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# COMBINATION OF BIOAUGMENTATION AND BIOSTIMULATION IN WWTP, LAMONGAN FISH MARKET AS A CONTROL EFFORT OF KALIOTIK RIVER POLLUTION

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## ABSTRACT

*Bioaugmentation and biostimulation are In Situ bioremediation with an engineered degradation model. Each of these method models has advantages and disadvantages, so a combination of the two is needed as an alternative that can accelerate the degradation process. This study aims to determine whether biostimulation and bioaugmentation or a combination of both can accelerate the degradation of the Lamongan Fish Market liquid waste. The parameters that will be analyzed quantitatively in this study are physics and chemistry which refers to the quality standards of PermenLHK N0. P.68 of 2016. Quantitative analysis tests include turbidity, TDS, TSS, pH, DO, Nitrate, Ammonia, BOD, COD. The treatments consisted of biostimulation (BS), bioaugmentation (BG) and a mixture of both (BB), K- (without aeration), K+ (with aeration). The test sample was taken from wastewater at the inlet of the Lamongan Fish Market WWTP. This research method will be carried out using a laboratory experiment using a completely randomized design with a factorial pattern with three replications with a batch reactor processing system. The first factor is the variation of the tested treatment samples (BS, BG, BB, K-, and K+) with the second factor being the incubation time (0, 1, 2, 3 weeks). The implementation of augmentation is by adding as much as 2% (v/v) per 100 mL of water sample (bacterial suspension with Aλ: 610nm = 0.5) of EM4. Meanwhile, the addition of liquid fertilizer to biostimulation was carried out at a concentration of 0.5% (v/v) to determine its effect on the combination of bioaugmentation and biostimulation. Incubation was carried out at room temperature aerobically by applying an aerator in each batch. In the biostimulation treatment, the parameter that experienced the largest percentage decrease was ammonia, which was 97%. And in the augmentation treatment, the parameters that experienced the largest percentage decrease were ammonia by 99% and nitrate by 74%. While the combination of the two experienced the largest percentage decrease, namely in ammonia 96% and nitrate at 85%. The high percentage of ammonia reduction was due to the high protein content of organic waste in wastewater at the Lamongan Fish Market WWTP, which was 115 mg/L, which did not meet the quality standards. The best ammonia reduction was in the bioaugmentation treatment which reached 0.6 mg/L on incubation day 21 when compared to the negative control (without treatment).*

***KEYWORDS:*** *biostimulation, bioaugmentation, Lamongan Fish Market WWTP, batch reactor*

## 1 INTRODUCTION

According to Donoriyanto, DS. (2011) Lamongan Regency is drained by the Bengawan Solo river along 68 Km, Lamong River 33 Km and Blawi River 27 Km, most of which are used for agricultural and residential needs. In addition to agriculture, river water is also used as a source of drinking water for some people in Lamongan Regency. However, the pollution of the Bengawan Solo Watershed (DAS) around the Lamongan Fish Market or known as the Kaliotic River (included in Lamong River) is an unresolved problem so far.

The Waste Water Treatment Plant (WWTP) at the Lamongan Fish Market is not functioning, as a result an unpleasant odor occurs because the fish waste management is not running properly. The impact of the strong stench and rancidity spread to the residents of the Tumenggungan Village and the residents of the Sidokumpul Village. The unpleasant smell also occurs along the road to the city and vice versa. This happens due to the absence of drainage, not functioning WWTP and waste quality control that is not carried out properly. In addition, the non-functioning WWTP causes the sewer to be clogged with plastic waste and the waste that comes out does not go through the WWTP process. As a result, the wastewater goes directly to the Bengawan Solo tributary (Kaliotik River) and pollutes the river water. This fish market waste also has a negative impact on farmers in the northern area of Lamongan if it is not immediately anticipated. Based on data from the Performance Report of the Environment Agency (2016), the potential of water resources originating from the Solo River can be utilized to irrigate 46,035 hectares of rice fields which are divided into 7 (seven) irrigation areas. Organic materials contained in fish market liquid waste can deplete dissolved oxygen in the waste, and cause unpleasant odors, and will be dangerous if the material is a toxic material. The occurrence of the process of oxidation of organic matter by microorganisms in wastewater, will cause the wastewater to change color to blackish brown or foul smelling. If this wastewater seeps into the ground close to the well, the well will be potentially polluted. Meanwhile, if the liquid fish waste is diverted to the river, it will cause itching, diarrhea, and environmental pollution (Pamungkas, MT., 2016). Therefore, it is necessary to control watershed pollution at the Lamongan Fish Market WWTP, one of the alternatives is bioremediation.

