



The Effectiveness of Probiotics in a Wastewater Treatment Plant (WWTP) For Reducing Environmental Pollution Parameters at the Tanah Merah Animal Slaughterhouse (RPH)

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Abstract

The characterization of non-treated liquid waste is essential in designing an effective waste management system. This study explores the profile of wastewater contamination from Slaughterhouses based on the regulation of the Minister of Environment No. 5 of 2014. In addition, this study analyzed the fluctuation of Chemical Oxygen Demand (COD) for 14 days with treatment variations of P0 to P5. Laboratory testing at Trunojoyo University Madura showed a very high level of organic contamination. The BOD reached 246.66 mg/L, exceeding the 100 mg/L threshold. COD of 286.93 mg/L also exceeded the 200 mg/L threshold. During testing, the COD degradation response showed statistically significant variation in the initial (days 0 and 3) and late (day 14) phases. However, there was no significant variation in the intermediate phase (days 5 and 7). P2 treatment on day three showed the best results with the lowest COD of 119.57 mg/L. This signifies the elimination of reactive simple organic compounds. In the intermediate phase, stagnation occurs due to difficulties in the biotransformation of complex compounds into intermediate products. In the final phase, P0 treatment resulted in the most optimal reduction of COD to 116.33 mg/L, indicating system stabilization. Other treatments, namely P1, P2, P3, and P5, experienced persistent organic compound accumulation with a fixed COD above 270 mg/L. This study concluded that the effectiveness of the treatment in reducing COD was influenced by the structural complexity of the pollutant and the process of re-release of dissolved metabolites in the system. This study confirms that waste management approaches require attention to temporal dynamics and the composition of pollutants.

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1. Introduction

Beef is a product of livestock produced from the slaughterhouse. Activities at slaughterhouses are one part of veterinary public health activities. One type of meat that is widely consumed by the people of East Java is beef. The number of meat consumption in Bangkalan Regency every year has increased. According to the Central Statistics Agency (BPS), the average per capita consumption per week according to the Meat Group per Bangkalan Regency (Commodity Unit) is 0.013 in 2021, in 2022 it will reach 0.015, in 2023 it will reach 0.019, and in 2024 it will reach 0.014. This certainly affects the number of cows slaughtered at the Slaughterhouse in Bangkalan Regency and the highest number of slaughters in Bangkalan Regency is in the Tanah Merah

District slaughterhouse, which in 2025 will reach as many as 3,155 heads (Animal Husbandry and Animal Health Service, 2025). In the livestock industry, especially in meat-producing livestock, the existence of slaughterhouse is very important because all products produced from a broiler must come out through slaughterhouse. slaughterhouse must have minimum standards that are met from various aspects such as physical, technological, and environmental. The animal slaughter industry, if not managed properly, can have negative impacts that damage the environment and disrupt human life. Slaughterhouse wastewater is very harmful to the environment. Waste disposal from slaughterhouses can result in oxygen depletion from water bodies and groundwater contamination and cause health problems for surrounding residents. Studies show (Herman, 2023) ^[3] that the waste generated from slaughterhouses contaminates surface water bodies and groundwater due to the cutting process, blood, fat, urine, and undigested food are produced and added to nearby streams. Slaughterhouse waste is easily degraded because it is an organic waste that contains proteins, carbohydrates, fats, and mineral salts that can serve as a substrate for the growth and reproduction of pathogenic microbes. The environmental impact of slaughterhouse activities was once revealed by Singh and Sachan that people living in the neighborhood around slaughterhouse experienced a decline in health quality such as excessive cough, diarrhea, typhoid fever, and malaria due to polluted air and water sources.

The Wastewater Treatment Plant (WWTP) at slaughterhouse Tanah Merah is designed to treat liquid waste before it is discharged into the environment. The construction of WWTP at slaughterhouse Tanah Merah in fact causes many problems, especially in terms of the surrounding environment. The large number of complaints from the community living around the slaughterhouses location provides an early indication of problems that need attention, especially by policy makers. The complaints that have arisen include the smell of dirt that spreads up to a radius of 100 meters which disturbs the comfort of people living around the slaughterhouse's location. The wastewater produced enters sewers and rivers (dry) which only functions during the rainy season. Wastewater that seeps into the soil pollutes the water of residents' wells, thereby reducing the quality of clean water which has been the main source for meeting daily clean water needs. This is suspected to be due to the lack of optimal functioning of WWTP. Based on the above background, the researcher felt the need to conduct a study related to the extent of the effectiveness of WWTP at Tanah Merah slaughterhouses.

