.

The data were analyzed quantitatively by using the analysis of variance and correlation regression analysis. The analysis of variance was conducted to assure the effect of treatment to liquid waste production. While the regression analysis was conducted in order to find out the pattern and relationship closeness between treatment with ammonia, nitrogen and sulfide concentration variables in the water of fish culture media.
3. Results and discussion

3.1. Results

Analysis water sample of media of fish test resulted data of ammonia concentration (NH\textsubscript{3}-N) as presented in Table 2.

Table 2. Average and standard deviation of ammonia concentration (NH\textsubscript{3}-N, mg / l) of fish test media culture during the experiment

| Treatment | Average and SD | The duration of fish maintenance (Day) |
|-----------|----------------|---------------------------------------|
| A         |                | 0 | 7  | 14 | 21 | 28 | 35  |
| A         | Average        | 0.2225 | 5.6627 | 2.715 | 2.4332 | 0.2239 | 0.2195 |
| A         | SD             | 0.1642 | 0.3138 | 2.1662 | 1.9231 | 0.1478 | 0.0922 |
| B         | Average        | 0.2909 | 5.2973 | 4.6869 | 3.6208 | 0.0549 | 0.1704 |
| B         | SD             | 0.0967 | 1.1535 | 3.0353 | 3.4540 | 0.0217 | 0.0261 |
| C         | Average        | 0.1932 | 5.276  | 8.2153 | 9.7967 | 0.103  | 0.1893 |
| C         | SD             | 0.0452 | 0.8309 | 1.3927 | 5.3897 | 0.0815 | 0.0360 |
| D         | Average        | 0.1541 | 5.4795 | 5.5372 | 7.6083 | 0.112  | 0.1682 |
| D         | SD             | 0.1103 | 0.7528 | 4.0558 | 7.7192 | 0.0731 | 0.0336 |
| K         | Average        | 0.3315 | 5.966  | 5.8095 | 4.2125 | 0.1321 | 0.2398 |
| K         | SD             | 0.1106 | 0.6967 | 3.9225 | 4.3897 | 0.0617 | 0.0766 |

The result of analysis of variance shows that the different dosage of enzyme in feed has no significant effect on ammonia concentration produced by fish test, but maintenance duration has significantly effect on the ammonia concentration produced. However, the combination of different dosage of enzyme in feed together with duration of maintenance of fish test, both of them do not have any significantly effect on ammonia concentration produced by the fish test.

Although the dosage was not significantly effect on ammonia concentration, but the Figure 1 shows that the highest concentration of ammonia found in the control treatment and the lowest in treatment A. The ammonia concentration during the study tends to increase from the beginning to maximum on day 12, then drops to the end of the study. As shown in Figure 2, the relationship between age /duration of fish culture with ammonia concentration in the test media stated in the equation \( y = 0.001x^3 - 0.0698x^2 + 1.2078x + 0.1617; R^2 = 0.4323. \)
Figure 1. The curve of average Ammonia concentration in the test media during the experiment.

Figure 2. Curve of relationship between age/duration of fish culture with ammonia concentration in test media.

The analysis of water samples conducted during the study resulted nitrite concentration (NO2-N) as presented in Table 3. The result of analysis of variance shows that the enzyme giving has no significantly effect on nitrite concentration in the test media, but the duration of culture has significant effect. As well, the combination of both the enzyme treatment and duration of culture do not have significant effect on the nitrite concentration in the test media.

Although the enzyme concentration was not significantly effect on the nitrite concentration in the test media, but the average data indicates that the highest nitrite concentration is in control (without enzyme) and the lowest is in treatment B. The nitrite concentration tends to increase by the increasing of fish maintenance duration, the nitrite concentration during the experiment ranges 0.03 up to 13.24 mg / l. The relationship between the nitrite concentration and the duration of fish maintenance stated by exponential equation $y = 0.0447e^{0.1036x}$, $R^2 = 0.368$. 
Table 3. Average and standard deviation of nitrite concentration (NO2-N, mg / l) of fish maintenance media during the experiment

