

# Eco-Friendly Bioremediation of Batik Dye Waste Using Indigenous Bacterial Consortia

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## ABSTRACT

This study explores the potential of indigenous bacteria in bioremediating batik dye wastewater, a major environmental pollutant that threatens aquatic ecosystems and human health. The research aimed to identify and characterize bacterial isolates capable of degrading natural dyes (*Indigofera tinctoria* L., *Caesalpinia sappan* L.) and synthetic dyes (methyl, naphthol, remazol, indigosol). The variable investigated in this study is the degradation rate of batik dye wastewater by bacteria, both individually and in bacterial consortia. Using isolation, purification, and 16S rRNA sequencing, 15 bacterial isolates were identified, with *Stenotrophomonas maltophilia* (D), *Stutzerimonas stutzeri* (H, I), *Micrococcus* sp. (J), and *Pseudomonas* sp. (N) exhibiting high effectiveness. *Micrococcus* sp. (J) achieved degradation rates of 99.62% for *Indigofera*, 85.74% for red remazol, and 83.05% for blue naphthol, while *Pseudomonas* sp. (N) degraded remazol red at 94.72%. A bacterial consortium (INJ: *Stutzerimonas stutzeri*, *Pseudomonas* sp., and *Micrococcus* sp.) efficiently degraded indigosol blue at 90.29%. Statistical analyses revealed no significant differences in decolorization between natural and synthetic dyes or dye colors. These bacteria demonstrated strong enzymatic activity under diverse environmental conditions, such as differences in pH, temperature, dye concentration, and chemical composition, providing an eco-friendly and cost-effective solution for batik dye waste bioremediation. The findings support SDG 6 (Clean Water and Sanitation) and SDG 12 (Responsible Consumption and Production) by improving wastewater quality and promoting sustainable waste management in the batik industry.

## INTRODUCTION

Batik, acknowledged by the United Nations Educational, Scientific, and Cultural Organization (UNESCO) as an Indonesian cultural heritage in 2009, is commemorated annually on National Batik Day every October 2nd, as decreed by Indonesian Presidential Decree No. 33 of 2009. The intricacy of Batik patterns lies in their vivid coloration, ornamental display, and scale, rendering each piece uniquely distinguished (Kasim et al. 2016). Nevertheless, the production process of Batik involves the utilization of hazardous and enduring chemicals, resulting in elevated levels of pH, biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), and heavy metals. This effluent contributes to the contamination of aquatic ecosystems (Syamwil et al. 2021).

The wastewater from Batik dye comprises deleterious elements such as chromium (Cr), sulfide (S<sup>2-</sup>), ammonia (NH<sub>3</sub>), phenol, and oil-fat (Dewi et al. 2019). These substances encompass wax, dye, soda ash, metal, and fabric (Noviar et al. 2019). The utilization of synthetic dyes in Batik production has been associated with environmental pollution and health hazards, including the risk of skin cancer (Utami

2024). Dyeing one meter of Batik fabric can necessitate 25 to 50 cubic meters of water, often discharged untreated into rivers and soil, resulting in significant pollution (Indrayani & Triwiswara 2020). Batik waste significantly contributes to water, soil, and air pollution through the release of toxic chemicals, heavy metals, and emissions. Effective waste management and treatment strategies are essential to mitigate these environmental impacts and protect public health (Budiyanto et al. 2019, Linggawati et al. 2022).

Given the adverse environmental repercussions, it is imperative to conduct research and enhance public awareness regarding the management of Batik dye effluent before its release into the environment. Various methodologies have been explored, such as electrochemical degradation utilizing carbon-polyvinyl chloride (PVC) electrodes to disintegrate Batik waste through electrolysis, achieving up to 97.68% waste degradation and substantial reductions in lead (Pb) and COD (Riyanto & Nisaa 2017). Another approach involves the utilization of natural dyes and cleaner production methods to minimize pollution from synthetic dyes (Elsahida et al. 2020). However, these techniques are costly, necessitating more economical and environmentally sustainable solutions.

Bioremediation, employing microorganisms such as bacteria and fungi to break down, eliminate, immobilize, or detoxify pollutants, presents a promising alternative (Peer et al. 2022). This process involves biosorption, biodegradation, and bioaccumulation to eradicate contaminants from the environment (Kumar et al. 2019), aiming to transform harmful elements into less toxic alternatives (Paladhi et al. 2022). Bioremediation with indigenous microorganisms effectively decomposes waste, offering a viable solution for managing Batik dye effluent, particularly for small-scale Batik enterprises lacking appropriate waste treatment facilities.

This study aims to address Batik dye effluent, particularly methyl, naphthol, remazol, indigosol, and natural dyes through bioremediation. These variables were chosen because the dyes used, remazol, naphthol, indigosol, and natural dyes such as *Indigofera* and *Caesalpinia*, are the most dominant and commonly employed in the batik-making process in Indonesia. Remazol dyes are known for their water solubility and vibrant colors, naphthol dyes produce bright and long-lasting hues, indigosol dyes are widely used for their strong adherence to fabric and rich coloration, while natural dyes like *Indigofera* and *Caesalpinia* provide environmentally friendly options with *Indigofera* offering a rich blue hue and *Caesalpinia* producing vibrant red tones essential for traditional and sustainable batik production (Muchtasjar et al. 2019, Elshahida et al. 2020, Dewi et al. 2019, Rakhmania et al. 2022).