slaughterhouses is a place used to slaughter and process farm animals, such as cows, goats, sheep, chickens, and pigs, for human consumption. Slaughterhouses, such animals are slaughtered in a manner that complies with animal welfare and food health standards. slaughterhouses also plays a role in maintaining the quality of the meat produced so that it is safe, suitable for consumption, and meets hygiene and sanitation standards. The slaughterhouse produces large volumes of waste for slaughter and cleaning slaughter animals to meet the consumption needs of the community. Requirements for Ruminant Slaughterhouses and Meat Handling Units In Article 4, the slaughterhouses unit functions to serve the community in providing safe, healthy,

whole, and halal meat. In addition to ensuring that the slaughtering process is carried out in the right way, slaughterhouses is also tasked with ensuring that the meat produced does not become a vector of disease. (Minister of Agriculture Indonesia, 2010) ^[7].

The definition of slaughterhouses according to the Indonesian National Standard (SNI) 01-6159-1999 concerning slaughterhouses is a community service unit in the provision of Safe, Healthy, Whole and Halal meat (ASUH), a place for monitoring and surveillance of animal diseases and zoonoses. In the livestock industry, especially in meat-producing livestock, the existence of slaughterhouses is very important because all products produced from a broiler must come out through slaughterhouses. Slaughterhouses must have minimum standards that are met from various aspects such as physical, technological, and environmental. If not managed properly, the slaughterhouses industry can have negative impacts that damage the environment and disrupt human life. Tanah Merah Slaughterhouse (slaughterhouses) is one of the animal slaughter facilities operating in Bangkalan Regency, East Java Province. Administratively, slaughterhouses Tanah Merah is located in Petrah Village, Tanah Merah District, Bangkalan Regency. This location is not far from community settlements, so slaughterhouses's operational activities have the potential to have a direct impact on the surrounding environment if not managed properly.

Slaughterhouse industry The slaughterhouse industry, if not managed properly, can have negative impacts that damage the environment and disrupt human life. slaughterhouses wastewater is very harmful to the environment. Animals, if not managed properly, can have negative impacts that damage the environment and disrupt human life. slaughterhouses wastewater is very harmful to the environment.

The WWTP at the Tanah Merah Slaughterhouse has a volume capacity of 10 M³ and 15 cows. The number of cuts that sometimes exceed capacity so that the WWTP does not work optimally. Providing treatment to WWTP is possible to reduce or help the performance of WWTP. WWTP (Wastewater Treatment Plant) Slaughterhouse (slaughterhouses) is a facility designed to treat wastewater produced from activities in slaughterhouses. Liquid waste from slaughterhouses typically contains organic matter, blood, fat, meat residues, and other chemicals that have the potential to pollute the environment if disposed of directly without treatment (Herman *et al.*, 2023) ^[3]. Slaughterhouse Liquid Waste slaughterhouses waste has the potential to damage the environment due to its high concentration of organic pollutants. The consequences posed by slaughterhouses wastewater are usually the result of blood from animals and intestines as well as the rest of the feces from the cleaning of the cage containing suspended solids, proteins and fats. This waste will result in a high increase in the variety of types and amounts of dissolved residues that can contaminate waterways, rivers, and water bodies. If this waste is not managed properly, it will damage the ecosystem which can reduce oxygen, cause the development of foul-smelling gases and allow disease-carrying organisms to nest (Rahayu & JAR, 2019) ^[4].

Waste generated from slaughterhouses itself must go through waste handling procedures and proper management.

This aims to ensure that the waste produced is safe when flowing into the environment and does not cause pollution because it does not exceed the predetermined wastewater quality standard limit (Lubis *et al.*, 2020) ^[5].

slaughterhouses waste contains suspended blood solutions, proteins, fats and solids which cause high levels of organic matter and nutrients, the high variety of types and dissolved residues will have the effect of polluting rivers and water bodies (Sanjaya Kiki & Wahid, 2024) ^[6]

The waste that arises from the animal slaughter process at slaughterhouses consists of feces, urine, rumen or stomach contents, blood, meat or fat, and water from the room washing process. Risks or dangers arising from activities at slaughterhouses with less effective wastewater management or no wastewater treatment plant (WWTP) have the potential to pose threats, such as the emergence of pathogenic bacteria that cause disease as well as increased levels of BOD, COD, TSS, oils and fats, and pH.

