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Utilization of Bacillus Sp. Bacteria on Reducing Pollutant Levels of Leather Tanning Industry Wastewater

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ABSTRACT: The leather tanning industry generates wastewater that can potentially pollute the environment, thus requiring effective treatment. This study aimed to analyze changes in BOD, TSS, sulfide, and pH concentrations before and after treatment, as well as to determine the optimal retention time. The method applied was an aerobic biofilter system using bioball media and the addition of Bacillus sp. with retention times of 0, 24, 48, and 72 hours. The results showed that the aerobic biofilter system with bioball media and Bacillus sp. reduced BOD from 2,680 mg/L to 142 mg/L (94.7% efficiency) and TSS from 1,670 mg/L to 320 mg/L (80.9% efficiency). The pH value increased from 7.34 to 8.60, while sulfide levels remained below the detection limit. The optimal retention time was 72 hours, indicating that this system is effective in improving the quality of leather tanning wastewater.

KEYWORDS: Aerobic biofilter, bacillus sp., leather tanning, wastewater

I. INTRODUCTION

In the global economy, the leather tanning industry plays an important role in meeting the needs of various sectors such as fashion and automotive. However, the production process produces liquid waste that is rich in organic and inorganic pollutants. Based on DIY Regional Regulation No. 7 of 2016, tanning is an activity that processes raw materials into finished leather goods. Each stage requires large amounts of water and additional chemicals, resulting in liquid waste with high pollutant potential. Liquid waste from the leather tanning industry generally contains sulfides, TSS, BOD, and extreme pH which can reduce water quality and endanger the ecosystem (Setiyono & Yudo, 2014; Susila & Sumasto, 2005). Treatment of tannery wastewater — which is generally known to contain many sulfur compounds (including sulfides), organic materials, and poses potential environmental/health hazards if discharged without adequate treatment (Valeika et al., 2006)

BPS (2024) shows that only 77% of industrial wastewater in Indonesia is treated safely, while some is still discharged directly into water bodies due to the limitations of conventional treatment technology. One alternative treatment is bioremediation using an aerobic biofilter reactor with bioball media and the addition of Bacillus sp. bacteria, which is known to be effective in reducing pollutants through biological activity.

Aerobic biofilters are a wastewater treatment technology that uses aerobic microorganisms to break down organic pollutants with the help of oxygen (Apelabi et al., 2021). One effective medium used is bioball, which is spherical and hollow with a high surface area (200–240 m^2/m^3) that supports the growth of microbial biofilm. Bioballs have the advantages of being lightweight, durable, easy to clean, and efficient in enhancing pollutant degradation.

Bacillus sp. is a gram-positive bacterium from the Bacillaceae family that is catalase-positive, motile with flagella, and capable of living both aerobically and facultatively anaerobically. This bacterium is commonly found in soil, water, and the rhizosphere, with the ability to produce enzymes that break down organic matter and antimicrobial compounds, thereby playing an important role in nutrient cycling and having potential in bioremediation (Sumardi et al., 2012).

Based on Azzahra's research (2024), the resistance test of Bacillus sp. against iron (Fe) at a concentration of 10,000 mg/L decreased to 3,172 mg/L at hour 0. The bioremediation ability of Bacillus sp. affects the concentration of Fe in the growth medium. Over the incubation period, bacterial performance decreases as it reaches saturation, resulting in a reduced ability to bind metals and the potential to release previously absorbed metals.

Considering the importance of managing wastewater from the leather tanning industry and the potential of Bacillus sp. as a solution in bioremediation, this study aims to analyze changes in Sulfide, TSS, BOD, and pH levels in leather tanning industry wastewater before and after treatment, as well as to determine the most effective retention time using an aerobic biofilter reactor with bioball media and the addition of Bacillus sp. bacteria.

II. METHODOLOGY

A. Time and Place of the Research

This research was conducted on a laboratory scale in April 2025. The research location was at the Environmental Quality

Laboratory of AKPRIND University Indonesia and the analysis of Sulfide and BOD was carried out at the Balai Kesehatan Masyarakat Yogyakarta.