The use of waste degradation biotechnology or bioremediation is always increasing in its application. One of the bioremediation methods is bioaugmentation. The principle of biougmentation is the addition of certain microbes in a polluted place which functions as a contaminant cleaner, this method is most often used in removing contamination in a place. However, there are several obstacles encountered when this method is used, namely it is very difficult to control the condition of the polluted site so that microorganisms can develop optimally. All of the mechanisms involved in the bioremediation, microorganisms released into an unfamiliar environment may be difficult to adapt. Therefore, a combination of biostimulation is needed in biougmentation applications. Considering that the Lamongan Fish Market WWTP is a type of WWTP with an anaerobic waste treatment system, it has weaknesses, namely the effluent requires additional treatment, the reduction efficiency of pathogenic bacteria is

low, to achieve a long start-up process. Therefore, additional treatment to minimize the weaknesses of the existing Lamongan Fish Market WWTP is to add compost and exogenous bacteria to it to support the growth and microbial activity in the area. According to Xie, F. et al. (2020) the most common microbes found in the denitrification section of anaerobic WWTPs are archaea, Candidatus Methanoperedens nitroreducens (M. nitroreducens) from the new family Candidatus Methano-peredenaceae and reduce nitrate to nitrite. Therefore, it is possible that the bacteria M. oxyfera and M. nitroreducens archaea can work together in the natural environment and coexist. Commamonas tertosteroni (Wu, Y. et al. 2020), added the bacteria as exogenous in WWTP as implications for bioaugmentation. Meanwhile, biostimulation is done by adding compost to increase pollutant reduction. In addition, the addition of oxygen which is already present in the Lamongan Fish Market WWTP system, according to Pagoray, H. (2009), the optimal degradation process at week 12 in a combination of biostimulation and bioagmentation can degrade the total waste up to 83%.

Utilization of microbes, including bacteria and fungi, in utilizing enzymes produced naturally by both. According to Aniriani, GW (2014) enzymes are proteins that are produced and used by living cells, one of which is to convert substrates into simpler molecules. Utilization of microbial consortium obtained from biougmentation techniques derived from exogenous bacteria. The combination of bioaugmentation and biostimulation is intended so that the more types of microbes added in the formulation will be directly proportional to the large number of degraded pollutant compounds.

The microbial consortium can be used for bioremediation in both industrial and domestic (or other environmental) wastes, for example the WWTP liquid waste from the fish market in Lamongan Regency. The alternative use of microbial consortium in bioaugmentation and the addition of compost or organic fertilizer in the biostimulation process, when combined, can accelerate the rate of degradation of organic liquid waste in WWTP.

## 2 MATERIALS AND METHODS

This research was conducted on a laboratory scale with a completely randomized design pattern RAL (4x5), with the first factor being treatment (BS, BG, BB, Control + and Control -) with the second factor being incubation time (H0, H7, H14 and H21).