The research entails the identification and characterization of native bacteria in Batik dye effluent, measuring bacterial proliferation, testing their degradation capabilities in the laboratory, and molecularly identifying the most efficient bacteria via 16S rRNA sequencing. Additionally, degradation tests using combinations of bacterial isolates or bacterial consortia will be conducted to ascertain their potential to degrade Batik dye effluent. The hypothesis posits that indigenous bacteria can effectively decompose these dyes, mitigating the adverse effects of batik dye effluent on aquatic ecosystems. Furthermore, it is anticipated that this laboratory-based research can be translated into practical applications, leading to an effective, cost-efficient, and environmentally friendly Batik dye effluent management system.

This study addresses key research gaps in previous works by adopting a holistic approach that integrates microbial proliferation rate measurements, molecular identification via 16S rRNA sequencing, and degradation tests using bacterial consortia. Earlier studies, such as Mutiara et al. (2023), demonstrated the effectiveness of microbial consortia in degrading synthetic dyes but did not consider microbial growth dynamics, which are critical for understanding biodegradation efficiency. Similarly, Hussain et al. (2024) and Prabhakar et al. (2022) focused on specific bacterial strains but lacked assessments of consortia-based degradation and molecular-level characterization. By highlighting the high efficiency of indigenous bacteria in degrading both natural (*Indigofera*, *Caesalpinia*) and synthetic dyes (methyl, naphthol, remazol, indigosol), this research not only fills these gaps but also provides practical, cost-effective, and environmentally sustainable solutions. These findings advance prior studies by emphasizing the synergistic potential of bacterial consortia and their application in managing batik wastewater, particularly for small-scale enterprises lacking proper treatment facilities.

By harnessing the innate capabilities of local bacteria, this study seeks to develop sustainable, efficient, and cost-effective bioremediation strategies to alleviate pollution stemming from the Batik industry, ultimately safeguarding ecosystems and public health. The objectives are to identify and characterize predominant indigenous bacteria in Batik dye wastewater and determine the most effective bacteria for decolorizing these dyes through 16S rRNA gene sequencing. This research was conducted in the Yogyakarta Special Region, Indonesia, in 2023. Although this study was conducted in Yogyakarta, the use of synthetic dyes, such as remazol, naphthol, and indigosol, is widespread in Indonesia, particularly in commercial batik industries across major production areas like Pekalongan, Solo, and Yogyakarta, due

to their practicality and vibrant, long-lasting colors (Dewi et al. 2019, Indrayani & Triwiswara, 2020). In contrast, natural dyes like indigofera and caesalpinia are more commonly used in specific regions, such as rural areas in Yogyakarta and Central Java, where there is better access to raw materials and a focus on sustainability.

## MATERIALS AND METHODS

### Study Area and Sample Collection

This experimental research was conducted on batik dye residue acquired from the batik industry in Bantul, Special Region of Yogyakarta, Indonesia. Residual samples were directly collected from the site and preserved in sterile containers for subsequent analysis.

### Isolation of Indigenous Bacteria

Isolation of autochthonous bacteria from batik wastewater was conducted utilizing the spread plate technique. The wastewater specimens were progressively diluted up to  $10^{-8}$  and  $10^{-15}$  with sterile isotonic sodium chloride solution (NaCl) for liquid wastewater and soil samples, respectively. The use of the spread plate technique for the isolation of indigenous bacteria in this study is associated with the selection of aerobic bacteria. Meanwhile, the sample dilution levels were determined based on the specific conditions of each sample. For instance, samples predicted to contain high concentrations of organic matter, such as batik dye effluent, are often densely populated with microbes. Therefore, dilution is required to a level that allows the separation of bacterial colonies within the sample. This approach ensures accurate isolation and characterization of bacteria for further analysis, particularly in complex environments like dye effluents, where microbial diversity and abundance are typically high (Madigan, 2018, Cappuccino, 2021). The diluted specimens were subsequently spread onto Nutrient Agar (NA) plates and incubated at 37°C for 24 hours to foster bacterial proliferation (Muchtasjar et al. 2019). The experimental samples in this study were replicated twice.

### Purification of Bacterial Isolates

Individual bacterial colonies were purified utilizing the continuous streak inoculation method on nutrient agar slant media. Isolated colonies were aseptically transferred to fresh slant media to ensure the presence of single strains. The inoculated media were then placed in an incubator at a temperature of 37°C for 24 hours to facilitate optimal bacterial growth (Mambe et al. 2016). During the preliminary phase, each bacterial isolate is assigned an isolate code represented by an alphabetical letter for easy documentation.

Subsequently, following the screening process based on its capacity to degrade diverse batik dyes, the chosen bacterial isolate will undergo molecular characterization, after which the isolate code, represented by an alphabetical designation, will be replaced with the species name.

### Phenetic Characterization of Bacteria

The phenotypic characterization of isolated bacteria comprised: a. Macroscopic observations: Examination of colony morphology (size, shape, color, texture) was conducted on agar plates (Rattray et al. 2022). b. Microscopic observations: Gram staining discriminated between Gram-positive and Gram-negative bacteria (Rohde, 2019). and c. Biochemical analyses: Various biochemical assays evaluated metabolic capacities, encompassing carbohydrate fermentation, starch hydrolysis, gelatin hydrolysis, catalase testing, citrate utilization, hydrogen sulfide ( $H_2S$ ) production, indole production, motility, temperature resilience, pH tolerance, and Methyl red-Voges Proskauer (MR-VP) tests on media (Carr 2017).

