



## Metagenomic Analysis of Bacterial Communities in Batik Wastewater from Zie Semarang

<sup>1</sup>Diah Rahma Safitri, <sup>2</sup>\*R. Susanti

<sup>1,2</sup>Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Semarang, Indonesia.

\*Corresponding Author e-mail: [basanatha8@mail.unnes.ac.id](mailto:basanatha8@mail.unnes.ac.id)

Received: March 2026; Revised: April 2026; Accepted: May 2026; Published: June 2026

**Abstract:** This study aims to analyze the abundance profile, diversity of bacterial communities, and their potential as bioremediation agents in batik wastewater. The research method includes analysis of heavy metal levels (Cr, Pb, Cd, and Cu) using the Atomic Absorption Spectrophotometer (AAS) method and metagenomic analysis based on Next Generation Sequencing (NGS) in the target region V3-V4 of the 16S rRNA gene. The AAS test results showed a chromium (Cr) concentration of 0.035 ppm. The metagenomic analysis results produced alpha diversity, namely the Shannon index of 2.749 and Simpson of 0.865, which means that bacterial diversity is in the moderate category with the dominance of certain groups. The bacterial community was dominated by the phyla Pseudomonadota (62.38%) and Actinomycetota (28.61%), which are known to have high adaptation and tolerance to Cr in degrading organic compounds. At the genus level, the highest percentages were found in Rickettsia (30.2%) and Xanthobacter (24.58%). Xanthobacter has potential as a bioremediation agent, but the presence of Rickettsia needs further study because the sample was mixed with other sources containing Rickettsia vectors. This study provides a basis for optimizing bioremediation by utilizing bacteria that have potential as pollutant degraders based on analysis of previous studies.

**Keywords:** Metagenomics; microbial community; wastewater; 16S rRNA

**How to Cite:** Safitri, D. R., & Susanti, R. (2026). Metagenomic Analysis of Bacterial Communities in Batik Wastewater from Zie Semarang. *Bioscientist: Jurnal Ilmiah Biologi*, 14(2), 541–552. <https://doi.org/10.33394/bioscientist.v14i2.20180>



<https://doi.org/10.33394/bioscientist.v14i2.20180>

Copyright© 2026, Safitri et al

This is an open-access article under the [CC-BY-SA](https://creativecommons.org/licenses/by-sa/4.0/) License.



### INTRODUCTION

As both a cultural identity and heritage, batik plays an important role in the Indonesian economy. However, its production process, particularly the dyeing stage, generates wastewater. Many small- to medium-scale batik industries still discharge liquid waste directly into the environment without adequate treatment. This practice poses serious environmental risks because it can lead to pollution and environmental degradation (Zammi et al., 2018). Such degradation is reflected in declining water quality, discoloration, unpleasant odors, and reduced dissolved oxygen levels in aquatic ecosystems (Hannan et al., 2024).

Batik wastewater contains various pollutants, including organic solvents, pigments, heavy metals, and sulfate (Tio et al., 2023). It may also contain surfactants, chloride, and aromatic amines (Wei et al., 2020; Kumar et al., 2023). Regarding heavy metal contamination, Assauqi et al. (2024) reported that Cr was present at higher concentrations than Pb and Cu in batik wastewater. The presence of heavy metals in textile wastewater can reduce microbial diversity and alter community composition. This is consistent with the findings of El-Liety et al. (2022), who reported that heavy metals influence amplicon sequence variants (ASVs) and decrease microbial diversity. Although the most affected bacterial groups may not disappear completely, they can persist at very low relative abundances, as observed in the phyla Acidobacteriota and Chloroflexota. In contrast, the dominance of certain bacterial groups may increase due

to the presence of taxa that are tolerant to heavy metals (Li et al., 2020). Therefore, wastewater treatment is necessary to reduce the load of organic pollutants, metals, and xenobiotic compounds, while also maintaining bacterial diversity, particularly bacteria that function as pollutant degraders in aquatic ecosystems.

Wastewater treatment can be conducted through mechanical, physical, and biological processes. Mechanical and physical treatments include screening, sedimentation, and filtration, whereas biological treatment can involve the use of bacterial consortia (Sharma et al., 2023). Biological treatment is considered more environmentally friendly because it utilizes bacteria capable of degrading pollutants in wastewater into less toxic compounds (Saeed et al., 2021). Fidiastuti et al. (2022) reported that indigenous bacteria found in wastewater were able to degrade pollutants. The identified bacterial groups included *Saccharospirillaceae* bacterium S5C81, *Sulfurospirillum cavolei* S2C617, *Desertifilum* sp. S8C857, *Shewanella xiamenensis* S2C505, *Saccharospirillaceae* bacterium S4C81, and *Bacillaceae* bacterium S6C1905. These bacterial communities can be comprehensively analyzed using modern molecular biology approaches, particularly metagenomics.

This study employed a metagenomic approach to analyze wastewater samples from Zie batik production. Using metagenomics, Kumar et al. (2021) reported the identification of *Pseudomonadaceae*, which has natural potential for degrading textile dyes. One major advantage of metagenomics is its ability to explore the biological potential of microorganisms that cannot be cultivated under laboratory conditions. Many environmental bacteria are difficult to culture using conventional methods, resulting in limited information on bacterial community profiles in aquatic ecosystems. Fidiastuti et al. (2022), for example, used conventional methods to analyze bacterial isolates from batik wastewater. Similarly, Citrapancayudha and Soetarto (2016) applied conventional isolation and characterization methods to identify bacteria with potential roles in wax degradation. Therefore, a metagenomic approach enables a more comprehensive analysis of bacterial community profiles in environmental samples (Saragih et al., 2023).

