Peculiar growth of *Pseudomonas* sp. LS3K with the addition of untreated tannery wastewater

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**Abstract.** The fresh untreated tanner wastewater contains high organic and chemical pollutants. This study aims to determine the growth characters of *Pseudomonas* sp. LS3K in high polluted medium from fresh untreated tannery wastewater. The growth characterization was observed in the solid medium (containing 0;25;50;75 and 100% sterile tannery wastewater) and liquid medium that contained tannery wastewater at 0;25 and 50%). The growth of *Pseudomonas* sp. LS3K was determined by observing the colony in the agar medium, the optical density (OD600) of the liquid medium, and the cell viability. The growth of the bacteria *Pseudomonas* sp. LS3K could not be observed at both agar and liquid medium containing tannery wastewater ≥50%. The growth activity could be observed in the agar medium containing 25% tannery wastewater on the second day incubation time. The growth curve was also observed at a liquid medium containing tannery industrial liquid waste at a concentration of 25% during 36 hours of observation. Based on the results of this study, it can be concluded that the *Pseudomonas* sp. LS3K had the potency adapted to live and grow at the medium that contaminated up to 25% fresh tannery wastewater. This strain could be a bioremediation agent of tannery wastewater.

**Keywords:** Growth characterization, leather industry, tannery wastewater, *Pseudomonas* sp. LS3K

1. **Introduction**

The tannery wastewater from the leather tanning industry has great potential in environmental pollution due to the high number of Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and high concentrations of chemicals pollutant in the waste. The leather tanning industry mostly uses the chrome tanning process, which produces liquid waste containing chromium. Heavy metals cannot be easily degraded, so remediating the wastewater needs complex steps, including physical, chemical, and biological treatments [1].

Bioremediation is the process of biodegradation the organic or inorganic pollutants under controlled conditions. The aim of the controlling system and reducing unwanted pollutants before disposed to the environment. Bioremediation utilizes the living organisms to degrade the hazardous pollutant and contaminants in the wastewater to obtain less hazardous and toxic waste. Bioremediation occurs because of microorganisms' addition to the waste to carry out biotransformation and lead to biodegradation. A group of microorganisms, either single or consortia such as *Chlorella* spp., *Spirogyra* spp., *Aspergillus* spp., *Acinetobacter* spp., *Pseudomonas* spp., *Vibrio* spp., *Arthrobacter* spp., and *Bacillus* spp. has been reported to be a biological agent for bioremediation of the tannery industrial wastewater [2]. These microbes have the potency to reduce odors through nitrification and denitrification processes aerobically to the content of...
organic materials and reduce Cr metal contained in the liquid wastewater from the leather tanning industry [3].

In previous studies, we isolated nitrifying and denitrifying bacteria from the soil around the local chicken farm and were able to grow on the medium with high ammonium content [4]. One of these isolates is Pseudomonas sp. LS3K has been identified and reported to be capable of the aerobic nitrification process [5] and aerobic denitrification [6]. Isolate Pseudomonas sp. LS3K becomes interesting to be studied and develop its potential further. In this study, we explore the resistance and viability of Pseudomonas sp. LS3K in a medium containing fresh tannery wastewater. Furthermore, we expect that Pseudomonas sp. LS3K has a role as a bioremediation agent, which is used to reduce contaminant mass and toxicity in tannery wastewater through either biodegradation (of organics) or biotransformation (of metals).

2. Materials and Methods

2.1. The Sample and bacterial preparation

The sample of fresh tannery wastewater in this study was obtained from one of the local tannery industries located in Bantul District, Special Region of Yogyakarta, Indonesia (DMS 7°50'04.0"S 110°23'49.1"E) in July 2020. The isolate of Pseudomonas sp. LS3K was obtained from the bacterial collection at the Laboratory of Leather, Waste, and By-Products Technology, Faculty of Animal Science, Universitas Gadjah Mada.