### Creation of Color Standard Curves

To quantify the removal of dyes, standard color curves were established for each dye under investigation: crimson naphthol, azure naphthol, scarlet remazol, cobalt remazol, crimson indigosol, azure indigosol, methyl crimson, methyl azure, *Caesalpinia*, and *Indigofera*. Initial solutions of 100 parts per million (ppm) were meticulously prepared for every dye in the nutrient broth media and subsequently diluted serially to concentrations varying from 10 ppm to 80 ppm. The optical density of each diluted solution was measured using ultraviolet-visible (UV-Vis) spectroscopy by a Thermo Scientific Genesys spectrophotometer to construct the standard curves.

### Decolorization Assay and Bacterial Growth Measurement

The capacity of individual bacteria to decolorize and break down specific dyes was evaluated. Starter cultures were established by introducing single bacterial isolates into sterile nutrient broth media (20 milliliters/mL) and incubating for 24 hours at ambient temperature with agitation (Subashkumar, 2014). Subsequently, 1 mL (20%) of this initial culture was introduced into fresh media containing distinct dye concentrations, such as 50 ppm for red naphthol, red remazol, blue remazol, red indigosol, methyl red, methyl blue, and secang (*Caesalpinia*), and 100 ppm for blue naphthol, indigosol blue, and *Indigofera*. The cultures were once again incubated at room temperature with agitation for a duration of one week. Following the incubation period, bacterial cells

were isolated through centrifugation, and the remaining liquid was assessed utilizing a UV-Vis spectrophotometer to determine absorbance and compute the percentage of dye decolorized. Bacterial proliferation was tracked by measuring the absorbance of the culture at 600 nanometers (nm). The decolorization outcomes were derived using the following formula in Equation 1.

$$\% \text{ Decolorisation} = \frac{\text{Value of start Absorbance} - \text{Value of Absorbance Finish}}{\text{Value of start Absorbance}} \times 100\% \quad \dots(1)$$

### McFarland Standard Curve Preparation

To quantify bacterial cell density, a McFarland standard curve was established. This standard was created by mixing specific proportions of 1% sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and 1% barium chloride (BaCl<sub>2</sub>) to produce solutions with varying levels of turbidity. The optical density of each McFarland standard was assessed at 600 nm using UV-Vis (Dalynn Biologicals, 2014).

### Measuring the Growth Rate of Bacterial Isolates ( $\mu$ )

A bacterial inoculum was meticulously prepared by introducing a single dose of bacterial isolate into a sterile nutrient broth medium (20 mL). Subsequently, the medium underwent an incubation process within a shaker incubator at ambient temperature for a duration of 24 hours. The bacterial inoculum was then extracted using a micropipette under aseptic conditions and transferred into a sterile nutrient broth medium (50 mL). The initial Optical Density (OD) of the bacterial culture was determined against the 0.5 McFarland standard, yielding approximately 0.110-0.120 A at a wavelength of 600 nm using a UV-Vis spectrophotometer (Duedu & French, 2017). The blank utilized for this procedure consisted of sterile nutrient broth medium. Following this, the medium was once again subjected to incubation in a shaker incubator at room temperature. OD values were recorded at specific time intervals of 3, 6, 8, 16, 24, 32, 40, 48, 72, and 96 hours. The absorbance values obtained were then transformed into Klett Units by multiplying the absorbance value by 500 (OD  $\times$  500). Subsequently, a growth curve was constructed based on the results of the absorbance values converted into Klett Units (Cuevas and Edwards, 2017) and (Kochan et al. 2020). The rate of bacterial growth ( $\mu$ ) was determined using the following formula in Equation 2.

$$\mu = \frac{(\ln(X_t) - \ln(X_0))}{t} \quad \dots(2)$$

Where:

$\mu$  = Bacterial Growth Speed

$X_t$  = Klett Value Bacterial Incubation Unit at t-th hour

$X_0$  = Klett Value Bacterial Incubation Unit at Hour 0

t = Bacterial Incubation Time (Hours)

ln = Natural Logarithm

### 16S rRNA Sequencing

For bacterial identification, 16 Svedberg ribosomal ribonucleic acid (16S rRNA) sequencing was conducted. Genomic deoxyribonucleic acid (DNA) was extracted from the bacterial isolates using a standardized extraction protocol. The 16S rRNA gene was amplified through polymerase chain reaction (PCR) with specific primers. Following amplification, the DNA fragments were sequenced bidirectionally. Subsequently, the acquired sequences were cross-referenced with known sequences in databases to ascertain the bacterial species present in the samples (Zemb et al. 2020).

### Antagonist Test

An antagonist assay was undertaken to ascertain the bacterium's capacity to produce secondary metabolites possessing antibiotic properties that can impede the proliferation of other bacteria. This examination also assesses the efficacy of bacterial combinations or consortia in decomposing batik dye waste (Bosmans et al. 2016).

### Bacterial Consortium Test (Combination of Bacteria) Degrading Batik Dye Waste

In nature, microbial communities are more commonly found in the form of consortia rather than single bacterial species, particularly when engaging in complex activities such as bioremediation. In a consortium, the metabolic by-products of one bacterial population can serve as substrates or resources for another population within the same community, creating a synergistic system. This interaction facilitates comprehensive bioremediation processes, such as those required for batik wastewater treatment, ensuring that the final products are environmentally safe (Ramaswamy et al. 2022, Barathi et al. 2022).