| Treatment | Average and SD | The duration of fish maintenance (Day) |
|-----------|---------------|----------------------------------|
| A         |               | 0                  | 7    | 14   | 21   | 28   | 35   |
| Average   | 0.3093        | 0.2611             | 0.7378 | 0.4085 | 9.6642 | 9.234 |
| SD        | 0.3791        | 0.2709             | 0.0881 | 0.2805 | 13.0338 | 6.5711 |
| B         |               |                    |       |       |       |       |       |
| Average   | 0.0958        | 0.2199             | 0.1495 | 0.6643 | 4.6713 | 0.9945 |
| SD        | 0.1028        | 0.2338             | 0.1716 | 0.1788 | 5.3524 | 0.3327 |
| C         |               |                    |       |       |       |       |       |
| Rataan    | 0.0379        | 0.0247             | 0.025  | 0.54  | 6.636  | 0.592  |
| Average   | 0.0066        | 0.0142             | 0.0043 | 0.1879 | 5.8034 | 0.1254 |
| SD        | 1.2018        | 0.1643             | 0.1113 | 0.6718 | 2.8178 | 2.6288 |
| D         |               |                    |       |       |       |       |       |
| Average   | 1.6710        | 0.1823             | 0.0829 | 0.0635 | 2.5005 | 2.1132 |
| SD        | 0.0577        | 0.1564             | 0.3181 | 0.1742 | 13.2472 | 6.4487 |
| K         |               |                    |       |       |       |       |       |
| Average   | 0.0374        | 0.1617             | 0.0982 | 0.0901 | 8.7028 | 8.8187 |
| SD        | 0.0577        | 0.1564             | 0.3181 | 0.1742 | 13.2472 | 6.4487 |

Figure 3. The average curve of nitrite concentration in the test media during the experiment

Figure 4. Curve of relationship between age / duration of fish culture with nitrite concentration in test medium.
The analysis of water samples conducted during the study resulted sulfide concentration (H2S, mg/l) as presented in Table 4.

Table 4. Average and standard deviation of sulfide concentration (H2S, mg/l) on culture media of fish test during the experiment.

| Treatment | Average and SD | Duration of fish maintenance (Day) |
|-----------|----------------|-------------------------------------|
|           | Average        | 0  | 7  | 14 | 21 | 28 | 35 |
| A         | 0.002          | 0.013 | 0.057 | 0.054 | 0.034 | 0.018 |
|           | SD             | 0   | 0.013 | 0.001 | 0.003 | 0.016 | 0.003 |
| B         | 0.002          | 0.033 | 0.069 | 0.051 | 0.032 | 0.023 |
|           | SD             | 0.0005 | 0.003 | 0.008 | 0.009 | 0.028 | 0.007 |
| C         | 0.002          | 0.041 | 0.073 | 0.058 | 0.036 | 0.017 |
|           | SD             | 0.0005 | 0.001 | 0.005 | 0.006 | 0.029 | 0.003 |
| D         | 0.002          | 0.034 | 0.048 | 0.048 | 0.039 | 0.019 |
|           | SD             | 0.001 | 0.011 | 0.034 | 0.004 | 0.031 | 0.009 |
| K         | 0.002          | 0.055 | 0.055 | 0.052 | 0.022 | 0.022 |
|           | SD             | 0.0005 | 0.007 | 0.008 | 0.005 | 0.014 | 0.008 |

The result analysis of variance shows that enzyme has no significant effect on the sulfide concentration in the test media, but the duration of maintenance has significant effect. As well, the combination of both treatment enzyme given and duration of maintenance do not significantly effect on the sulfide concentration in the test media. Nevertheless, the average of each treatment shows that the lowest H2S concentration in treatment A and the highest in treatment C (Figure 5). Moreover, the concentration of Hydrogen Sulfide in the fish culture media tends to increase at the beginning of the experiment and reaches the highest concentration on the 14th day of fish culture, then drops until the end of the experiment. The H2S concentration during the experiment ranges from 0.002 to 0.055 mg/l. The relationship between the nitrite concentration and the duration of fish maintenance stated by the equation \( y = -3E-08x5 + 3E-06x4 - 9E-05x3 + 0.0011x2 + 6E-05x + 0.005 \), \( R^2 = 0.5832 \).

Figure 5. Curve of H2S average concentration in the test media during the experiment
3.2. Discussion

This study indicates that enzyme which given into the feed can improve the water quality of culture media of fish test. Although it is significantly effect, but the enzyme may appeared to improve the water quality of test media. The ammonia, nitrite and sulfide concentrations of test media for test fish fed with enzymes are generally better than test media for test fish fed without enzymes, especially test media for test fish fed with low enzyme content (0.0125% - 0.025%). The improvement of water quality in the test media which was given was low-dose enzyme may occurred as a result of the increasing efficiency on feed utilization, therefore the solid waste in the form of feed and feces residual became lower.

Several types of enzymes have been utilized in the activity of fish cultivation, such as complex enzymes which consist of fungal xylanase, β-glucanase, pentosonase, β-amylase, fungal β-glucanase, hemicellulase, pectinase, cellulase, and cellubiase [8]. F isate enzyme for improving phosphor digestibility on Tilapia [9], complex enzyme EZplus containing protease, lipase, amylase, pepsin, trypsin and chemotrypsin on tilapia [10]. The complex enzyme EZplus has also been tested on milkfish by [7].