The tools used for the research include: aerobic biofilter tank, aerator, bioball media, waste collection tank, petri dish, inoculating needle, incubator, stationery. The research materials consist of: leather tanning industry liquid waste, Bacillus sp. isolate, nutrient agar (NA), nutrient broth (NB), distilled water.

B. Wastewater Sampling Techniques

The sampling of liquid waste from the leather tanning industry is based on SNI 6989.59:2008 concerning Methods for Sampling Water Samples. A industry that does not yet have a wastewater treatment plant (WWTP) can have wastewater samples taken if there is an equalization tank with batch processes originating from several discharge channels. The quality of wastewater can vary due to the production process. All wastewater from various stages of production is collected in an equalization tank before being discharged. Therefore, samples in this study will be taken as grab samples in the channel before the wastewater enters the receiving waters National Standardization Body (2008).

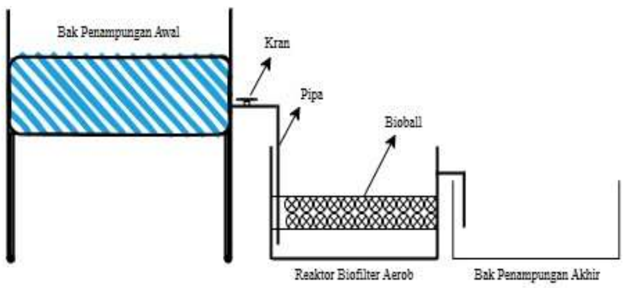

Figure 1. Reactor design schematic

C. Isolate Rejuvenation Process

The bacterial isolate will be regrown on solid nutrient agar (NA) medium and then incubated at room temperature for 2 x 24 hours. After that, the colonies cultured for 2 x 24 hours will be refreshed by taking a single colony using a sterile inoculating needle and then performing a streak plate on the surface of the Bacillus sp. nutrient agar (NA) medium, followed by re-incubation for 2 x 24 hours. The subculture is considered successful if colonies appear on the nutrient agar (NA) medium.

The results of the first-stage subculture purification were followed by a second-stage subculture in liquid medium. A single colony of microorganisms separated on the surface of the solid medium was taken with an inoculating loop and inoculated into 300 ml of nutrient broth (NB) (Shovitri, 2013). A total of 1 mL of the subculture was diluted in sterile distilled water.

D. Seeding and Acclimatization Process

Seeding (microorganism cultivation) can be carried out by adding bacteria, by flowing the wastewater to be treated into a reactor containing bioball media and adding Bacillus sp. bacteria until a biofilm layer forms on the biofilter media used. The results obtained must be stable, so the cultivation time required is 2 weeks. Wastewater is added to the culture so that the medium can oxidize the organic pollutants in the wastewater. In addition, seeding is carried out to cultivate microorganisms so that they can be conditioned in an initial environmental adaptation site, serving as a breeding ground for microorganisms that will be tested in the reactor. At this stage, the microorganisms will grow on the medium, indicated by a slimy layer that has formed, which is dark to brown in color and not easily detached from the medium. In addition, in this process, the initial sulfide levels are measured under the assumption that Bacillus sp. bacteria have grown and developed in the medium.

After seeding, the next step is acclimatization, which involves replacing the new wastewater in the reactor that contains bioball media in order to obtain a good culture and microorganisms that are able to adapt to the wastewater. The acclimatization process is carried out by gradually replacing the wastewater from the seeding with tanning wastewater over 4 days at mixing concentrations of 30%, 50%, 80%, and 100%. This acclimatization process is carried out to prevent the previously seeded bacteria from dying because they have not yet had the chance to adapt to the new environment. In this process, the wastewater is replaced by continuously flowing the wastewater into the reactor until the water from the seeding process is completely replaced with industrial tanning wastewater.