Naturally formed bacterial consortia are inherently stable when they are well-adapted to their environmental conditions. Recognizing this, the present study constructed a bacterial consortium tailored for potential application as a bioremediation agent in batik wastewater treatment. Such engineered consortia can mimic natural microbial dynamics while being optimized for specific environmental applications (Ihsanullah et al. 2020).

Bacterial consortium test for degrading batik dye waste involves the following steps:

- a. Establishment of a bacterial consortium: The amalgamation of bacterial consortia is formulated using three highly effective bacteria. To determine the bacterial consortium combinations from five effective bacteria, the combination theory is applied as shown in Equation 3.

$${}^5_3C = \frac{5!}{(5-3)!3!} \quad \dots(3)$$

By applying the combination theory, the resulting combinations consist of DHI, DHN, DHJ, DIN, DIJ, DNJ, HIN, HIJ, HNJ, and INJ bacteria.

- b. Discoloration of batik dye by the indigenous bacterial consortium: A bacterial starter culture is meticulously prepared by inoculating a single dose of bacterial isolate into a sterile nutrient broth medium. This medium is subsequently incubated in a shaker incubator at ambient temperature for a duration of 24 hours. Subsequently, 3 mL of the starter culture, each composed of 1 mL + 1 mL + 1 mL of pure starter culture based on the aforementioned combination of bacteria, is inoculated onto a medium consisting of Nutrient Broth and various dyes (naphthol red, naphthol blue, remazol red, remazol blue, indigosol red, indigosol blue, methyl red, methyl blue, *Caesalpinia*, and *Indigofera*). The medium is then subjected to incubation in a shaker incubator at room temperature with a speed of 121 revolutions per minute (rpm) for a span of 168 hours (equivalent to 1 week). Following this incubation period, the medium is transferred to a 15 mL capacity Eppendorf tube and centrifuged to precipitate the bacterial biomass using a centrifuge operating at 3000 rpm for 30 minutes. The discoloration of the dye is quantitatively assessed utilizing a UV-Vis spectrophotometer.

$$\% \text{ Decolorisation} =$$

$$\frac{\text{Value of start Absorbance} - \text{Value of Absorbance Finish}}{\text{Value of start Absorbance}} \times 100\% \quad \dots(4)$$

### Data Analysis

The ability of bacterial isolates to degrade batik dye wastewater was assessed using Equation 1. The degradation capacity of these isolates for batik dye residues, including methyl, naphthol, remazol, indigosol, and natural dyes, was categorized into two color groups: blue and red. If the data met the assumptions of normality and homogeneity, an Analysis of Variance (ANOVA) was conducted to evaluate differences in degradation rates among bacterial isolates for blue and red dyes separately. Additionally, a t-test was conducted to compare the degradation rates of each bacterial isolate in breaking down red and blue dyes, both

synthetic (methyl, naphthol, remazol, indigosol) and natural (*Indigofera tinctoria* L., *Caesalpinia sappan* L.). However, if the data did not meet the normality or homogeneity assumptions for either ANOVA or the t-test, the Kruskal-Wallis test was used as a non-parametric alternative, as suggested by Kim (2019) and Morgan et al. (2019). These statistical approaches are widely applied in biological and environmental research to determine significant differences between independent groups.

## RESULTS AND DISCUSSION

### Characterization and Identification of Indigenous Bacteria

The isolation procedure resulted in a total of 15 bacterial isolates extracted from batik dye wastewater. Each isolate was assigned a unique alphabetical code, and the specific type of batik dye incorporated into the nutrient media is detailed in Table 1.

The principal aim of this research was to characterize and identify predominant autochthonous bacteria from batik dye wastewater containing remazol, naphthol, and indigosol dyes. This extensive isolation effort highlighted the diverse microbial community inherent in batik dye wastewater.

### Characterization of Isolated Bacteria

The phenotypic characterization consisted of macroscopic observations of colony morphology and microscopic examinations (Gram staining) as delineated in Table 2,

Table 1: Bacterial isolation results from batik dye wastewater.

| Bacterial Isolate Code | Isolate Name | Discription               |
|------------------------|--------------|---------------------------|
| A                      | RZ T 10      | Remazol selective media   |
| B                      | RZ T 10      | Remazol selective media   |
| C                      | NP AT 7      | Naphthol selective media  |
| D                      | NP AT 7      | Naphthol selective media  |
| E                      | RZ T 8       | Remazol selective media   |
| F                      | NP T 13      | Naphthol selective media  |
| G                      | NP T 13      | Naphthol selective media  |
| H                      | RZ ID 4      | Remazol selective media   |
| I                      | NP ID 4      | Naphthol selective media  |
| J                      | RZ NP 6      | Remazol selective media   |
| K                      | RZ ID 4      | Remazol selective media   |
| L                      | ID T 10      | Indigosol selective media |
| M                      | ID T 13      | Indigosol selective media |
| N                      | RZ AT 7      | Remazol selective media   |
| O                      | NP AT 7      | Naphthol selective media  |

while the biochemical and physiological characterization of bacterial isolates is detailed in Table 3.