### 2.1 Batch Reactor Preparation

The manufacture of batch reactors is carried out in plastic containers with a volume of 5 liters, by filling 2 liters of waste water from the Lamongan Fish Market WWTP each. For each batch reactor, an aerator was applied, while the control (-) reactor was not applied. In the Biostimulation (BS) treatment, it was done by adding 0.5% (v/v) liquid fertilizer, the Bioaugmentation (BG) treatment was done by adding a microbial consortium of EM4 as much as 2% (v/v) per 100 mL. And in the combination of both, namely Bioaugmentation and Biostimulation (BB) by adding 0.5% (v/v) and 2% (v/v) respectively. While in Control (+) only aerator is applied. Each treatment was repeated three times, and the incubation time was 21 days.

2.2 Wastewater Measurement and Test

Inadequate wastewater treatment can reduce pollutant parameters, especially physical and chemical parameters, which are found in liquid waste. In addition, the parameter testing carried out also refers to the Minister of Environment and Forestry Regulation No. 68 of 2016, namely, among others, TSS, TDS, turbidity, pH, DO, nitrate, ammonia, and COD. Parameter measurements were carried out on incubation H0, H7, H14, and H21.

## 3 RESULTS AND DISCUSSION

This study was conducted on a laboratory scale with a completely randomized design pattern RAL (5x4), with the first factor being treatment (BS, BG, BB, Control + and Control -) with the second factor being incubation time (H0, H7, H14 and H21). with 3 repetitions.

Table 1. Data on Biostimulation Treatment Results during 21 Days of Incubation

| Parameters | Days to | | | |
|---|---|---|---|---|
| | H0 | H7 | H14 | H21 |
| pH | 7.8 | 8.13 | 15.98 | 7.97 |
| TSS (mg/L) | 100 | 41 | 41 | 176 |
| TDS (mg/L) | 4.85 | 6.42 | 8.98 | 6.47 |
| Turbidity (NTU) | 47.9 | 34 | 15.98 | 143 |
| DO | 1.8 | 7.5 | 5.1 | 20 |
| Nitrate (mg/L) | 133 | 41.8 | 133 | 0.92 |
| Ammonia (mg/L) | 115 | 9.6 | 0.4 | 3 |
| COD (mg/L) | 572 | 856 | 1033 | 1500 |

Based on Table 1, the biostimulation treatment experienced a significant decrease in the parameters measured were nitrate and ammonia from 133 mg/L to 0.92 mg/L and 115 mg/L to 3 mg/L on day 21. According to PermenLHK number: p.68/menlhk-setjen/2016, there are parameters that do not meet the maximum level standards, including pH, COD, TSS. Meanwhile, the parameter value that has decreased significantly and has met the quality standard is ammonia of 3 mg/L when compared to the maximum standard of 10 mg/L. Biostimulation with the addition of 0.5% liquid fertilizer can reduce the percentage of nitrate levels by 99.3% and ammonia by 97.3%.

According to Zhyahrial et al. (2014) the addition of nutrients such as fertilizer, in biostimulation of liquid waste can reduce the content of organic compounds. Meanwhile, the parameters that experienced the highest to lowest increase in sequence were DO, turbidity, COD, TSS, TDS, and pH. Excess TSS concentration is characterized by water turning increasingly cloudy, while high COD values occur due to environmental factors that influence such as the dissolved oxygen content in the reactor is sufficient to help

bacteria decompose pollutant compounds in the reactor (Nevya Rizki et al., 2017). The high or low pH of the water is influenced by the compounds contained in the water, while the concentration of TDS and TSS in river water is influenced by domestic waste, industrial waste, market waste or public facilities (Rinawati et al., 2016). The turbidity value increased from 47.9 NTU to 143 NTU on the 21st day (Table 1), this was due to the growth of microorganisms, both bacteria and fungi in wastewater and microorganisms from liquid fertilizer to the death phase, leaving cell debris that causes wastewater is getting cloudy.

