Survival ability of *Bacillus cereus* LS2B in the presence of tannery wastewater

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Abstract. The leather industry's production activity affects liquid waste containing a high amount of organic and chemical compounds. This study aims to determine *Bacillus cereus* LS2 B's survival ability in the medium with the presence of fresh untreated tannery wastewater. The growth characterization was made and observed in the agar medium with 0; 25; 50; 75 and 100% tannery wastewater. Growth profile in a liquid medium was observed with the addition of 0; 25; and 50% tannery wastewater. The survival ability of *Bacillus cereus* LS2B was observed by a visual colony formed in the agar medium, the optical density (OD600) of cell bacteria in the liquid medium, and the cell viability. The growth of *Bacillus cereus* LS2B could not be confirmed at both solid and liquid medium with tannery wastewater more than 25%. The survival ability and cell activity were observed in the agar medium containing 25% tannery wastewater after incubation time at 48 hours. The growth curve was also observed at a liquid medium containing tannery wastewater at 25% during 36 hours observation since at the 12th hour. Thus, *Bacillus cereus* LS2B could tolerate up to 25% of fresh untreated tannery wastewater in the solid and liquid medium.

1. Introduction

Nowadays, tanning processing becomes the creative industry that grew rapidly. However, the tannery will always produce a very significant polluted wastewater with a high content of organic matters, salinity, ammonia, organic nitrogen, and also other pollutants such as chromium and sulfide. The tannery industry has become a serious source of wastewater, plays a role as a serious threat to human health and the aquatic environment. Some organic compounds in the effluent of tannery wastewater are challenging to be handled by biological methods [1]. Tannery wastewater containing several heavy metals pollutants such as Al, Pb, Cd, Hg and Ag do not have any biological role and are toxic to living organisms. Various microorganisms, either single or consortium, such as *Bacillus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Aspergillus* sp., Etc., have been identified to have the ability to survive and degrade the presence of metals in the wastewater, opening opportunities to maximize biological methods in the tannery wastewater treatment, which is more effective, efficient and environmentally friendly [2].
Bacillus cereus LS2B was isolated from odorous soil around the chicken farm and tolerant in the high presence of ammonia stressor in the medium [3]. This isolate was assumed may be one of the potential bacteria for the bioremediation process in tannery wastewater. The previous study showed that the Bacillus cereus LS2B had the ability to produce alkaline protease [4] and it had the potency as a de-hairing agent in the leather tanning at a laboratory scale [5]. This study aims to determine the survival ability of growth profile Bacillus cereus LS2B in a medium containing untreated industrial tannery wastewater. By knowing the survival ability of this bacterium, it is hoped that it can be used as a bioremediation agent for handling tannery wastewater, especially to degrade the organic matter content.

2. Materials and methods
2.1. Sample and bacterial preparation
Untreated tannery wastewater in this study was taken from equalizer flock of the leather industries located in Bantul District, D. I. Yogyakarta, Indonesia in July 2020. The Bacillus cereus LS2B was collected at the Laboratory of Leather, Waste, and By-Products Technology, Faculty of Animal Science, Universitas Gadjah Mada.

2.2. Nutrient medium for bacterial culture
The Nutrient liquid medium for bacterial culture (100 ml) made by 1 gram of meat extract, 1 gram of microbiological peptone, 0.5 gram of NaCl, and aquadest into a beaker glass, (just added as much as 1.5 gram of agar powder to make solid medium), stir until homogenized, pH adjusted to 7.2 with the addition of 0.1 N NaOH or 0.1 N HCl and then sterilized by autoclave (121°C, 15 psi, 15 minutes).

2.3. Bacterial stock isolate
The bacterial stock isolate was made by one Ose of Bacillus cereus LS2B isolate from the agar plate medium added into 10 mL of the sterile nutrient liquid medium and then incubated on a rotary shaker at 120 rpm at ambient temperature for 24 hours.

2.4. The medium of tannery wastewater
The fresh tannery wastewater was taken and then filtered used filter paper Whatman (2 Mikron) and then sterilized by autoclave (121°C, 15 psi, 15 minutes). The medium of tannery wastewater was made into 2 medium (the solid medium and the liquid medium) with the different level of tannery wastewater concentrations. The solid medium in 100 ml made by the composition including the sterile fresh tannery wastewater (0, 25, 50, 75 and 100%), aquadest, 1 gram of meat extract, 1 gram of microbiological peptone, 0.5 gram of NaCl, and 1.5% of agar powder. The solution was stirred until homogenized, pH adjusted to 7.0 with the addition of 40% NaOH solution, and then sterilized by autoclave (121°C, 15 psi, 15 minutes). After that, the medium was plating on the petridish. The liquid medium in 100 ml made by the composition including the sterile fresh tannery wastewater (0, 25 and 50%), aquadest, 1 gram of meat extract, 1 gram of microbiological peptone, 0.5 gram of NaCl, stir until evenly dissolved using a magnetic stirrer, pH adjusted to 7.0 with the addition of 40% NaOH solution and then sterilized by autoclave (121°C, 15 psi, 15 minutes).

2.5. The observation of survival ability
Growth characterization of Bacillus cereus LS2B against the presence of tannery wastewater in the solid medium of tannery wastewater was conducted by taking as much as 10μL bacterial stock isolate and then added into the plate of the solid medium, spread it used drigalski, and incubated at 30°C in an incubator for 5 days and then the growth of the bacterial colony was observed every day. Growth characterization in liquid medium was conducted by taking 2% (v/v) bacterial stock isolate and then added into 100 ml liquid
medium of tannery wastewater, placed in a rotary shaker at 120 rpm, and incubated for 36 hours at ambient temperature. Every 6 hours the medium was tested the growth activity used spectrophotometry method (OD600). The liquid medium was treated by treatment in triplicate containing T0 (0% tannery wastewater), T1 (25% tannery wastewater + isolate Bacillus cereus LS2B), T1 blank (25% tannery wastewater without the addition of bacteria), T2 (50% tannery wastewater + Bacillus cereus LS2B) and T2 blank (50% tannery wastewater without the addition of bacteria).