The bacterial isolates displayed a diverse array of colony shapes, colors, and configurations, showcasing the morphological richness within the microbial community. For example, isolate J, identified as *Micrococcus* sp., exhibited a circular yellow colony with a convex elevation and an entire colony edge. Besides isolating J, the details for all other bacterial isolates, identified by their respective codes, can be found in Table 2. Gram staining further distinguished these isolates, unveiling a combination of gram-positive and gram-negative bacteria, crucial for comprehending their structural and functional roles in dye degradation (Calgin & Kalayci 2024). The integration of advanced materials such as nanocomposites derived from natural waste can significantly enhance the degradation capabilities of indigenous bacteria. Haroon et al. (2023) demonstrated that nanomaterials synthesized through green processes, such as those derived from plant waste and fly ash, exhibit high biocompatibility and stability. These properties enable them to adsorb heavy metals and organic dyes effectively, thus complementing bacterial enzymatic activities in wastewater remediation. As indicated in Table 2, 11 are gram-positive, while 4 are gram-negative. The fundamental disparities between gram-positive and gram-negative bacteria reside in their cell wall composition, quorum-sensing signal molecules, and ecological functions. Gram-positive bacteria possess a thicker peptidoglycan layer and utilize peptides for quorum sensing, whereas gram-negative bacteria feature a thinner peptidoglycan layer, an outer membrane, and employ N-acyl homoserine

lactone molecules for quorum sensing (Bishop & McCue 2021).

The biochemical and physiological analysis (Table 3) offered profound insights into the metabolic capacities of these isolates. Assessments such as citrate utilization, starch hydrolysis, and fermentation of diverse sugars illuminated the enzymatic potential of these bacteria. Significantly, isolates J and N, which demonstrated the highest efficiency in dye degradation, also displayed favorable outcomes in multiple biochemical examinations, indicating their robust metabolic frameworks. This comprehensive assessment emphasizes the significance of comprehending bacterial metabolism for both environmental and industrial purposes, particularly in the realm of dye pollutant bioremediation.

The biochemical and physiological evaluation of bacteria is indispensable for grasping their roles and utility. This process aids in understanding bacterial physiology, pathogenesis, and metabolism (Malarney & Chang 2023) and is pivotal for the recognition and characterization of advantageous microbial strains through microbiological, biochemical, and molecular methodologies (Hossain & Sultana 2018). In industrial settings, fundamental enzyme assays and biochemical analysis are essential for various industrial enzymes and applications (Salwan et al. 2023). Bacteria may coordinate numerous unrelated genes concurrently in response to extreme environments like batik wastewater containing synthetic chemical dyes. This coordination entails global regulatory systems such as the lactose operon and the maltose regulon, which react to these

Table 2: Bacterial colony morphology.

| Isolate code | Shape            | Color           | Elevation     | Colony Edge     | Configuration                  | Gram +/- |
|--------------|------------------|-----------------|---------------|-----------------|--------------------------------|----------|
| A            | <i>Circular</i>  | Yellowish white | <i>Convex</i> | <i>Entire</i>   | <i>Round</i>                   | +        |
| B            | <i>Circular</i>  | Yellowish white | <i>Convex</i> | <i>Entire</i>   | <i>Round</i>                   | +        |
| C            | <i>Circular</i>  | Brownish white  | <i>Convex</i> | <i>Entire</i>   | <i>Round</i>                   | +        |
| D            | <i>Circular</i>  | Brownish white  | <i>Convex</i> | <i>Undulate</i> | <i>Serrate</i>                 | +        |
| E            | <i>Circular</i>  | White           | <i>Convex</i> | <i>Entire</i>   | <i>Round</i>                   | +        |
| F            | <i>Circular</i>  | Yellow          | <i>Convex</i> | <i>Entire</i>   | <i>Round</i>                   | +        |
| G            | <i>Irregular</i> | Brownish white  | <i>Convex</i> | <i>Lobate</i>   | <i>Irregular and Spreading</i> | +        |
| H            | <i>Irregular</i> | White           | <i>Raised</i> | <i>Undulate</i> | <i>Round and Scalloped</i>     | -        |
| I            | <i>Irregular</i> | White           | <i>Raised</i> | <i>Undulate</i> | <i>Round and Scalloped</i>     | -        |
| J            | <i>Circular</i>  | Yellow          | <i>Convex</i> | <i>Entire</i>   | <i>Round</i>                   | -        |
| K            | <i>Irregular</i> | White           | <i>Raised</i> | <i>Undulate</i> | <i>Round and Scalloped</i>     | -        |
| L            | <i>Circular</i>  | Yellowish white | <i>Convex</i> | <i>Entire</i>   | <i>Serrate</i>                 | +        |
| M            | <i>Irregular</i> | Brownish white  | <i>Convex</i> | <i>Undulate</i> | <i>Round and Scalloped</i>     | +        |
| N            | <i>Circular</i>  | Brownish white  | <i>Convex</i> | <i>Undulate</i> | <i>Serrate</i>                 | +        |
| O            | <i>Irregular</i> | White           | <i>Convex</i> | <i>Undulate</i> | <i>Round and Scalloped</i>     | +        |

Table 3: Results of biochemical and physiological characterization of bacterial isolates from batik dye waste.