E. Waste Processing

In this process, the waste is treated using an aerobic biofilter reactor made of glass, equipped with a tap and media support. A total of 10 L of waste will be treated. The wastewater is collected in an initial holding tank and then flows upward into the aerobic biofilter reactor containing bioball media. In this treatment, the retention time variations used are 24 hours, 48 hours, and 72 hours. After the wastewater is treated according to the specified retention time variations, the next step is to examine parameters such as sulfide, TSS, BOD, and pH.

F. Metode Analisis

The method used is descriptive analysis by examining the The reduction of the tested parameters will be analyzed descriptively by looking at the comparison before and after for each parameter of tannery industrial wastewater. This is done by calculating using the reduction efficiency formula and creating a graph as follows:

$$Ep = \frac{C1-C2}{C1} \times 100\%$$

Description:

Ep: Reduction efficiency

C1: Content before processing

C2: Content after processing

The analytical analysis used in this study is linear regression analysis using correlation tests with SPSS software. This analysis aims to identify the influence between variables and measure how strong the relationship is between the dependent variable and the independent variables used through the correlation coefficient.

III. RESULTS AND DISCUSSION

A. Waste Processing Results

Waste is collected at the initial collection tank of the Bantul Communal WWTP and has been treated using an aerobic biofilter reactor with bioball media and the addition of Bacillus sp. Bacteria.

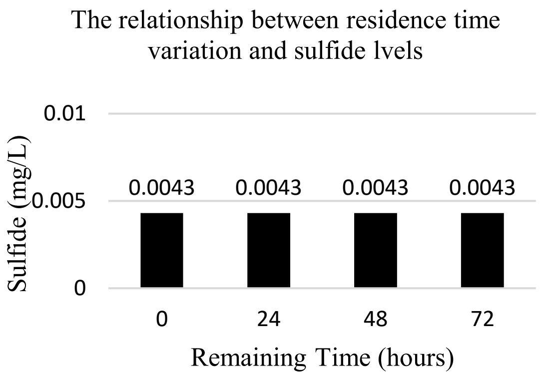

Figure 1. Graph of the relationship between residence time variation and sulfide concentration

Based on Table 1, the liquid waste from the leather tanning industry has the highest pollution levels in the BOD and TSS parameters, which have exceeded the quality standards according to DIY Regional Regulation No. 7 of 2016, so treatment is required before it is discharged into the environment.Waste from the equalization tank is physically dark gray to black, has a pungent odor, and contains oil and fat, which have the potential to pollute the environment.

Table 1. Results of sample tests before processing

Parameter	Hasil Uji (mg/L)	Baku Mutu (mg/L)
Sulfida	<0,0043	0,5
BOD	2.680	50
TSS	1.670	50
pH	7,34	6,0-9,0

One of the processing alternatives applied is an aerobic biofilter with bioball media combined with the addition of Bacillus sp. bacteria.

B. Effect of Residence Time on Sulfide Levels

The efficiency of sulfide reduction is calculated from the ratio of concentration before and after treatment. The results for residence time variations of 0, 24, 48, and 72 hours with an aerobic biofilter based on bioballs and Bacillus sp. bacteria are shown in the following table 2.

The processing results showed that the sulfide level remained <0.0043 mg/L from the beginning to the end of the treatment, so no significant decrease was observed. This is because the sulfide concentration in the waste is already very low before treatment due to natural biodegradation processes by microorganisms. Sulfide is described as a toxic, flammable, colorless gas with a 'rotten egg' smell at low concentrations (Chou, 2016). Sulfide is related to industrial activities (e.g., animal feed factories, seafood processing) and suggests a more conservative threshold to protect the community (Puspasar et al., 2019).

Thus, the aerobic biofilter reactor based on bioball with Bacillus sp. did not show a significant effect due to substrate limitations. However, the results still meet the quality standards of DIY Regional Regulation No. 7 of 2016 (0.5 mg/L), making it safe for the environment.