The metagenomic approach used in this study targeted the V3–V4 region of the 16S rRNA gene. This molecular approach is suitable because the 16S rRNA gene contains stable conserved regions as well as variable regions that allow differentiation among species and accurate detection. In addition, this approach is considered more sensitive than conventional culture-based and characterization methods (Bukin et al., 2019).

Based on the background described above, this study aimed to comprehensively identify the bacterial community profile in Zie batik wastewater and assess its potential as a source of bioremediation agents using a 16S rRNA gene-based metagenomic approach targeting the V3–V4 region. The findings are expected to provide a foundation for developing the use of pollutant-degrading bacteria as bioremediation agents, particularly in aquatic environments affected by batik wastewater contamination.

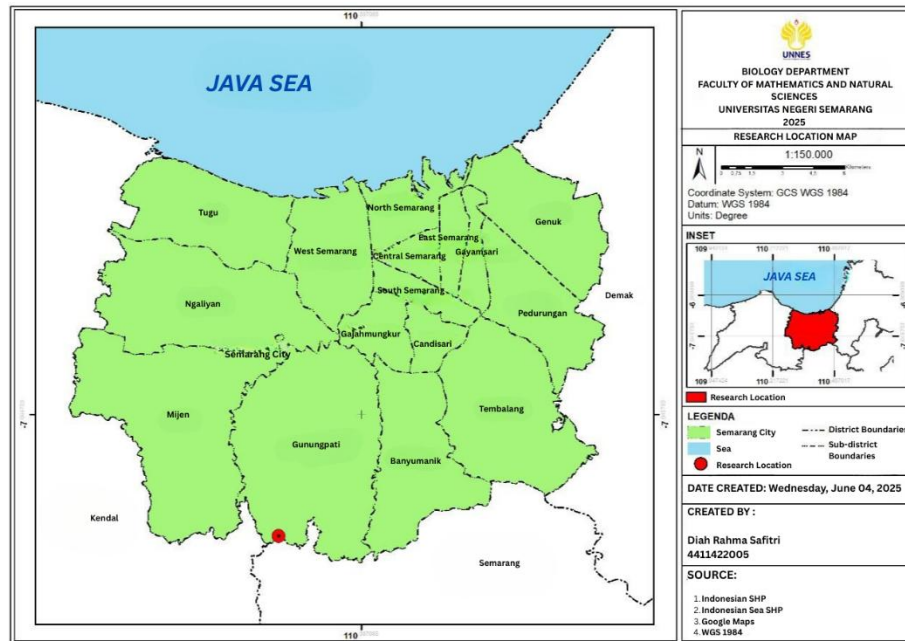
## METHOD

This study was an environmental microbiology investigation using a metagenomic approach to characterize the diversity and abundance of bacteria in wastewater with potential relevance as bioremediation agents.

### Study Site and Period

Batik wastewater samples were collected from Zie Batik Industry, located in Sekaran Village, Gunungpati District, Semarang City, Central Java, Indonesia, at the

coordinates 7.1029984, 110.3609564. The study was conducted from May to August 2025.



**Figure 1.** Map of the sampling location

## Sampling

Batik wastewater samples were collected from three different points along the wastewater channel at a depth of 0.6 m using the grab sampling method. A total of 200 mL of wastewater was collected from each sampling point. The samples were labeled with the collection date and stored at approximately 4°C to maintain microbial stability during transport to the laboratory. For initial storage, the samples were kept at -20°C. The samples were subsequently pooled before being sent to PT Genetika Science Indonesia for metagenomic analysis.

## Heavy Metal Analysis

Heavy metals, including Cr, Pb, Cd, and Cu, were analyzed using an Atomic Absorption Spectrophotometer (AAS; PerkinElmer Analyst 400) equipped with hollow cathode lamps and supplied with air and acetylene gas. Sample preparation was performed using a wet digestion method in accordance with SNI 6989-84:2019. Briefly, 100 mL of sample was heated with concentrated HNO<sub>3</sub>, with HCl added when necessary, until a clear solution was obtained. The digested sample was then diluted to 100 mL in a volumetric flask using demineralized water. Measurements were conducted under optimized conditions by adjusting the gas flow rate, wavelength, and lamp current. Method validation included the determination of sensitivity, precision, working range, and the limits of detection and quantification (LoD and LoQ), which were calculated based on the standard deviation of the blank and the calibration curve to ensure the accuracy of the analytical results (Asmorowati et al., 2025).

## Metagenomic Analysis

### DNA extraction and purification

DNA extraction was performed using the MagBead Plus Kit (Zymo Research, D4082). A total of 200 µL of batik wastewater suspension was mixed with 200 µL of BioFluid & Solid Tissue Buffer and 20 µL of Proteinase K. The mixture was homogenized and incubated at room temperature, approximately 20–30°C, for 20 min.

This step was intended to lyse the cell walls and release DNA from the sample into the solution.