Wastewater Parameters of Slaughterhouses according to the Regulation of the Minister of Environment of the Republic of Indonesia Number 5 of 2014 explains that the parameters of wastewater for slaughterhouses consist of:

1. Biochemical Oxygen Demand (BOD) is the amount of oxygen in wastewater that bacteria or microorganisms need to carry out aerobic decomposition of organic materials under certain time and temperature standard conditions. The decomposition of organic waste through the oxidation process by microorganisms in water is a natural process that easily occurs if the wastewater contains enough oxygen. In wastewater organic pollutants are naturally broken down by the bacteria present. When there is enough oxygen, bacteria will decompose aerobically. If they run out of oxygen, decomposition is carried out by anaerobic bacteria. Biochemical Oxygen Demand (BOD) is an important indicator to find out the organic substances in wastewater, the more organic substances are contained, the higher the BOD level. The maximum BOD level allowed for slaughterhouse activities is 100 mg/L.

2. Chemical Oxygen Demand (COD) To find out the amount of organic matter in water, a faster test can be carried out than the BOD test, which is based on the chemical reaction of an oxidant material. COD (Chemical Oxygen Demand) test, which is a test that determines the amount of oxygen needed by oxidant materials, such as potassium dichromate, to oxidize organic matter in water. The COD test usually results in a higher oxygen requirement than the BOD test because materials that are stable to biological reactions and microorganisms can be oxidized in the COD test. The maximum COD level allowed for slaughterhouse activities is 200mg/L.

In Liquid Waste, the standard quality of wastewater produced by businesses or activities such as industry and so on has been determined by the government in determining the size of the level or limit of pollutant particles in the water. Wastewater quality standards (BMAL) are often called effluent standards. To determine BMAL, there are two stages that must be carried out, namely the determination of the target water quality and then the determination of the pollution burden on the carrying capacity of the sample being tested (PerMen LH 5, 2014).

Table 1: Parameters and Maximum Rate

Parameter	Units	Up to Maximum
COD	Mg/L	200
BOD	Mg/L	100

Source: Regulation of the Minister of Environment of the Republic of Indonesia Number 5 of 2014 concerning wastewater quality standards for Slaughterhouse (slaughterhouses) activities

According to the Regulation of the Minister of Environment Number 5 of 2014 concerning Wastewater Quality Standards for slaughterhouses activities, there are several parameters of slaughterhouses waste that must meet the applicable quality standards, which can be seen in Table 2.1.

Research conducted by (Vinsensius *et al.*, 2022) ^[8] on the Evaluation of the Feasibility of a Liquid Waste Treatment Plant at the Oeba Slaughterhouse, Kupang City. The methods used in this study are observation methods and laboratory tests. The data from the research results were analyzed descriptively by describing the results of the analysis obtained in the form of tables, graphs, and drawings and compared with the regulation of the Minister of Environment Number 05 of 2021. Based on the results of the research that has been conducted, it can be concluded that the WWTP system implemented at the Oeba Slaughterhouse, Kupang City with the parameters used including pH, temperature and total coliform parameters meet the quality standards that have been set, while the TSS, BOD, and COD parameters exceed the quality standards that have been set.

Research conducted by (Sari, 2020) ^[9] on Liquid Waste Treatment with the Addition of Probiotics (ANPRO) at the Ruminant Slaughterhouse (slaughterhouses-R) in Pemembang City. This study is a quantitative research with a Pretest True Experiment (Posttest Control Group Design) method approach. This method is a research design that uses two groups of subjects. The administration of Anpro bacteria in liquid waste treatment can be done by adding 5 times the dose with a time of 14 days, while at a dose of 10 times it is very effective because the process of decomposition of bacteria is even better.