| Biochemical Test                | Isolate Code |   |   |   |   |   |   |   |   |   |   |   |   |   |   |  |
|---------------------------------|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|--|
|                                 | A            | B | C | D | E | F | G | H | I | J | K | L | M | N | O |  |
| Simmons Citrate                 | -            | - | - | - | - | + | + | + | + | - | + | + | + | + | + |  |
| Motility                        | -            | - | - | - | - | - | - | - | - | - | - | - | - | - | - |  |
| Starch Hydrolysis               | -            | - | - | - | - | - | + | + | + | - | + | - | - | - | - |  |
| Glucose Fermentation            | -            | - | - | - | - | - | - | + | + | + | + | + | + | + | - |  |
| Sucrose Fermentation            | -            | - | - | - | - | - | - | - | - | + | - | - | + | - | - |  |
| Galactose Fermentation          | -            | - | - | - | - | - | - | - | - | + | - | - | + | - | - |  |
| Maltose Fermentation            | -            | - | - | - | - | - | - | - | - | + | - | - | + | - | - |  |
| Lactose Fermentation            | -            | - | - | - | - | - | - | - | - | + | - | - | + | - | - |  |
| Facultative Anaerobe            | +            | + | + | + | + | + | + | - | - | + | - | - | + | + |   |  |
| Anaerobe                        | -            | - | - | - | - | - | - | + | + | - | + | + | - | - | + |  |
| Gram Negative                   | +            | + | + | + | + | + | + | - | - | - | - | + | + | + | + |  |
| Gram Positive                   | -            | - | - | - | - | - | - | + | + | + | + | - | - | - | - |  |
| Growth pH 4                     | -            | - | - | - | - | - | - | + | + | + | + | - | - | - | - |  |
| Growth pH 7                     | +            | + | + | + | + | + | + | + | + | + | + | + | + | + | + |  |
| Growth pH 9                     | +            | + | + | + | + | + | + | + | + | + | + | + | + | + | + |  |
| Growth NaCl 5%                  | +            | + | + | + | + | + | + | + | + | + | + | + | + | + | + |  |
| Growth NaCl 10%                 | +            | + | - | + | + | + | + | + | + | + | + | + | + | + | - |  |
| Catalase                        | +            | + | + | + | + | + | + | + | + | + | + | + | - | + | + |  |
| H <sub>2</sub> S production     | -            | - | - | - | - | - | - | - | - | - | - | - | - | - | - |  |
| Indole                          | -            | - | - | - | - | - | - | - | - | - | - | - | - | - | - |  |
| Growth Temperature -4°C         | +            | + | + | + | + | + | + | - | - | + | - | + | + | + | + |  |
| Growth Temperature 25°C         | +            | + | + | + | + | + | + | + | + | + | + | + | + | + | + |  |
| Growth Temperature 55°C         | -            | - | - | + | + | + | + | + | + | + | - | + | - | - | + |  |
| Gelatin                         | -            | - | - | - | - | - | - | - | - | - | - | - | + | - | - |  |
| Methyl Red                      | -            | - | - | - | - | - | - | - | - | - | - | - | + | - | - |  |
| +P                              | -            | - | - | - | - | - | - | - | - | - | - | - | - | - | - |  |
| Growth on Echinulate Slant Agar | -            | - | - | - | - | - | - | + | + | - | + | - | - | - | - |  |

Note: + = Positive Test - = Negative Test

conditions, enabling bacterial genes to acclimate to the synthetic dyes present in batik wastewater.

### Bacterial Growth Rate

Quantifying bacterial growth rates not only provides insights into their physiological behavior but also holds practical implications for industrial, environmental, and medical applications. The table presented here (Table 4) summarizes the growth rates of various bacterial isolates under standardized conditions.

Understanding bacterial proliferation is crucial in environmental biotechnology, particularly for bioremediation applications. Certain bacterial isolates exhibit exceptional abilities to degrade environmental pollutants, such as dye wastes, even under growth-limiting conditions, emphasizing

the importance of metabolic capabilities over growth rates.

Growth rates ( $\mu\mu$ ) of various bacterial isolates, along with key observations, reveal significant diversity in their proliferation potentials. Isolate J demonstrates the lowest growth rate due to inefficient substrate utilization, whereas isolate O exhibits the highest growth rate. Despite isolate J's sluggish proliferation, it shows remarkable dye-degrading efficacy, efficiently breaking down diverse dye wastes. This highlights the significance of functional capabilities beyond growth rates in bioremediation (Basan et al. 2020).

Bacterial proliferation rates are influenced by nutritional status, environmental factors, temperature, protein synthesis capability, and cellular dimensions. The interplay between these factors and waste degradation efficacy is determined by bacterial species, environmental conditions, and

Table 4: Bacterial growth rate.

| No. | Isolate Code | Growth Rate [ $\mu$ ] |
|-----|--------------|-----------------------|
|     |              | Average               |
| 1   | A            | 0.2733                |
| 2   | B            | 0.2770                |
| 3   | C            | 0.2892                |
| 4   | D            | 0.2856                |
| 5   | E            | 0.2766                |
| 6   | F            | 0.2848                |
| 7   | G            | 0.2895                |
| 8   | H            | 0.2233                |
| 9   | I            | 0.2704                |
| 10  | J            | 0.1817                |
| 11  | K            | 0.2246                |
| 12  | L            | 0.3067                |
| 13  | M            | 0.2986                |
| 14  | N            | 0.3009                |
| 15  | O            | 0.3100                |

waste composition, all of which impact the efficiency of bioremediation efforts (Zainol et al. 2020). Isolate J's substantial dye waste degradation ability, despite its slow growth, underscores its value as a bioremediation agent, demonstrating that functional efficiency can outweigh growth constraints in such applications (Santal et al. 2024).