Following lysis, 400  $\mu\text{L}$  of MagBinding Buffer was added to the sample mixture and homogenized. Subsequently, 33  $\mu\text{L}$  of magnetic beads was added, and the mixture was incubated for approximately 10 min to allow DNA binding to the beads. The mixture was then placed on a magnetic rack to separate the supernatant from the beads. Sequential washing was performed using MagBinding Buffer, Pre-Wash Buffer, and g-DNA Wash Buffer, with three washing steps in total. The magnetic beads were then dried at 55°C for approximately 10 min, followed by elution with 50  $\mu\text{L}$  of Elution Buffer and incubation for 5 min. The eluted DNA was separated again using a magnetic rack and stored at -20°C. The concentration and purity of the purified DNA were assessed using a NanoDrop 2000 spectrophotometer. Absorbance was measured at 260 and 280 nm to determine DNA concentration and purity ratio.

### DNA amplification by PCR

PCR amplification targeted the V3–V4 region of the 16S rRNA gene using Phusion Plus PCR Master Mix (F631S). Amplification was performed using the primer pair 341F, 5'-CCTAYGGGRBGCASCAG-3', and 806R, 5'-GGACTACNNGGGTATCTAAT-3'. The PCR produced amplicons of approximately 470 bp. The PCR program began with initial denaturation at 98°C for 40 s, followed by amplification cycles consisting of denaturation at 98°C for 20 s, annealing at 53°C for 40 s, and extension at 72°C for 40 s. The reaction was completed with a final extension at 72°C for 2 min (Abellan-Schneyder et al., 2021).

### Visualization of PCR products

A total of 2  $\mu\text{L}$  of amplified PCR product was loaded into the wells of a 1% agarose gel prepared with TBE buffer. A 100 bp DNA ladder, 2.5  $\mu\text{L}$ , was also loaded as a molecular size marker to estimate the size of the PCR fragments. Electrophoresis was performed at a constant voltage, typically 80–120 V, for 30–60 min until the DNA bands migrated according to fragment size. The gel was then stained and placed on a UV transilluminator to visualize the DNA bands. The results were documented using a gel documentation system.

### DNA quantification

DNA quantification was performed using the Qubit dsDNA HS Assay Kit according to the manufacturer's protocol. This method was used to obtain an accurate DNA concentration. A minimum DNA concentration of 10 ng/ $\mu\text{L}$  was required for library preparation for Illumina sequencing.

### Sequencing

Sequencing was performed using the Illumina MiSeq 2500 platform. Next-generation sequencing (NGS) technology was selected because it enables large-scale DNA sequence read processing and provides detailed information on the bacterial community present in the samples. Sequencing was conducted in paired-end mode. The resulting sequence files were subsequently subjected to bioinformatic analysis.

### Bioinformatic Analysis

The paired-end sequencing reads were first processed using Cutadapt (Martin, 2011) to remove adapter sequences and PCR primers, thereby ensuring the quality and biological relevance of the data. The processed reads were then analyzed using the DADA2 plugin (Callahan et al., 2017), which was used for sequencing-error correction, denoising, filtering of low-quality sequences, and chimera removal to generate valid amplicon sequence variants (ASVs). After ASV generation, taxonomic

classification was performed using the SILVA database version 138.2, silva\_nr99\_v138.2, as the reference database. All downstream data analyses were conducted using packages in RStudio with R version 4.2.3. Final visualizations, including taxonomic bar plots showing bacterial proportions at the phylum and genus levels, were prepared and refined using Microsoft Excel 2019.

## RESULTS AND DISCUSSION

### Heavy Metal Content

Analysis of heavy metal content in batik wastewater showed that chromium (Cr) was detected at a concentration of 0.035 ppm, whereas Pb, Cd, and Cu were not detected in the sample (Table 1). The presence of Cr in batik wastewater is likely associated with the use of sodium dichromate as a mordant, which is commonly applied to improve color fastness in textile fibers (Velusamy et al., 2021). Although the detected Cr concentration was below the textile industry wastewater quality standard of 1.0 ppm established by Indonesian Minister of Environment Regulation No. 5 of 2014, its presence remains environmentally relevant because chromium is persistent and non-degradable in aquatic ecosystems.

**Table 1.** Heavy metal content in batik wastewater

Heavy metal	Concentration (ppm)
Cr	0.035
Pb	0.000
Cd	0.000
Cu	0.000

Even at low concentrations, Cr may exert ecological pressure on aquatic microorganisms and other biota. Heavy metals in textile wastewater can increase oxygen demand, while the turbid color of wastewater may reduce sunlight penetration and inhibit photosynthesis (Kencana & Radityaningrum, 2022). Such environmental stressors can alter microbial community structure by reducing sensitive bacterial groups and favoring taxa that are more tolerant of heavy metals and dye-derived pollutants. This pattern is consistent with El-Liety et al. (2022), who reported that heavy metal contamination can affect ASV composition and reduce microbial diversity.

### Alpha Diversity

The metagenomic analysis based on 16S rRNA amplicon sequencing of the V3–V4 region generated several alpha diversity parameters, including ASVs, observed taxa, Chao1, ACE, and diversity indices such as Shannon, Simpson, and Fisher indices (Table 2). These values provide an overview of the richness, diversity, and dominance structure of the bacterial community inhabiting batik wastewater.