Table 2. Results of sulfide processing

Variasi waktu tinggal (jam)	Sulfida (mg/L)	Efisiensi penurunan (%)
0	<0,0043	0
24	<0,0043	0
48	<0,0043	0
72	<0,0043	0

The research data was tested using linear regression to determine the relationship between variations in retention time and sulfide parameter levels. This test was conducted using the SPSS program. The analysis results indicate that the correlation coefficient and its significance value cannot be obtained because there are no variables that vary, since if one of the variables in the analysis does not vary, it is impossible to calculate the relationship between the two. The values of the sulfide parameter levels are the same, so there are no changes that can be analyzed.

C. TSS Parameter

The efficiency of TSS reduction is calculated based on the comparison of concentrations before and after treatment, with results at residence time variations of 0, 24, 48, and 72 hours shown in the following table.

Table 3. TSS processing results

Variation of residence time (hours)	TSS (mg/L)	Reduction efficiency(%)
0	1.670	0
24	990	40,7
48	370	77,8
72	320	80,9

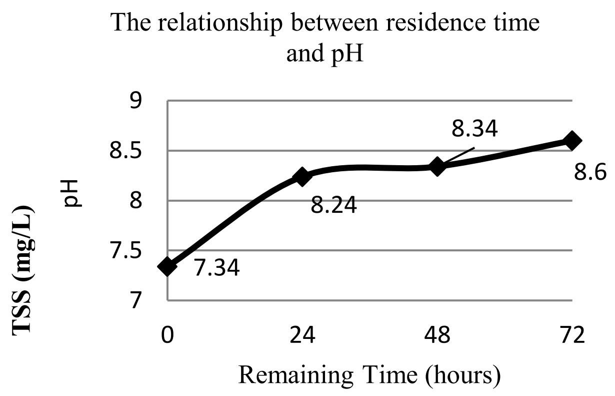

Figure 2. Graph of the relationship between residence time variation and TSS levels

The processing results showed that the TSS levels experienced a significant decrease from 1,670 mg/L at 0 hours of residence time to 320 mg/L at 72 hours, with the highest removal efficiency reaching 80.9%. This decrease is due to the contact process between the waste and the biofilm on the bioball media, which plays a role in the decomposition of suspended solids. Nevertheless, the final TSS concentration still does not meet the quality standard set in the DIY Regional Regulation No.7 of 2016, which is 50 mg/L.

The research data were analyzed using linear regression testing to determine the relationship between retention time variations and TSS parameter levels; this test was conducted using the SPSS program.The analysis results showed that the correlation coefficient for the variation in residence time with the decrease in TSS parameter levels was -0.952, indicating a negative relationship. This suggests that the longer the residence time used, the greater the reduction in the TSS parameter levels. It can be concluded that statistically there is a significant effect of residence time variation on the reduction of TSS levels.

D. BOD Parameter

The efficiency of BOD reduction calculated based on the comparison of concentrations before and after treatment, with results at residence time variations of 0, 24, 48, and 72 hours using an aerobic biofilter with bioball media supplemented with Bacillus sp. presented in the following table.

Table 4. BOD processing results

Variation in length of stay (hours)	BOD (mg/L)	Reduction efficiencyn (%)
0	2680	0
24	296	89,5
48	216	92,4
72	142	94,7

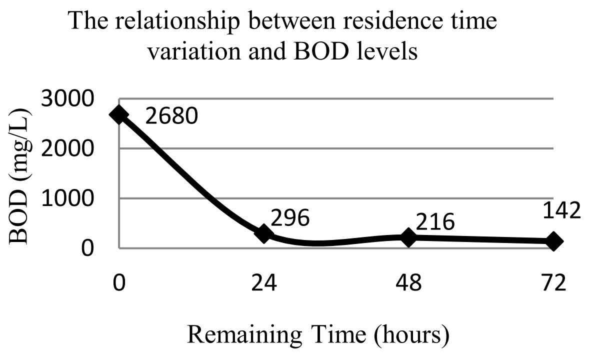

Figure 3. Graph of the relationship between residence time variation and BOD levels

The decrease in BOD was significant with increasing residence time, from 2,680 mg/L at 0 hours to 142 mg/L at 72 hours, with the highest efficiency of 94.7%. This process is influenced by the activity of microorganisms, especially Bacillus sp., which forms biofilm on the bioball media and breaks down organic matter. Although there was a significant decrease, the final BOD level is still 142 mg/L, so it does not yet meet the quality standard of 50 mg/L according to DIY Regional Regulation No. 7 of 2016.