### Decolorization Assay and Bacterial Growth Measurement

The efficacy of the isolates in degrading batik dye waste was assessed through decolorization assays. Figs. 1 and 2

depict the degradation percentages for azure and crimson batik dyes, respectively.

Fig. 1 illustrates the levels of degradation of blue batik dye waste by 15 bacterial strains. Isolate J (*Micrococcus* sp.) demonstrated the highest degradation efficacy, achieving 99.62% for *Indigofera* and 83.5% for naphthol blue. This remarkable efficiency can be attributed to its robust enzymatic prowess, encompassing a variety of oxidases and reductases, enabling the bacterium to metabolize a broad spectrum of dye molecules (Ikram et al. 2023). In addition to bacterial-based decolorization, the application of nanocomposites has been shown to provide significant advantages in treating dye-laden wastewater. According to Haroon et al. (2023), nanocomposites synthesized from agricultural waste achieve high dye adsorption efficiencies, effectively removing pollutants that are otherwise resistant to biological degradation. This synergy between adsorption and biodegradation presents a promising strategy for optimizing batik dye wastewater treatment.

Isolate I (*Stutzerimonas stutzeri*) also exhibited commendable performance, particularly in the degradation of blue remazol dye with an efficiency of 79.40%. The production of enzymes such as azoreductase, laccase, and peroxidase by this isolate plays a pivotal role in the decolorization and breakdown of dye molecules (Joshi et al. 2020).

Isolate N (*Pseudomonas* sp.) achieved an 85.26% degradation efficiency for *Indigofera*. *Pseudomonas* sp. is renowned for its metabolic versatility and capacity to produce an array of enzymes, including azoreductase and dioxygenase, crucial for the breakdown of complex organic pollutants like synthetic dyes (Patel et al. 2017).

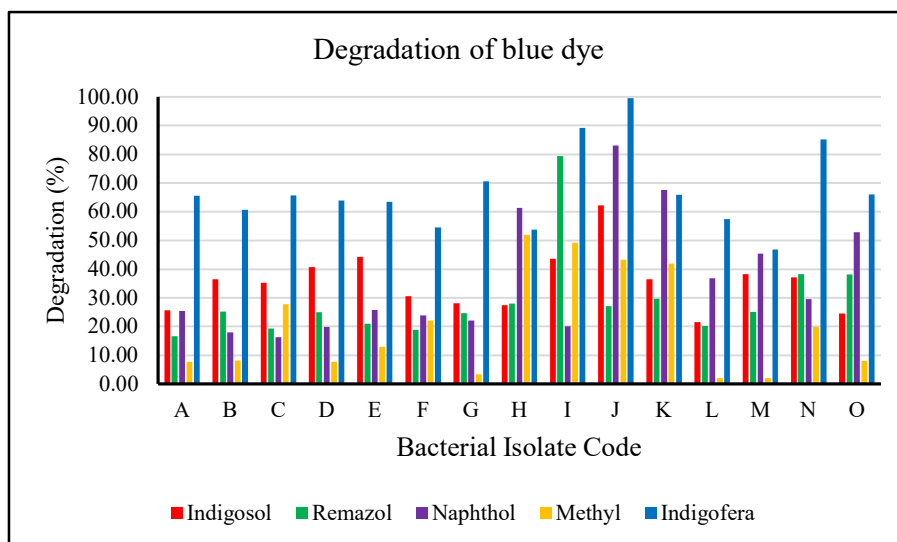


Fig. 1: Bar diagram of degradation of blue batik dye waste.

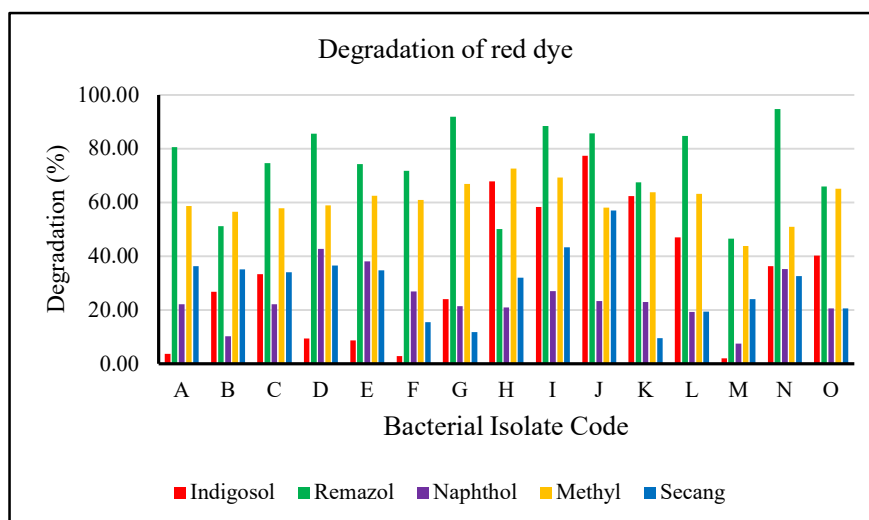


Fig. 2: Bar diagram of degradation of red batik dye waste.

Comparatively lower degradation efficiencies were observed in isolates H, with each achieving approximately 52.04-61.28% for methyl and naphthol blue dyes, respectively. These disparities can be attributed to variations in enzymatic capabilities and the environmental adaptability of the respective isolates (Misha 2022).