**Table 2.** Alpha diversity of the bacterial community in batik wastewater

Parameter	Value
ASVs	20,110
Observed taxa	139
Shannon index	2.749
Simpson index	0.865
Chao1	139.25
ACE (Abundance-based Coverage Estimator)	139.867
Fisher index	20.121

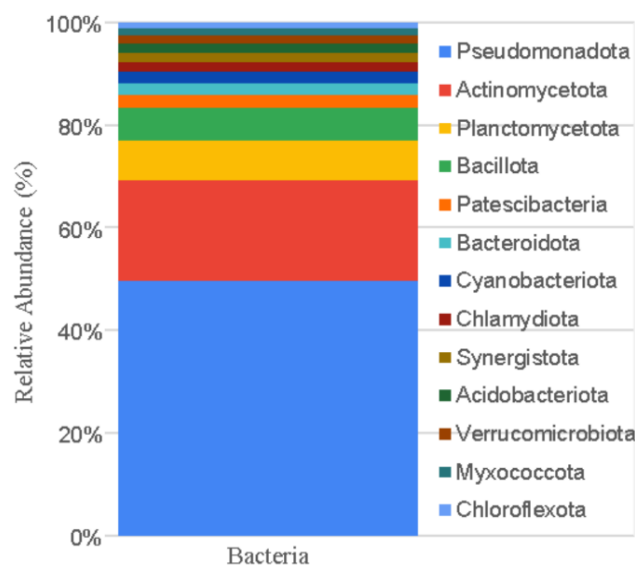
The Shannon index value of 2.749 indicates a moderate level of bacterial diversity, as values within the range of  $1 < H' \leq 3$  are generally interpreted as moderate diversity (Nuraina et al., 2018). This result suggests that the batik wastewater contained a relatively diverse bacterial community, although the distribution of individuals among taxa was not fully even. This interpretation is supported by the Fisher index value of 20.121, which indicates the presence of many different bacterial taxa within the community.

However, the Simpson index value of 0.865 indicates high dominance, suggesting that only certain bacterial groups were highly abundant, while others were present in lower proportions. This uneven distribution reflects selective pressure in the wastewater environment. In aquatic ecosystems contaminated by textile dyes and heavy metals, sensitive bacterial groups tend to decline, whereas bacteria capable of tolerating or degrading toxic compounds may become dominant (Wang et al., 2025). Resistant genera, including *Acinetobacter*, *Clostridium*, *Pseudomonas*, and *Enterobacter*, have been reported to increase in polluted aquatic environments, while less tolerant groups persist at lower abundance (Custodio et al., 2022). Therefore, the alpha diversity profile in this study indicates that batik wastewater supports a moderately rich bacterial community, but its structure is shaped by pollutant-driven dominance.

The high dominance reflected by the Simpson index may also be related to reduced ecological stability under pollutant stress. Textile dyes and heavy metals can create environmental conditions that favor bacteria tolerant to high pollutant loads and reduced oxygen availability (Zheng et al., 2023). Thus, although many bacterial taxa were detected in the batik wastewater, their abundance was uneven, with several dominant phyla and genera contributing substantially to the overall community structure.

### Taxonomic Profile of the Bacterial Community

At the phylum level, 13 bacterial phyla were identified in the batik wastewater. The community was dominated by Pseudomonadota, which accounted for 62.38% of the total bacterial abundance, followed by Actinomycetota (28.61%), Planctomycetota (4.86%), Bacillota (3.37%), and Patescibacteria (0.26%) (Figure 2). The dominance of Pseudomonadota indicates that the wastewater environment favored bacterial groups with high metabolic flexibility and strong tolerance to pollutant stress.

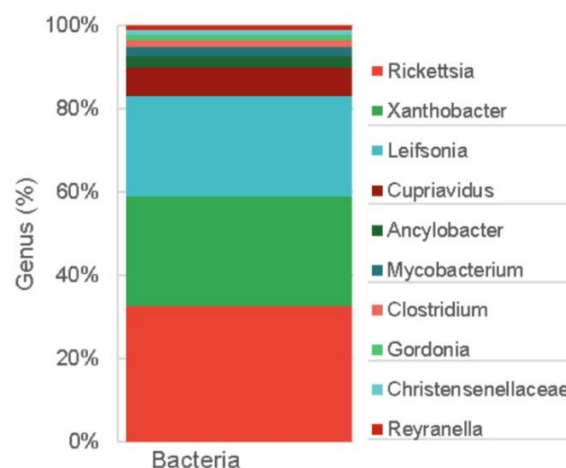


**Figure 2.** Relative abundance of the bacterial community at the phylum level

*Pseudomonadota* is widely recognized for its metabolic versatility and ability to utilize various organic compounds, including dye-derived pollutants (Chobert et al., 2024). Members of this phylum are also resistant to heavy metals and can survive under variable oxygen conditions commonly found in wastewater channels (Malik et al., 2024; Isah et al., 2024). Their ecological importance in contaminated environments is related to the production of enzymes such as azoreductases, laccases, and hydrolases, which contribute to the degradation of azo dyes, phenolic compounds, and other hazardous substances (Sastroredjo et al., 2023).