The research data were tested using linear regression to determine the relationship between retention time variations and BOD parameter levels; this test was conducted using the SPSS program. The analysis results showed a correlation coefficient between retention time and the decrease in BOD parameter levels of -0.806, indicating a negative relationship. This suggests that the longer the retention time used, the greater the reduction in BOD parameter levels. It can be concluded that statistically there is no significant effect of residence time variation on BOD reduction. This may occur due to external factors such as temperature, which affect bacterial activity and the biodegradation process, because if environmental conditions are unstable or not properly controlled, the results can be inaccurate.

E. pH Parameter

pH efficiency was analyzed to determine the pH value before and after treatment. The results of pH changes at residence time variations of 0, 24, 48, and 72 hours in an aerobic biofilter with bioball media and the addition of Bacillus sp. are shown in the following table.

Table 5. pH processing results

Variation in length of stay (hours)	pH
0	7,34
24	8,24
48	8,34
72	8,60

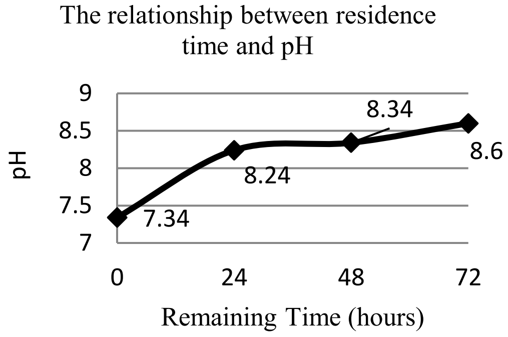

Figure 4. Graph of the relationship between residence time variation and pH levels

The acidity level (pH) indicates the chemical condition of the water, which affects the activity of microorganisms. Observation results showed an increase in pH values over time, from an initially neutral condition to a range of 7.0–8.0. This increase indicates that the aerobic biofilter reactor environment with bioball media and added Bacillus sp. supports the biological decomposition process. The continued pH increase was tested using forecasting, with the maximum pH peak occurring at 17 days. Based on DIY Regional Regulation No.7 of 2016, the standard pH range is 6.0–9.0, and the treatment results are still within this standard. This indicates that microbial activity is proceeding optimally, as the pH is within the suitable range for the metabolism of decomposing bacteria.

The research data was tested using linear regression to determine the relationship between residence time variations and sulfide parameter levels; this test was conducted using the SPSS program. The results of the analysis above show a correlation coefficient of 0.914 between residence time and the decrease in pH parameter levels with a positive relationship. This indicates that the longer the residence time, the higher the measured pH parameter level. It can be concluded that statistically there is no significant effect of residence time variations on the decrease in pH levels. The pH levels that continue to increase need to be predicted or forecasted for when they reach their maximum; forecasting tests indicate that the peak increase in pH levels occurs at 17 days.

IV. CONCLUSIONS

Based on the results of data processing and analysis that have been carried out, it is concluded that:

1. The research results show that the initial levels of leather tanning industry wastewater before treatment were sulfide <0.0043 mg/L, BOD 2,680 mg/L, TSS 1,670 mg/L, and pH 7.34. After treatment using an aerobic biofilter reactor with bioball media and the addition of Bacillus sp., the obtained levels were sulfide <0.0043 mg/L, BOD 142 mg/L, TSS 320 mg/L, and pH 8.60, respectively. This indicates that the biofilter system is only effective in reducing BOD and TSS parameters.
2. The residence time for Sulfide, BOD, TSS, and pH after treatment using the addition of Bacillus sp. bacteria in an aerobic biofilter reactor with bioball media showed the most effective results at a residence time of 72 hours for the BOD parameter at 142 mg/L with a reduction efficiency of 94.7%, and a residence time of 72 hours for the TSS parameter at 320 mg/L with a reduction efficiency of 80.9%.

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