2.6. Viability cell bacteria by the presence of tannery wastewater
Bacterial isolates of Bacillus cereus LS2B was grown on a liquid nutrient medium with the treatments at P0, P1, and P2 containing concentrations of tannery wastewater at 0, 25, and 50%. The isolate bacteria were taken from the preculture after incubated in 24 hours. As much as 5 ml of liquid Nutrient Medium was added 1% (v/v) of bacterial stock preculture then shaked at rotary shaker at 120 rpm for 24 hours. After 24 hours as much as 1 µL was taken, dropped on the plate solid medium containing the nutrients medium and the bacterial growth viability was observed.

2.7. Analisis data
The observed data of growth activity, and characterization of Bacillus cereus LS2B in the medium contains tannery wastewater has performed triplicates, and it was analyzed by descriptive in figure and graphical data.

3. Results and discussions

3.1 The growth and survival ability of Bacillus cereus LS2B with the presence of tannery wastewater on solid medium
The results of the growth of Bacillus cereus LS2B with the presence of tannery wastewater on the solid medium can be seen in Figure 1. Figure 1 shows the visualization of the growth of Bacillus cereus LS2B on agar medium after 5 days of observation. The bacterial growth activity was clear formed and could be seen in the medium contains 0% and 25% tannery wastewater medium. Bacillus cereus LS2B were able to grow optimally since the 1st day on the medium 0% tannery wastewater while on the medium 25% tannery wastewater was seen on the 2nd day. Until the 5th day, the growth visualization of Bacillus cereus LS2B were not observed in the medium with the addition of 50, 75, and 100% of tannery wastewater. This phenomenon was assumed that the Bacillus cereus LS2B had not tolerant in the presence of pollutant content in the medium with an additional ≥50% of tannery wastewater. The presence of high levels of pollutant heavy metal like chromate in the arround of the living medium has an inhibitory effect on most microorganisms [6]. The results of the growth observation indicated that the bacteria Bacillus cereus LS2B were assumed to have the ability to grow by utilizing both organic or inorganic nutrients in the medium sources and still available to adaptive in the addition of tannery wastewater up to 25%.
3.2 The growth and survival ability of *Bacillus cereus* LS2B with the presence of tannery wastewater on liquid medium

The result of bacterial growth (OD600) from *Bacillus cereus* LS2B grown in liquid medium with the presence of tannery wastewater for 36 hours of observation can be seen in Figure 2.

Based on the result of the observation OD600, *Bacillus cereus* LS2B still can grow in a medium containing up to 25% of tannery wastewater. In a medium with 0% tannery wastewater, the growth curve was clear formed where the OD value has continued to increase among 36 hours. In a medium with 25% tannery wastewater, the growth curve of *Bacillus cereus* LS2B was seen initially increased after the 12th hour, while in the control there were no increases in the OD value. The growth ability of *Bacillus cereus* LS2B in the medium with the addition of tannery wastewater up to 25% indicates the adaptive potential for the presence of tannery wastewater and 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. [7] stated that an increase in the absorbance value of the medium reflects an increase in the number of cells in the medium.
Figure 2. The bacterial growth of *Bacillus cereus* LS2B in the presences of tannery wastewater in liquid.

The growth of *Bacillus cereus* LS2B on the medium 50% tannery wastewater was not visible, during 36 hours of observation there was no visible increase in OD values in the control or with the addition of bacteria. Since the beginning of the observation, the absorbance value (OD600) of the medium with the addition of 25% and 50% tannery wastewater showed high values OD ranging λ at 0.8 and 2.0 nm. This phenomenon shows that in the reality condition of the fresh tannery wastewater is high in pollutant content, thus increasing the level of turbidity. [8] The fresh tannery wastewater characterization is the high content of organic, inorganic, nitrogen compound, and chemical compounds that causing the solution with high suspended solids and dissolved solids.

3.3 Cell viability of *Bacillus cereus* LS2B

The results of the viability test of *Bacillus cereus* LS2B is presented in Figure 3. The results of the viability test of *Bacillus cereus* LS2B is presented in Figure 3. The viability results show that the treatment of the addition of tannery wastewater in the cultivation medium at P1 and P2 gives the inhibition effect on the *Bacillus cereus* LS2B 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 on the inhibition activity against *Bacillus cereus* LS2B. That phenomenon was assumed because the effect of the presence of pollutants continued in the fresh tannery wastewater.
Figure 3. The viability of cell bacteria of *Bacillus cereus* LS2B after cultivation on the presence medium contains tannery wastewater.

4. Conclusion
Based on the results of this study, it can be concluded that the *Bacillus cereus* LS2B had the potency adapted to lived and grow at the medium containing up to 25% fresh tannery wastewater.

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Acknowledgment
This research was financially supported by grant aid from the Directorate General of Higher Education (DGHE), Ministry of Education and Culture, the Republic of Indonesia through The Program Penelitian Dasar Unggulan Perguruan Tinggi 2020 under Directorate of Research, Gadjah Mada University management with grant number 2789/UN1.DITLIT/DIT-LIT/PT/2020 and the Program Rekognisi Tugas Akhir Universitas Gadjah Mada Tahun 2020 with grant number 732/UN1.P.III/KPT/HUKOR/2020.