The dominance of *Pseudomonadota* in this study may be explained by several adaptive mechanisms. Members of this phylum can tolerate heavy metal exposure through efflux systems that remove metal ions from cells and enzymatic reduction mechanisms that reduce metal toxicity (Fardami et al., 2023). These mechanisms allow *Pseudomonadota* to persist in toxic environments where less adapted bacteria may decline. In addition, environmental factors such as pH, temperature, oxygen concentration, and nutrient availability may further support their persistence. Some members of *Pseudomonadota* are facultative aerobes, enabling them to adapt to fluctuating oxygen conditions in wastewater ecosystems (Aljuaid et al., 2026).

The high abundance of *Pseudomonadota* observed in this study is consistent with previous findings. El-Liethy et al. (2022) reported that *Pseudomonadota* dominated textile wastewater, reaching 70.38% relative abundance. Similarly, Isah et al. (2024), using 16S rRNA-based metagenomic analysis, found that *Pseudomonadota* was the dominant phylum in textile wastewater, with a relative abundance of 60.45% under aerobic conditions. These comparable findings suggest that *Pseudomonadota* is a common and functionally important bacterial group in textile wastewater environments. At the genus level, 56 genera or genus-level taxa were identified. The ten most abundant taxa were *Rickettsia* (30.21%), *Xanthobacter* (24.56%), *Leifsonia* (22.46%), *Cupriavidus* (6.23%), *Ancylobacter* (2.58%), *Mycobacterium* (2.11%), *Clostridium* (1.48%), *Gordonia* (1.44%), Christensenellaceae-related taxa (1.03%), and *Reyranella* (0.83%) (Figure 3). This genus-level profile further supports the alpha diversity results, showing that the community contained many bacterial taxa but was strongly dominated by a limited number of genera.



**Figure 3.** Genus-level profile of the most abundant bacterial taxa

Among the identified genera, *Rickettsia* showed the highest relative abundance, accounting for 30.21% of the bacterial community. However, this finding should be interpreted cautiously because *Rickettsia* is an obligate parasitic genus and is not typically found as a free-living bacterium in wastewater. Its primary habitats are

vertebrate hosts and arthropod vectors (Wittmers et al., 2025). Therefore, the high abundance of *Rickettsia* in batik wastewater is not fully consistent with its known ecological characteristics. This result may indicate possible mixing of batik wastewater with other wastewater sources containing biological materials from animals, tissues, or vectors carrying *Rickettsia* (Salje, 2021). Consequently, the high proportion of *Rickettsia* may not represent the native bacterial community of batik wastewater.

In contrast, several other dominant genera were more directly associated with pollutant degradation and environmental adaptation. *Xanthobacter*, which accounted for 24.56% of the community, is known for its ability to degrade hazardous chemicals and perform nitrogen fixation in complex industrial environments (Tikhonova et al., 2021). *Cupriavidus* and *Ancylobacter* also showed relevant ecological functions in contaminated environments. *Cupriavidus* can degrade complex compounds and contribute to denitrification and bioprecipitation processes for contaminant removal, including fluoride and nitrate (Liu et al., 2021). Meanwhile, *Ancylobacter* can utilize organic compounds such as oxalate, phenol, and 1,2-dichloroethane as nutrient sources, indicating its adaptive potential in pollutant-rich environments (Kumar et al., 2020).

The second most dominant phylum, Actinomycetota, accounted for 28.61% of the bacterial community. This phylum is commonly found in aquatic environments and plays an important role in wastewater biodegradation, particularly through its ability to degrade synthetic dye compounds. Members of Actinomycetota produce enzymes such as azoreductases, peroxidases, and laccases, which can break down azo dye structures into simpler compounds. Isolates from contaminated wastewater have also been reported to decolorize dyes such as methylene blue and remazol blue through biofilm formation and interactions with other microorganisms (Adenan et al., 2020).

The dominance of Actinomycetota was mainly represented by *Leifsonia*, which accounted for 22.46% of the genus-level community. *Leifsonia* is a Gram-positive bacterium with strong adaptive potential for chromium removal from wastewater. Its physical and chemical adsorption mechanisms can precipitate up to 95.4% of Cr(VI) on the biosorbent surface (Tan et al., 2018; Billah et al., 2020). This finding is particularly relevant because Cr was the only heavy metal detected in the batik wastewater sample. Therefore, the presence of *Leifsonia* may indicate a bacterial response to chromium-containing wastewater conditions.

Other Actinomycetota-associated genera, including *Mycobacterium* and *Gordonia*, also have potential ecological roles in contaminated environments. *Mycobacterium* possesses strong detoxification mechanisms and heavy metal tolerance and has been reported to survive on microplastics in wastewater containing Cr and Cu (Zhao et al., 2021). Several species are also capable of degrading complex organic compounds that are difficult to decompose (Li et al., 2025). Similarly, *Gordonia* can adapt to heavy metal-exposed environments and has potential metal-reducing capabilities (Tamura et al., 2020). These findings suggest that Actinomycetota contributed to both metal tolerance and organic pollutant degradation in batik wastewater.

Bacillota was also detected in the bacterial community, although at a lower relative abundance of 3.37%. Despite its lower abundance, this phylum is ecologically important because many of its members can form spores, allowing them to survive under highly toxic environmental conditions (Bressuire-Isoard et al., 2018). Bacillota also includes thermophilic bacteria that remain stable at high temperatures, making them potentially useful in industrial wastewater treatment systems with fluctuating temperatures (Guo et al., 2021).