Research conducted by (Katrina Purba *et al.*, 2022) ^[10] on the effect of giving different microorganisms (EM4) effectiveness on laundry wastewater quality. This study aims to evaluate the effect of the effectiveness of microorganisms (EM4) in neutralizing pH and reducing phosphate, ammonia, nitrite, and nitrate levels in laundry wastewater. This study was conducted for 14 days at the Aquatic Resources Management Laboratory (MSP), Department of Fisheries, University of Palangkaraya using a Complete Random Design (RAL) with four treatments: treatment K (without dose), treatment A (20 ml), treatment B (40 ml), and treatment C (60 ml), each repeated three times, with observation and water sampling on days 0, 1, 7, and 14. Research shows that microorganisms (EM4) are effective in neutralizing pH and lowering levels of phosphates, ammonia, nitrites, nitrates, although the effect is not significant. The optimal treatment dose is found in treatment C (60 ml). The average values of phosphate, ammonia, nitrite, and nitrates showed a more significant decrease than the K, A, and B treatments.

2. Method

This research was conducted at the Integrated Laboratory,

Trunojoyo University Madura. The research period was carried out from July to August 2025.

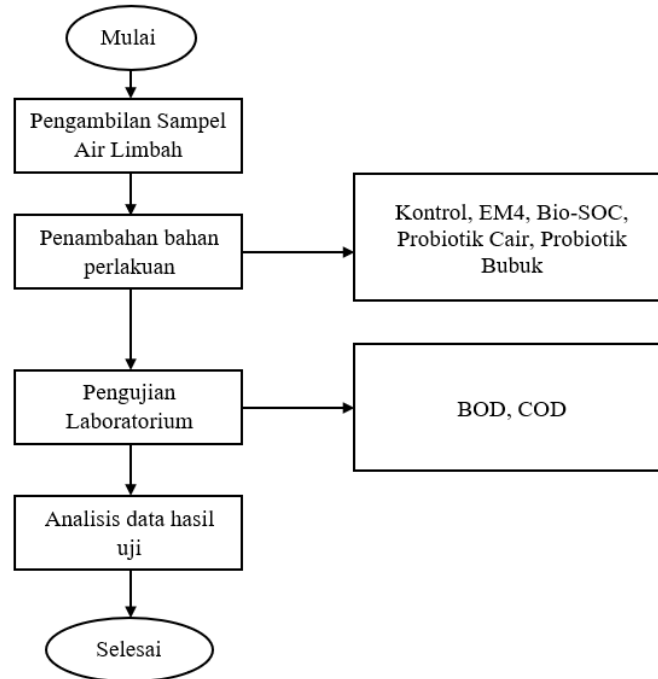


Fig 1: Research Methodology Flow

Liquid waste sampling was carried out at the Tanah Merah Slaughterhouse (slaughterhouses) located in Petrah village, Tanah District, Merah Regency.

The treatment scheme is carried out by putting each probiotic product into a liquid waste sample bottle using an anaerobic system. After the data is collected, the next stage is to select data which is then processed and analyzed. The data presentation method is one of the steps in compiling a research report that aims to make the data easy to understand, analyze, and draw conclusions so as to provide a clear understanding of the research being conducted. The data obtained from this study are the results of laboratory tests on the levels of BOD, COD, TSS, oils and fats, and pH. Data were processed using SPSS to compile a variance analysis table (ANOVA) with a significance level of 0.05. If there is a difference between samples, then it is further analyzed using the Duncan method. ANOVA (Analysis of Variance) is a parametric statistical test used to find out if there is a significant mean difference between two or more treatment groups.

3. Results and Discussion

Wastewater contamination testing before treatment is an important stage in the study of liquid waste management, this

test provides a preliminary overview of the level of pollution and characteristics of the waste to be treated. This information is the basis for determining the type and effectiveness of the appropriate processing technology. The parameters analyzed in this study include Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD). These parameters were chosen because they are generally used as the main indicators of wastewater quality. Laboratory examination of liquid waste was carried out at the Integrated Laboratory, Trunojoyo University of Madura (UTM). To measure the BOD rate, COD. After the laboratory results come out, the following test results data are obtained. Data were taken from control samples without treatment, to determine the results of slaughterhouses liquid waste contamination levels:

The test results were compared with the wastewater quality standards stipulated in the Regulation of the Minister of Environment Number 5 of 2014. Although this regulation was issued before 2020, until now it is still valid and has become a national reference in wastewater management in Indonesia. This regulation serves as a legal standard that must be complied with by business and industrial activities in the management of liquid waste before it is discharged into the river