Those Various enzymes are proven to improve the digestibility of feed, and reduce the feces volume produced by fish. [10], resulted that tilapia (Oreochromis niloticus) which is fed with lower protein content (26%) produces a faster growth than fish fed with higher protein content (32%) with the addition of enzyme to fish feed with the highest enzyme level (5%). The enzyme used is EZplus containing protease, lipase, amylase, pepsin, trypsin and chemotrypsin. Study on the addition of Farmazyme enzyme (containing enzyme fungal xylanase, β-glecanase, pentosonase, β-amylase, fungal β-glecanase, hemicellulose, pectinase, cellulose and cellubiase) performed by [8] on African catfish, Clarias gariepenus, shows better SGR (Specific Growth Rate), FCR (Food Conversion Ratio) and PER (Protein Efficiency Ratio). In this study, enzymes used containing protease, lipase, amylase, pepsin, trypsin and chemotrypsin.

Soni et al [7] combines the use of feed with low protein level and the complex enzyme EZplus. The use of feed with low protein level aimed to press the production cost of milkfish cultivation, while the application of biotechnology product is undertaken to improve the efficiency of milkfish digestibility, so that more feed converted into meat than wasted with fish feces and reduce the contamination / waste generated from feces done by cultivated fish [7].

The residue of inedible feed and fish feces containing the protein will be overhauled by the bacterial decomposer contained in the water into toxic organic compounds namely ammonium

\[ y = -3E-08x^5 + 3E-06x^4 - 9E-05x^3 + 0.0011x^2 + 6E-05x + 0.005 \]

\[ R^2 = 0.5832 \]
(unionized ammonia NH₄⁺) and ammonia (NH₃-N). Unionized ammonia is harmful to fish, but ammonia is highly toxic. *Nitrosomonas sp* bacteria will convert ammonia to nitrite (NO₂⁻-N), and *Nitrobacter* converts nitrite to nitrate in the nitrogen cycle. This process occurs for 2 weeks until 2 months, so it can be understood from the various curves presented that the concentrations of ammonia, nitrite and sulfide in the test media tends to increase from the beginning of the trial to the second week and decrease after more than 2 weeks up to the end of the experiment.

Hydrogen Sulfide is known as a colorless toxic gas, flammable and smells like rotten egg is also found in the water. In the water this gas is found as a result of disintegration of organic matter, when bacteria analyzes the organic matter in an anaerobic atmosphere. The sulfides contained in the test media are presumed to be the result of feces decomposition and the remains of feed which are not consumed by the fish.

Concentrations of ammonia, nitrite and H₂S in the culture media of fish test tended to increase with the increasing of fish test age. This occurred as a result of the increase of volume, feces weight and the feed residuals produced, due to the increase of feed amount given along with the increase of the test fish weight. During the experiment, the test fish were given feed in form of pellet with a portion of 5% from body weight. The research results performed by the previous writer showed that the feces weight and the residual of inedible feed went up by the increase of the fish weight.

**4. Conclusion**

The results of this study indicated that the enzyme usage although it is not significantly effect on the quality of media culture of tilapia maintained for 35 days, but there is a tendency that the usage of low-dose enzymes (0.0125% to 0.025%) tended to have ammonia, nitrite and sulfide that were lower than the maintenance media of fish maintained with non-enzyme feed. The more increasing the fish age cultivated, the more increasing and different the ammonia, nitrites and sulfides concentrations in maintenance media in each week.

The results of this study open the hope of the possibility of water quality improvement on aquaculture by the application of cleaner production cultivation method using enzymes mixed into the feed. The method of cultivation with cleaner production methods should continue to develop, while improving the various deficiencies occurred.

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**References**

[1] Melo CV Neto R V R Costa A C de Freitas R T F Freato T A Souza U N 2013 *Acta Sci Anim Sci* 35 (4)

[2] Kurniashi S 2013 *Aquaticulture Research Journal* Vol 8 (1)

[3] Mashaii N Rajabipour F Mohammadi M Sarsang Hi Bitaraf A Hossein H Zadeh 2016 *Journal of Applied Aquaculture* Volume 28 Issue 1

[4] Jensen K O Denver S Zanoli R 2011 *J Life Sci* 58: 79 – 84.

[5] Unger J M Schumann A Brinker 2015 *Aquacult Environ Interact* vol 7: 223-238

[6] UNEP 2011 Towards a life cycle sustainability assessment: making informed choice on products

United Nation Environment Programme

[7] Sony AFM E Sutikno I.M Suitha 2014 *Milkfish (Chanos chanos Forskal) Culture in fish pond environment friendly*

[8] Yildirim Y B and Turam F 2010 *Journal of Animal and Veterinary Advances* 9: 327 – 331

[9] Amin M Yusadi D Mokoginta I 2011 *Fisheries Science & Technology Journal* Vol 6 (2): 52 – 60.

[10] Arafat M Y N Abdulgani R D Devianto 2015 *ITS Journal of Sciences and Arts* Vol 4 (1): 2337 – 3520