The functional relevance of Bacillota is further supported by its ability to produce extracellular enzymes, including laccases, peroxidases, and azoreductases. These enzymes can degrade complex synthetic dyes, including azo, anthraquinone, and triphenylmethane dyes, into simpler compounds (Ikram et al., 2022). In this study, one member of Bacillota identified at the genus level was *Clostridium*, which accounted for 1.48% of the bacterial community. *Clostridium* is known for its ability to ferment various organic compounds under anaerobic conditions, allowing it to persist in oxygen-limited wastewater environments such as batik wastewater (Nguyen et al., 2020).

Overall, the bacterial community structure in batik wastewater reflected the combined influence of heavy metal content, dye-derived pollutants, and wastewater environmental conditions. Although Cr was detected at a low concentration, its persistence and potential ecological effects may contribute to microbial selection. The moderate Shannon diversity, high Fisher index, and high Simpson dominance indicate that the wastewater contained diverse bacterial taxa but with uneven abundance. This pattern was reflected taxonomically by the dominance of Pseudomonadota and Actinomycetota, which include several genera with pollutant degradation, heavy metal tolerance, and environmental remediation potential.

## CONCLUSION

Batik industrial wastewater contains the heavy metal chromium (Cr). Based on the Shannon index, the bacterial community was classified as having moderate diversity. The wastewater was dominated by the phyla Pseudomonadota and Actinomycetota, which are known for their capacity to degrade organic compounds and tolerate Cr exposure. At the genus level, the community was dominated by *Xanthobacter*, which has potential for bioremediation applications based on previous studies. However, the presence of *Rickettsia* requires further investigation, as the sample may have been mixed with other sources containing vectors that carry *Rickettsia*. Overall, the findings of this study provide a basis for developing bioremediation strategies using bacteria with potential pollutant-degrading capabilities, as supported by analyses grounded in previous research.

## RECOMMENDATION

Further research should focus on laboratory-based evaluation of bacteria from the genus *Xanthobacter* to assess their ability to degrade dyes and reduce chromium (Cr) under controlled experimental conditions.

## ACKNOWLEDGMENT

The authors express their gratitude to the Dean of the Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, for funding this research scheme under contract number 1.21 4/UNS 7/PPK 04/2025 in April 2025. The authors also thank Batik Zie Semarang for granting permission and access for sample collection.

## REFERENCES

- Abellan-Schneyder, I., Matchado, M. S., Reitmeier, S., Sommer, A., Sewald, Z., Baumbach, J., List, M., & Neuhaus, K. (2021). Primer, pipelines, parameters: Issues in 16S rRNA gene sequencing. *mSphere*, 6(1), e01202-20.
- Adenan, N., Lim, Y., & Ting, A. (2020). Discovering decolorization potential of triphenylmethane dyes by actinobacteria from soil. *Water, Air, & Soil Pollution*, 231.

- Aljuaid, R. S., Alshareef, S. A., Jamal, B. T., Dhafeer, F. H., Alnahari, A. A., & Ashy, R. A. (2026). Spatial and vertical stratification of groundwater microbial communities reveals proteobacterial dominance and redox-driven ecological transitions. *Microorganisms*, *14*(1), 232.
- Asmorowati, D. S., Azmi, K. U., & Sumarti, S. S. (2025). Optimization of the AAS method with variations in air speed, wavelength, and light current. *Indonesian Journal of Chemical Science*, *14*(2), 57–65.
- Assauqi, J. D., Rusdi, H. O., Nursyaf, K. N., Ihya'Ulumuddin, M., Asha, N. P., & Siregar, W. A. (2024). Pengolahan limbah batik dari UKM Batik Blimbing Malang dengan metode elektrokoagulasi menggunakan elektroda besi dan alumunium. *JC-T (Journal Cis-Trans): Jurnal Kimia dan Terapannya*, *8*(2), 16–23.
- Billah, A., Moelyaningrum, A., & Ningrum, P. (2020). Phythoremediasi chromium total (Cr-T) menggunakan kayu apu (*Pistia stratiotes* L.) pada limbah cair batik. *[Nama jurnal tidak tersedia]*, *24*, 47–54.
- Bressuire-Isoard, C., Broussolle, V., & Carlin, F. (2018). Sporulation environment influences spore properties in *Bacillus*: Evidence and insights on underlying molecular and physiological mechanisms. *FEMS Microbiology Reviews*, *42*, 614–626.
- Bukin, Y. S., Galachyants, Y. P., Morozov, I. V., Bukin, S. V., Zakharenko, A. S., & Zemskaya, T. I. (2019). The effect of 16S rRNA region choice on bacterial community metabarcoding results. *Scientific Data*, *6*(1), 190007.
- Chobert, S.-C., Roger-Margueritat, M., Flandrin, L., Berraies, S., Lefèvre, C. T., Pelosi, L., Junier, I., Varoquaux, N., Pierrel, F., & Abby, S. S. (2025). Dynamic quinone repertoire accompanied the diversification of energy metabolism in Pseudomonadota. *The ISME Journal*, *19*(1), wrae253.
- Citrapancayudha, D. R., & Soetarto, E. S. (2016). Biodegradasi residu wax dari limbah industri batik oleh bakteri. In *Proceeding Biology Education Conference: Biology, Science, Environmental, and Learning* (Vol. 13, No. 1, pp. 800–806).
- Custodio, M., Espinoza, C., Peñaloza, R., Peralta-Ortiz, T., Sánchez-Suárez, H., Ordinola-Zapata, A., & Vieyra-Peña, E. (2022). Microbial diversity in intensively farmed lake sediment contaminated by heavy metals and identification of microbial taxa bioindicators of environmental quality. *Scientific Reports*, *12*(1), 80.
- El-Liethy, M. A., Hemdan, B. A., & El-Taweel, G. E. (2022). New insights for tracking bacterial community structures in industrial wastewater from textile factories to surface water using phenotypic, 16S rRNA isolates identifications and high through-put sequencing. *SSRN Electronic Journal*.
- Fardami, A. Y., Umar, B. I., Muntasir, S., Abduljalil, L., Mahdi, A. A., Aminu, A., Ibrahim, L., Abdullahi, I. D., Muhammad, S. Z., & Ahmadu, A. F. (2023). Mechanisms of bacterial resistance to heavy metals: A mini review. *UMYU Scientifica*, *2*(1), 76–87.
- Fidiastuti, H. R., Amin, M., & Sari, N. K. (2022). Biodegradation of batik wastewater: Study of isolation and characterization of indigenous bacteria in Tulungagung, East Java Province, Indonesia. *Jurnal Biologi Indonesia*, *18*(1), 1–9.
- Guo, G., Liu, C., Hao, J., Tian, F., Ding, K., Zhang, C., Yang, F., Liu, T., Xu, J., & Guan, Z. (2021). Development and characterization of a halo thermophilic bacterial consortium for decolorization of azo dye. *Chemosphere*, *27*(2), 12–25.
- Hannan, I. A., Witrie, S. E., & Adi, N. P. (2024). Dampak pencemaran air akibat limbah industri batik printing di Kecamatan Pekalongan Utara terhadap kualitas air sungai. *Gudang Jurnal Multidisiplin Ilmu*, *2*(8), 34–42.