Table 2: Results of Tanah Merah Slaughterhouses-R Liquid Waste Content Test

Yes	Parameter	Quality Standards (mg/L)	Test Results Before Treatment	Klasifikasi Quality Standards (Ministerial Regulation LH 5/2014)
1	BOD	100	246,66	Height
2	COD	200	286,93	Medium

Remarks: Numbers accompanied by different letters in the same column indicate a real difference between the treatments on the Duncan test 5% tn = unreal, * = real difference

After laboratory analysis of the parameters of BOD, COD before being treated, it is known that most of these parameters still do not meet the limits set in the Regulation of the Minister of Environment Number 5 of 2014. The BOD value was 246.66 mg/L, COD was 286.93 mg/L, indicating a very high level of organic pollution, far exceeding the set quality standard threshold, the amount of the main parameters exceeding the set quality standards, except for the oil and fat parameters. This shows that there are many organic materials that are easily decomposed in the waste so that they require a large amount of oxygen for the decomposition process. This situation can cause the level of dissolved oxygen in the water that receives the waste to decrease.

BOD is the main parameter used to describe the level of biodegradable organic pollution in wastewater. BOD indicates the amount of dissolved oxygen required by aerobic microorganisms to decompose organic matter during the biodegradation process. A high BOD value indicates a high concentration of organic matter that has the potential to reduce dissolved oxygen levels in water (Salimi *et al.*, 2021) [11]. The results showed that the BOD value of wastewater before treatment was 246.66 mg/L, more than double the quality standard set by the quality standard of 100 mg/L.

This condition indicates that wastewater contains a very high organic load. According to Ahmed *et al.* (2020), wastewater disposal with high BOD without treatment can cause hypoxic conditions in the receiving water bodies, leading to a decrease in biodiversity and disturbance of the balance of aquatic ecosystems. In addition to having an impact on the environment, a high BOD value is also a challenge in wastewater treatment. Recent studies show that wastes with high concentrations of BOD generally require intensive biological treatment, such as activated sludge systems or biofilm reactors, to ensure effective reduction in organic load (Li *et al.*, 2022).

COD is a parameter that measures the amount of oxygen

needed to oxidize all organic and inorganic materials that can be chemically oxidized. In contrast to BOD, COD includes compounds that are not biodegradable, thus providing an overview of the total pollutant load in wastewater (Liang *et al.*, 2020). The COD test result value was 286.93 mg/L, which exceeded the quality standard of 200 mg/L. High COD values indicate the presence of complex organic compounds and the possibility of reduced inorganic compounds contributing to the pollution load. This waste contains organic and/or inorganic compounds that are difficult to decompose biologically. This parameter is often used to assess more complex contamination in liquid waste.

Recent research shows that high COD values often correlate with low efficiency of a single biological processing, requiring a combination of biological and physical-chemical processes (Chen *et al.*, 2023) [13]. In this study, a high BOD value followed by a high COD value also indicates that although most organic matter is biodegradable, there is still a division or separation of pollutants that are difficult to decompose and require additional treatment.

Chemical Oxygen Demand (COD) is a parameter that indicates the amount of oxygen needed to oxidize all organic and inorganic compounds that can be chemically oxidized in wastewater. Unlike BOD, which only has a high biodegradable organic content, COD reflects the total pollutant load which includes biodegradable compounds and compounds resistant to biological processes. Therefore, COD is often used as a key indicator of overall wastewater pollution levels (Wang *et al.*, 2021). The results of the Chemical Oxygen Demand (COD) measurement showed that the treatment response to COD changes varied between observation times. Based on Duncan's follow-up test of 5%, a significant difference between treatments was identified on day 0, day 3, and day 14 ($p < 0.05$), while on day 5 and day 7 there was no significant difference ($p > 0.05$).

Table 3: Chemical Oxygen Demand (COD) Slaughterhouses-R Tanah Merah Test Results

Treatment	COD (mg/L) On Day To -				
	0	3	5	7	14
P0	287,00 c	210,33 b	198,33	232,67	116,33 a
P1	282,67 c	281,33 c	288,00	302,00	306,33 c
P2	223,00 bc	119,67 a	244,00	247,33	292,00 c
P3	175,00 from	178,00 from	201,67	274,67	275,00 c
P4	279,67 c	271,33 c	219,67	214,33	193,00 b
P5	147,00 a	243,00 bc	288,00	253,00	276,33 c
Uji Duncan 5%	*	*	tn	tn	*