2.2. Medium and bacterial culture.

The liquid medium for bacterial culture (100 ml) was made from 1% meat extract, 1% microbiological peptone, and 0.5% NaCl. Agar medium was made by adding 1.5% agar powder and stir until evenly dissolved using a magnetic stirrer. pH was adjusted to 7.2 using 0.1 N NaOH or 0.1 N HCl and continued by autoclave (121°C, 15 psi, 15 minutes).

2.3. Stock isolate

The bacterial stock was made by putting one loopful of Pseudomonas sp. LS3K from the agar plate medium was then added into the sterile liquid medium and incubated on a rotary shaker at 120 rpm for 24 h at room temperature.

2.4. The medium of tannery wastewater.

The fresh tannery wastewater from the local industry was taken and filtered using Whatman filter paper (2 μ) and sterilized by autoclave (121°C, 15 psi, 15 minutes). In the agar and liquid medium, tannery wastewater was added in different concentrations. The agar medium was made in 100 ml with the composition: The sterile fresh tannery wastewater (0; 25; 50; 75 and 100%), aquadest, 1% meat extract, 1% microbiological peptone, 0.5% NaCl, and 1.5% of agar powder. The medium was then stirred until evenly dissolved using a magnetic stirrer, and pH was adjusted to 7.0 with the 40% NaOH solution and then sterilized by autoclave (121°C, 15 psi, 15 minutes). After sterilization, the agar medium was prepared to pour into the Petri dish plate. The Liquid Medium was made in 100 ml with the composition: The sterile fresh tannery wastewater (0; 25 and 50%), aquadest, 1% meat extract, 1% microbiological peptone, 0.5% NaCl, stirred until evenly dissolved using a magnetic stirrer, pH was adjusted to 7.0 with the addition of 40% NaOH solution and then sterilized by autoclave (121°C, 15 psi, 15 minutes).
2.5. Growth characterization assay
The characterization of the agar medium's growth was conducted by taking 100 μl of isolate and added into the agar medium plate containing tannery wastewater, spread using Drigalski, and incubated at 30°C in the incubator for 5 days (every day the bacterial growth was observed). Growth characterization in liquid medium was conducted by taking a 2% (v/v) stock isolate of *Pseudomonas* sp. LS3K and added into 100 ml of tannery wastewater liquid medium. The liquid medium was made in triplicate containing P0 (0% tannery wastewater), P1 (25% tannery wastewater + isolate bacteria), P1 blank (25% tannery wastewater without the addition of bacteria), P2 (50% tannery wastewater + isolate bacteria) and P2 blank (50% tannery wastewater without the addition of bacteria). The medium was placed in a rotary shaker at 120 rpm and incubated for 36 h at room temperature. At every 6 h, the medium was observed using the spectrophotometer (OD600).

2.6. Viability of cell bacteria
Strain LS3K was grown on a liquid nutrient medium with different concentrations of tannery wastewater P0 (0%), P1 (25%), and P2 (50%). Bacteria were taken from the preculture, which had been cultivated for 24 hours. The amount of 5 ml liquid nutrient medium was then added by 1% (v/v) of bacterial stock. Cultivation has been performed at a rotary shaker (120 rpm) for 3 days. At every 24 h, 1 μL of the liquid medium was taken and dropped on the agar plate containing nutrient agar medium. The plate was then incubated for 3 d to observe the cell growth viability.

2.7. Analisis data
The growth activity and characterization of *Pseudomonas* sp. LS3K in the tannery wastewater medium was analyzed by a descriptive figure and graphic.