- Ikram, M., Naeem, M., Zahoor, M., Hanafiah, M., Oyekanmi, A., Islam, N., Ullah, M., Mahnashi, M., Ali, A., Jalal, N., Bantun, F., Momenah, A., & Sadiq, A. (2022). *Bacillus subtilis*: An efficient bacterial strain for the reclamation of water loaded with textile azo dye, Orange II. *International Journal of Molecular Sciences*, 11(2), 23–25.
- Isah, M. M., Taining, Z., Abd Jalil, N. K., Ibrahim, Z., & Abdul-Wahab, M. F. (2024). Insights into the microbiota of a textile wastewater treatment system towards sustainable bioremediation of industrial wastes. *Key Engineering Materials*, 1002, 119–131.
- Kencana, E. M., & Radityaningrum, A. D. (2022). Kombinasi filtrasi dan fitoremediasi untuk pengolahan limbah cair industri batik. *Dampak*, 19(2), 56–65.
- Kementerian Lingkungan Hidup dan Kehutanan Republik Indonesia. (2019). *Peraturan Menteri Lingkungan Hidup dan Kehutanan Republik Indonesia Nomor P.16/MENLHK/SETJEN/KUM.1/4/2019 tentang perubahan kedua atas Peraturan Menteri Lingkungan Hidup Nomor 5 Tahun 2014 tentang baku mutu air limbah*.
- Kumar, A., Pillay, B., & Olaniran, A. O. (2020). Genome sequence and metabolic analysis revealed the catabolic pathways for the degradation of 1,2-dichloroethane and other related xenobiotics in *Ancylobacter aquaticus* strain UV5. *Gene Reports*, 21, 100969.
- Kumar, D., Patel, Z., Pandit, P. R., Pandit, R., Puvar, A., & Patel, A. K. (2023). Textile industry wastewater microbiome: Recovery of metagenome-assembled genomes (MAGs) using shotgun sequencing approach from Gujarat, India. *Ecological Genetics and Genomics*, 26(5), 116–123.
- Kumar, V., Thukral, A., Sharma, A., & Bhardwaj, R. (2021). Extending the concept of entropy-negentropy for the assessment of ecological dominance and diversity at alpha, beta, and gamma levels. *Geology, Ecology, and Landscapes*, 7(1), 27–39.
- Li, C., Quan, Q., Gan, Y., Dong, J., Fang, J., Wang, L., & Liu, J. (2020). Effects of heavy metals on microbial communities in sediments and establishment of bioindicators based on microbial taxa and function for environmental monitoring and management. *Science of the Total Environment*, 749, 141555.
- Li, S., Liu, J., & Fang, P. (2025). Biodegradation of phenanthrene by *Mycobacterium* sp. TJFP1: Genetic basis and environmental validation. *Microorganisms*, 13.
- Liu, J., Su, J., Ali, A., Wang, Z., & Zhang, R. (2021). Potential of a novel facultative anaerobic denitrifying *Cupriavidus* sp. W12 to remove fluoride and calcium through calcium bioprecipitation. *Journal of Hazardous Materials*, 42(14), 156–167.
- Malik, S. S., Loganathachetti, D. S., Khan, M. A., Sadaiappan, B., & Mundra, S. (2024). Bacterial resistome in different stages of wastewater treatment plant is highly impacted by the abundance of the Pseudomonadota community. *Bioresource Technology Reports*, 26, 101814.
- Nguyen, T., Watari, T., Hatamoto, M., Sutani, D., Setiadi, T., & Yamaguchi, T. (2020). Evaluation of a combined anaerobic baffled reactor downflow hanging sponge biosystem for treatment of synthetic dyeing wastewater. *Environmental Technology and Innovation*, 19(4), 67–76.
- Nuraina, I., Fahrizal, & Prayogo, H. (2018). Analisa komposisi dan keanekaragaman jenis tegakan penyusun hutan tembawang Jelomuk di Desa Meta Bersatu, Kecamatan Sayan, Kabupaten Melawi. *Jurnal Hutan Lestari*, 6(1), 137–146.
- Saeed, M. U., Hussain, N., Sumrin, A., Shahbaz, A., Noor, S., Bilal, M., Aleya, L., & Iqbal, H. M. N. (2022). Microbial bioremediation strategies with wastewater