Table 2. Results of Bioaugmentation Treatment During 21 Days of Incubation

| Parameters | Days to | | | |
|---|---|---|---|---|
| | H0 | H7 | H0 | H21 |
| pH | 7.8 | 8.06 | 8.09 | 8.17 |
| TSS (mg/L) | 100 | 28 | 33 | 47 |
| TDS (mg/L) | 4.85 | 5.51 | 7.88 | 5.68 |
| Turbidity (NTU) | 47.9 | 12 | 14.83 | 5.68 |
| DO | 1.8 | 7.4 | 5.1 | 17.7 |
| Nitrate (mg/L) | 133 | 95.5 | 89 | 34.5 |
| Ammonia (mg/L) | 115 | 23.6 | 0.7 | 0.6 |
| COD (mg/L) | 572 | 706 | 1076 | 1032 |

Based on Table 2, the best decrease was in the nitrate and ammonia parameters, respectively, from 133 mg/L to 34.5 mg/L and from 115 mg/L to 0.6 mg/L on day 21. This decrease is also directly proportional to the percentage decrease in TSS of 53%. According to PermenLHK number: p.68/menlhk-setjen/2016, there are parameters that do not meet the maximum level standards, including pH, COD, TSS. Meanwhile, the parameter value that has decreased significantly and has met the quality standard is ammonia of 0.6 mg/L when compared to the maximum standard of 10 mg/L. Bioaugmentation with the addition of 2% EM4 can reduce the percentage of nitrate levels by 74% and ammonia by 99.4% on day 21. Exogenous bacteria from EM4 added are able to utilize ammonium and nitrate as a nitrogen source and can reduce ammonia levels in water (Khastini, RO ., 2022). Microorganisms contained in EM4 consist of several microbes, such as lignolytic, cellulolytic, proteulytic microbes, non-siliotic nitrogen fixation, Lubricus (lactic acid bacteria) and a few photosynthetic bacteria, Actinomycetes, Streptomyces sp., fermented fungi, and yeast that can be used as inoculants. to increase microbial diversity. Due to the diversity of the consortium in EM4 used, ammonia and nitrate can be degraded by existing microbes.

Table 3. Data on the results of the combination of biostimulation and bioaugmentation treatments during 21 days of incubation

| Parameters | Days to | | | |
|---|---|---|---|---|
| | H0 | H7 | H0 | H21 |
| pH | 7.8 | 8.06 | 8.13 | 8 |
| TSS (mg/L) | 100 | 68 | 79 | 70 |
| TDS (mg/L) | 4.85 | 6.7 | 8.83 | 8 |
| Turbidity (NTU) | 47.9 | 25.8 | 48.4 | 45 |
| DO | 1.8 | 7.3 | 4.6 | 4 |
| Nitrate (mg/L) | 133 | 67.4 | 19.4 | 19 |
| Ammonia (mg/L) | 115 | 16.9 | 4.7 | 4 |
| COD (mg/L) | 572 | 912 | 875 | 800 |

Based on Table 3, the best decrease in the combination of biostimulation and bioaugmentation was in the nitrate and ammonia parameters, respectively, from 133 mg/L to 19 mg/L and from 115 mg/L to 4 mg/L on day 21. This decrease is also directly proportional to the percentage decrease in TSS of 30%. According to PermenLHK number: p.68/menlhk-setjen/2016, there are parameters that do not meet the maximum level standards, including pH, COD, TSS. Meanwhile, the parameter value that has decreased significantly and has met the quality standard is ammonia of 4 mg/L when compared to the maximum standard of 10 mg/L. Bioaugmentation with the addition of 2% EM4 can reduce the percentage of nitrate levels by 85.7% and ammonia by 96.5% on day 21.