3. Results and Discussions

3.1 The growth characteristics of *Pseudomonas* sp. LS3K on the agar medium contains sterile tannery wastewater
Figure 1 shows the visualization of *Pseudomonas* sp. LS3K growth on agar medium for 5 days of cultivation. The bacterial growth could be seen in the medium containing 0% and 25% of the tannery wastewater medium. *Pseudomonas* sp. LS3K was able to grow optimally since the 1st day on the medium containing 0% tannery wastewater, while on the medium with 25% tannery wastewater level, the growth was seen on the 2nd day. The growth visualization of *Pseudomonas* sp. LS3K was not observed in the medium with the addition of 50, 75, and 100% of tannery wastewater. In a medium with 0% and 25% of tannery wastewater, the growth of *Pseudomonas* sp. LS3K with colonies forms a yellowish-white was visualized. The results of the visualization of *Pseudomonas* sp. LS3K was in line with the other study [4]. *Pseudomonas* sp. LS3K has a morphological colony observed to be round, high convex with yellowish-white colony color. The growth observation indicated that the *Pseudomonas* sp. LS3K was assumed to have the ability to grow by utilizing organic or inorganic nutrients in the medium sources and still available to adapt in the addition of tannery wastewater up to 25%. [7] stated that *Pseudomonas* is a microbe that has the ability to utilize inorganic nutrient sources for the living and metabolic activities of its cells. The previous study that several bacteria from *Pseudomonas* sp. had adaptive tolerance in the presence of heavy metals such as Pb, Cu, Hg, Ni, and Zn [2].
Figure 1. The growth of *Pseudomonas* sp. LS3K in agar medium containing sterile tannery wastewater at concentrations 0; 25; 50 and 100%, from 1st to 5th day cultivation period.

3.2 The growth characteristics of *Pseudomonas* sp. LS3K bacteria on the liquid medium contains sterile tannery wastewater

The result of bacterial growth (OD600) from *Pseudomonas* sp. LS3K grown in liquid medium with the addition of tannery wastewater for 36 hours of observation can be seen in Figure 2.

Figure 2. The bacterial growth of *Pseudomonas* sp. LS3K in liquid medium at different concentration of sterile tannery wastewater.
Based on the result of the observation by OD600, *Pseudomonas* sp. LS3K still can grow in a medium containing up to 25% of tannery wastewater. In a medium with 0% tannery wastewater, the growth curve was formed where the OD value continued to increase among 36 hours. In a medium with 25% tannery wastewater, the growth curve of *Pseudomonas* sp. LS3K was seen starting increase after the 18th hour, while in control, there were no increases in the OD value. The growth ability of *Pseudomonas* sp. LS3K in the medium with the addition of tannery wastewater up to 25% indicates the adaptive potential for utilizing nutrients in the medium, which are then used as a source of nutrients for life, growth, and cell metabolic activity, which can be observed based on the increase in the absorbance value of the medium. [8] stated that an increase in the absorbance value of the medium reflects an increase in the medium's number of cells.

The growth of *Pseudomonas* sp. LS3K on the medium 50% tannery wastewater was not visible, during 36 hours of observation, there was no visible increase in OD values in control or with the addition of bacteria. Since the beginning of the observation, the absorbance value (OD600) of the medium with 25% and 50% tannery wastewater showed high values OD ranging λ at 0.8 and 2.1 nm. This phenomenon shows that the fresh tannery wastewater's real condition is high in pollutant content, increasing the turbidity level. [1] the fresh tannery wastewater characterization is high in organic, inorganic, nitrogen compound, and chemical compounds that caused the solution with high suspended solids and dissolved solids.

### 3.3 Viability cell of *Pseudomonas* sp. LS3K

The results of the viability test of *Pseudomonas* sp. LS3K is presented in Figure 3. The viability results show that tannery wastewater treatment in the medium P1 and P2 gives the inhibition effect on the *Pseudomonas* sp. LS3K growth on the nutrient agar medium. The visual of colony bacteria formed after cultivation in the medium P1 and P2 were thinner and small compared to control (P0). The presence of tannery wastewater in the medium in this study was performed the inhibition activity against *Pseudomonas* sp. LS3K. That phenomenon was assumed because the effect of pollutants' presence continued in the fresh tannery wastewater used. The higher level concentration of tannery wastewater may be contained, the higher the pollutant substrate contained.

![Figure 3](image.png)

*Figure 3.* The viability of cell bacteria of *Pseudomonas* sp. LS3K after cultivation on the medium contains sterile tannery wastewater.
4. Conclusion

Based on the results of this study, it can be concluded that the *Pseudomonas* sp. LS3K had the potency adapted to live and grow at the medium that contaminated up to 25% fresh tannery wastewater, and this strain could be a bioremediation agent of tannery wastewater.

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