Remarks: Numbers accompanied by different letters in the same column indicate a real difference between the treatments on the Duncan test 5% tn = unreal, * = real difference

The results of the Chemical Oxygen Demand (COD) observation on day 0 showed the initial condition of the wastewater before the treatment process took place significantly. At this stage, the COD value reflects the total load of chemically oxidized organic and inorganic compounds, including biodegradable compounds, complex compounds, and compounds resistant to biological degradation (Wang *et al.*, 2021). COD values on day 0 showed considerable variation between treatments, with values ranging from 147.00 mg/L (P5) to 287.00 mg/L (P0). The results of the 5% Duncan test showed a marked difference between the treatments on day 0, indicating that each treatment had different initial characteristics in influencing the presence of oxidized compounds in the

wastewater.

P0 and P1 treatment showed relatively high COD values on day 0, which indicates the dominance of complex organic compounds or reduced inorganic compounds that are still intact and have not undergone the biodegradation process. According to Zhang *et al.* (2022), high initial COD values are often associated with the presence of aromatic compounds, complex proteins, as well as industrial byproducts that require intensive chemical oxidation. The opposite result in the P5 treatment showed the lowest COD value on day 0. This condition indicates that the treatment has an initial ability to reduce the fraction of oxidized compounds, possibly through physical mechanisms such as precipitation or early adsorption. However, low COD values in the early stages do

not necessarily guarantee the effectiveness of the treatment in the later stages, as fractions of resistant compounds can still accumulate and affect COD values in the later phases (Li *et al.*, 2022).

The difference in initial COD values can also be influenced by variations in the distribution of dissolved and suspended organic compounds, as well as the initial interaction between the waste and the treatment applied. Therefore, the results on day 0 provide an important picture of the initial state of the system and the potential response to the advanced processing process.

Day 3 is the initial phase of the process of transforming pollutants in the waste treatment system. At this stage, changes in COD values reflect the initial response to treatment, either through chemical reactions, physical processes, or the beginning of the biological activity of microorganisms (Chen *et al.*, 2023) ^[13]. On day 3, a decrease in COD began to be seen in some treatments. The P2 treatment produced the lowest COD value of 119.57 mg/L and based on the Duncan 5% test this value was significantly different from other treatments. This sharp decline indicates that P2 treatment is highly effective in eliminating chemically oxidized compounds in the early phases. The results showed a marked difference compared to P1 and P4 which showed the highest COD values. The other treatments were in the intermediate group and did not differ markedly, suggesting that the initial effectiveness of the treatment in degrading chemically oxidized organic compounds still varied.

A significant decrease in COD in the early phase is often associated with the removal of simple organic compounds and reduced inorganic compounds through oxidation reactions or adsorption mechanisms. Wang *et al.* (2021) stated that the initial phase of waste treatment is often dominated by the elimination of the reactive COD fraction, resulting in a rapid reduction in COD in a short period of time. On the contrary, P0, P1, and P4 treatments showed a relatively small or even stagnant decrease in COD on day 3. This condition shows that in this treatment, the complex organic compound fraction still dominates and has not undergone significant transformation. According to Chen *et al.* (2023) ^[13], complex organic compounds require longer contact times or additional treatments to be effectively oxidized.

After the initial phase of processing which is characterized by a noticeable difference in COD values on the 0th and 3rd days, the processing system enters the intermediate phase on the 5th and 7th days. This phase is an important transition stage that reflects a shift in the dominant mechanism from the elimination of oxidized compounds that react easily to the advanced transformation process of more complex and resistant organic compounds. This change in mechanism is generally characterized by a slowing rate of COD decline as well as an increase in uniformity of responses between treatments (Gheraout & Elboughdiri, 2020). On day 5 and day 7, the results of the statistical test showed no real difference between the treatments. Although numerically there are fluctuations in COD values, this condition indicates that the oxidation process of organic compounds takes place relatively uniformly throughout the treatment, so the specific effects of the treatment are not statistically detected in this phase.

On day 5, COD values across all treatments showed a relatively homogeneous trend, with a range of values that remained at medium to high levels. The absence of a

significant difference between treatments indicates that at this stage, the processing process is dominated by the transformation of complex organic compounds into intermediate products, which still contribute significantly to the value of COD. According to Dutta *et al.* (2021), in the intermediate phase of waste treatment, microorganisms begin to degrade complex organic compounds such as proteins, polysaccharides, and aromatic compounds into simpler molecules. However, the partial degradation product is still chemically oxidized, so the decrease in COD does not occur drastically. This condition explains why COD values tend to stagnate or experience small fluctuations on day 5.