Based on Table 1-5, Herrero and Stuckey (2015) explain that microbial dynamics and ecology in bioreactors that treat waste is a complex process. It is not only influenced by the microbial consortium added such as EM4, but also the method of inoculation and maintenance of the selected microbe and the design of the bioreactor must be considered. Therefore, the value of each parameter fluctuates at each sampling point, the incubation time is day zero to day 21. The decrease in nitrate and ammonia in the combination treatment of biostimulation and bioaugmentation (Table 3) was not greater than that of the bioaugmentation treatment alone (Table 2), this is This occurs because the microbial composition of liquid fertilizer for biostimulation and EM4 for bioaugmentation is experiencing competition in the dynamics of the use of nutrient sources. Both microbial secondary metabolites can inhibit the growth of other microbes and the microbial death phase occurs faster than the bioaugmentation treatment alone.

Table 4. Results of Positive Control Treatment During 21 Days of Incubation

| Parameters | Days to | | | |
|---|---|---|---|---|
| | H0 | H7 | H14 | H21 |
| pH | 7.8 | 8.03 | 8.18 | 8.06 |
| TSS (mg/L) | 100 | 31 | 33 | 105 |
| TDS (mg/L) | 4.85 | 5.55 | 8.18 | 6.43 |

| | | | | |
|---|---|---|---|---|
| Turbidity (NTU) | 47.9 | 8.61 | 11.76 | 188 |
| DO | 1.8 | 9.4 | 3.9 | 13.6 |
| Nitrate (mg/L) | 133 | 54 | 125 | 86.6 |
| Ammonia (mg/L) | 115 | 10.7 | 1.7 | 2.9 |
| COD (mg/L) | 572 | 723 | 691 | 978 |

The positive control treatment was carried out without the addition of liquid fertilizer or EM4, but there was still oxygen aeration as in the BS, BG and BB treatments. The highest percentage decrease was in ammonia at 97.4% and nitrate at 34.8% (Table 4). This decrease still occurs because in the treated wastewater there are already indigenous bacteria that have the potential to degrade organic compounds. The small percentage of nitrate reduction was caused by the indigenous bacteria in the wastewater being nitrate-degrading bacteria which were dominated by anaerobic bacteria, so that the ammonia-degrading bacteria were dominated by aerobic bacteria.

Table 5. Data on Negative Control Treatment Results During 21 Days of Incubation.

| Parameters | Days to | | | |
|---|---|---|---|---|
| | H0 | H7 | H14 | H21 |
| pH | 7.8 | 7.64 | 8.18 | 8.13 |
| TSS (mg/L) | 100 | 77 | 10 | 32 |
| TDS (mg/L) | 4.85 | 6.09 | 6.14 | 6.69 |
| Turbidity (NTU) | 47.9 | 33.5 | 4.86 | 28.9 |
| DO | 1.8 | 0.7 | 7.1 | 2 |
| Nitrate (mg/L) | 133 | 133 | 17.4 | 51 |
| Ammonia (mg/L) | 115 | 128.8 | 25.7 | 0.9 |
| COD (mg/L) | 572 | 710 | 619 | 987 |

Based on Table 5, the treatment was carried out without oxygen aeration, but the percentage of ammonia reduction was also high, namely 99.2% and nitrate 61.6%. Another decrease occurred in TSS, from 100 mg/L on day zero to 32 mg/L on day 21. This is presumably because the potential bacteria that degrade ammonia and nitrate in wastewater are dominated by anaerobic bacteria, so that even without any treatment, the value of ammonia and nitrate still drops. So that the decrease in organic compounds is directly proportional to the decrease in TSS by 68%, which is to 32 mg/L on the 21st day. Anaerobic waste treatment process has proven to be more effective (Hadiwidodo et al., 2012).

## 4 CONCLUSION

Based on the results of the study, the conclusions are as follows,

1. Biostimulation in Lamongan Fish Market wastewater, experienced the best decrease in the ammonia parameter, which was 97%.

2. Bioaugmentation in Lamongan Fish Market wastewater, experienced the best decrease in the ammonia parameter by 99% and nitrate by 74%
3. The combination of biostimulation and bioaugmentation in the Lamongan Fish Market wastewater, experienced the best decrease in the ammonia parameter by 96% and nitrate by 85%.

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