- treatment potentialities: A review. *Science of the Total Environment*, 818, 151754.
- Salje, J. (2021). Cells within cells: Rickettsiales and the obligate intracellular bacterial lifestyle. *Nature Reviews Microbiology*, 19, 375–390.
- Saragih, G., Hidayani, T. R., Mirnandaulia, M., Ginting, C. N., & Fachrial, E. (2023). *Mikroba endofit dalam dunia kesehatan: Manfaat dan aplikasi*. Unpri Press.
- Sastoredjo, S., Sausan, Z., & Riza, A. (2023). Ribotyping analysis of microbes producing hydrolase from batik liquid waste samples. *Journal of Science and Informatics for Society (JSIS)*.
- Sharma, M., Agarwal, S., Agarwal Malik, R., Kumar, G., Pal, D. B., Mandal, M., Sarkar, A., Bantun, F., Haque, S., Singh, P., Srivastava, N., & Gupta, V. K. (2023). Recent advances in microbial engineering approaches for wastewater treatment: A review. *Bioengineered*, 14(1), 2184518.
- Tamura, T., Saito, S., Hamada, M., Kang, Y., Hoshino, Y., Gono, T., Mikami, Y., & Yaguchi, T. (2020). *Gordonia crocea* sp. nov. and *Gordonia spumicola* sp. nov. isolated from sludge of a wastewater treatment plant. *International Journal of Systematic and Evolutionary Microbiology*.
- Tan, W., Wang, Y., Mumford, K., Li, J., Xu, X., & Ding, L. (2018). Performances of purified indigenous *Leifsonia* sp. and its mechanism in the removal of Cr(VI) under shaking condition. *International Journal of Environmental Science and Technology*, 1–8.
- Tikhonova, E., Grouzdev, D., & Kravchenko, I. (2021). *Xanthobacter oligotrophicus* sp. nov., isolated from paper mill sewage. *International Journal of Systematic and Evolutionary Microbiology*, 7, 1–8.
- Tio, A., Rahman, D., & Sulistyowati, R. (2023). Aplikasi fotokatalis TiO<sub>2</sub> dan alternatifnya untuk degradasi pewarna sintesis dalam limbah cair. *Environmental Science Journal (ESJO): Jurnal Ilmu Lingkungan*.
- Velusamy, S., Roy, A., Sundaram, S., & Mallick, T. (2021). A review on heavy metal ions and dye removal through graphene oxide-based adsorption strategies for textile wastewater treatment. *The Chemical Record*, 21.
- Wang, Z., Deng, G., Hu, C., Hou, X., Zhang, X., Fan, Z., Zhao, Y., & Peng, M. (2025). Microbial diversity and community assembly in heavy metal-contaminated soils: Insights from selenium-impacted mining areas. *Frontiers in Microbiology*, 16.
- Wei, F., Shahid, M. J., Alnusairi, G. S., Afzal, M., Khan, A., El-Esawi, M. A., Abbas, Z., Wei, K., Zaheer, I. E., & Rizwan, M. (2020). Implementation of floating treatment wetlands for textile wastewater management: A review. *Sustainability*, 12, 5801.
- Wittmers, F., Poirier, C., Bachy, C., Eckmann, C., Matantseva, O., Carlson, C., Giovannoni, S., Goodenough, U., & Worden, A. (2025). Symbionts of predatory protists are widespread in the oceans and related to animal pathogens. *Cell Host & Microbe*, 33(2), 182–199.
- Zammi, M., Rahmawati, A., & Nirwana, R. R. (2018). Analisis dampak limbah buangan pabrik batik di Sungai Simbangkulon, Kabupaten Pekalongan. *Walisongo Journal of Chemistry*, 1(1), 1–5.
- Zhao, Y., Gao, J., Wang, Z., Dai, H., & Wang, Y. (2021). Responses of bacterial communities and resistance genes on microplastics to antibiotics and heavy metals in sewage environment. *Journal of Hazardous Materials*, 4(2), 123–137.
- Zheng, H., Yang, X., Meng, K., Li, S., Yu, H., Peng, Q., Zhang, Y., Zhang, X., Xu, X., Zhang, Y., Xu, Z., Li, L., Ying, Q., & Elsheery, N. (2023). Textile dyes alter the bacterial community structure in contaminated soil. *Journal of Soil Science and Plant Nutrition*, 23, 2599–2609.