On day 7, the COD value still showed a relatively uniform pattern between treatments and remained statistically not significantly different. However, conceptually, day 7 marks the transition to an advanced phase, where some of the compounds between the degradation results begin to undergo further oxidation or transformation into a more stable form. According to Li *et al.* (2021), in this phase the microorganism has passed the initial adaptation stage and begins to operate under more stable metabolic conditions. However, complex organic compounds that are resistant to biological degradation still persist in the system and contribute to COD values. Therefore, the decline in COD on day 7 generally lasts more slowly than the initial phase.

In addition to biological mechanisms, physical-chemical factors also play an important role in this phase. Some organic compounds can undergo condensation reactions or the formation of new complexes that are stable and difficult to oxidize, thus maintaining COD values at some level. A study by Rodríguez-Narvaez *et al.* (2021) shows that the presence of aromatic compounds and partial oxidation by-products can inhibit the reduction of COD in the intermediate to advanced phase of waste treatment.

Thus, the conditions on day 7 reflect a temporary balance between the advanced degradation process and the formation of stable compounds, which explains why COD values have not shown a significant decrease and the differences between treatments are still not statistically visible.

At the end of observation (day 14), the difference in COD between treatments was again observed significantly. The P0 treatment showed the lowest COD value and was significantly different from the other treatments, while the P1, P2, P3, and P5 treatment were in the group with higher COD values and did not differ significantly from each other. These results show that the effectiveness of treatment in lowering COD is temporal and influenced by the dynamics of the processing process during the observation time. Based on the observations, the COD value on day 14 showed a marked difference between treatments based on the 5% Duncan test. The P0 treatment resulted in the lowest COD value of 116.33 mg/L, which was significantly different from all other treatments. The significant reduction in COD in this treatment indicates that the treatment mechanism that occurs is able to eliminate most of the chemically oxidized compounds to the final phase.

According to Abdelrahman *et al.* (2020), the continuous decline in COD until the final phase of processing is generally related to the subsequent oxidation of intermediate compounds as well as system stabilization, where fractions of complex organic compounds have been decomposed or transformed into simpler forms and no longer contribute significantly to COD. This suggests that P0 treatment has a

relatively good ability to deal with non-biodegradable compounds or resistant compounds that are not completely eliminated in the early and intermediate phases. In contrast, P1, P2, P3, and P5 treatments showed relatively high COD values on day 14, which was above 270 mg/L. This condition indicates that even though the processing process has lasted for two weeks, there are still fractions of organic or inorganic compounds that are persistent and difficult to oxidize completely. According to Li *et al.* (2021), the presence of aromatic compounds, humic acids, and partial degradation by-products is often the cause of high COD values in the final phase of waste treatment.

effect on COD changes in the early and late phases of observation, but not in the middle phase of the process.

Variations in response between treatments indicate differences in the oxidation mechanisms of organic compounds, as well as the possibility of re-release of dissolved organic compounds during the treatment period.

4. Conclusion

Initial Characterization and Quality Status of Wastewater: The characteristics of wastewater effluent prior to treatment show a high level of organic contamination and are above the safe threshold required by the Minister of Environment Regulation No. 5/2014. The BOD (246.66 mg/L) and COD (286.93 mg/L) parameters confirmed the high to medium category organic load. This condition is exacerbated by an acidic pH value (5.38) and a high TSS (166.63 mg/L), so this waste has the potential to damage aquatic ecosystems if it is immediately disposed of without treatment. In contrast, the oil & fat content (3.70 mg/L) is still within safe limits.

Temporal Dynamics of COD Degradation: The application of various treatment variations (P0 to P5) resulted in a dynamic, non-linear COD degradation profile over 14 days of observation. Based on the Duncan Multiple Distance Test (DMRT) at a significance level of 5%, the variation in treatment had a significant effect (*) in the initial phase (Day 0 and 3) and the final phase of the experiment (Day 14). However, in the mid-incubation phase (Day 5 and 7), differences between treatments were detected insignificant (\$tn\$). This indicates a transition phase of microbial kinetics or chemical stabilization within the system.

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