Fig. 2 illustrates the levels of degradation of red batik dye waste by the bacterial isolates. Isolate J (*Micrococcus* sp.) once again demonstrated exceptional performance, achieving an 85.74% degradation efficiency for remazol red dye. This achievement is consistent with prior research highlighting the extensive enzymatic capabilities of *Micrococcus* sp. (Ikram et al. 2023). Isolate H (*Stutzerimonas stutzeri*) attained a 72.68% degradation efficiency for methyl red dye. The presence of azoreductase, laccase, and peroxidase enzymes enables this isolate to efficiently break down various dye types (Joshi et al. 2020). In the case of remazol red dye, isolate D (*Stenotrophomonas maltophilia*) exhibited a noteworthy efficiency of 85.51%, comparable to isolate N with 94.72% and isolate J with 85.74%. This suggests that *Stenotrophomonas maltophilia* could be highly proficient in degrading specific dye varieties, particularly remazol red, possibly due to specialized enzymes targeting the molecular structure of this dye. *Pseudomonas* sp. (Isolate N) also excelled in degrading remazol red dye, achieving an efficiency of 94.72%. This indicates the significant potential of this isolate for practical applications in dye wastewater bioremediation (Ihsanullah et al. 2020). Lower degradation efficacy was observed in isolates A and B, particularly with secang red dye. This could be attributed to limitations in enzymatic activity or the incapacity of these isolates to adapt to the challenging conditions posed by synthetic batik dyes

(Salwan et al. 2023). Based on the decolorization capabilities of bacterial isolates towards batik dye waste as depicted in Figs. 1 and 2, isolates D, H, I, J, and N have been identified as effective. Moreover, these five efficacious bacterial isolates were discerned through 16S rRNA sequencing. To evaluate the degradation prowess of effective bacterial isolates (D, H, I, J, N) on blue batik dye waste containing natural dyes (*Indigofera*), methyl, naphthol, remazol, and indigosol, as well as red batik dye waste with natural red dyes (*Caesalpinia*), methyl, naphthol, remazol, and indigosol, an ANOVA analysis was performed. The outcomes are presented in Table 5 below.

The Anova results showed no significant differences in the decolorization abilities of bacterial isolates for blue ( $F = 1.288$ ,  $p = 0.308$ ) and red dyes ( $F = 0.308$ ,  $p = 0.869$ ), indicating comparable capacities among the isolates. Anova analysis was performed because the data met the assumptions of normality and homogeneity. Separate ANOVA analyses for blue and red dyes supported these findings, consistent

Table 5: The results of the ANOVA analysis on the ability of effective bacterial isolates to degrade various batik dye wastes.

| Degradation rate of blue batik dye waste |                |    |             |       |      |
|------------------------------------------|----------------|----|-------------|-------|------|
|                                          | Sum of Squares | df | Mean Square | F     | Sig. |
| Between Groups                           | 3085.841       | 4  | 771.460     | 1.288 | .308 |
| Within Groups                            | 11975.115      | 20 | 598.756     |       |      |
| Total                                    | 15060.956      | 24 |             |       |      |
| Degradation rate of red batik dye waste  |                |    |             |       |      |
| Between Groups                           | 806.592        | 4  | 201.648     | .308  | .869 |
| Within Groups                            | 13090.297      | 20 | 654.525     |       |      |
| Total                                    | 13896.889      | 24 |             |       |      |

Table 6: The results of the independent t-test and Kruskal-Wallis test between the ability of effective bacterial isolates in degrading blue and red batik dye waste.

| Types of batik waste dyes       | Information                  | The t-test results |       |        |       |                 |
|---------------------------------|------------------------------|--------------------|-------|--------|-------|-----------------|
|                                 |                              | F                  | Sig.  | t      | df    | Sig. (2-tailed) |
| Natural dyes                    | Equal variances assumed      | 3.616              | 0.94  | 3.941  | 8     | 0.004           |
|                                 | Equal variances not assumed. |                    |       | 3.941  | 6.208 | 0.007           |
| Indigosol                       | Equal variances assumed      | 3.783              | 0.088 | -0.403 | 8     | 0.697           |
|                                 | Equal variances not assumed. |                    |       | -0.403 | 5.354 | 0.702           |
| The Kruskal-Wallis test results |                              |                    |       |        |       |                 |
|                                 | Chi-Square                   | df                 |       |        |       | Asymp. Sig.     |
| Methyl                          | 5.771                        | 1                  |       |        |       | 0.016           |
| Naphthol                        | 0.011                        | 1                  |       |        |       | 0.917           |
| Remasol                         | 5.771                        | 1                  |       |        |       | 0.016           |

with studies by Rakhmania et al. (2022), which demonstrated the effectiveness of dye-degrading bacteria in disintegrating azo dyes commonly used in the batik industry. The outcomes of this investigation include a comparison of the degradation abilities of bacterial isolates on red and blue batik dye waste for each dye type, both natural and synthetic. For natural dyes and indigosol, an independent t-test was conducted as the data met normality and homogeneity assumptions. Meanwhile, methyl and naphthol dyes did not meet the homogeneity requirement, and remazol dye failed the normality assumption; thus, these were analyzed using the Kruskal-Wallis test, as shown in Table 6.

The t-test results revealed a significant difference in the degradation abilities of bacterial isolates for natural blue and red dyes ( $p = 0.004$ ), while no significant difference was observed for indigosol ( $p = 0.697$ ). This suggests that the chemical structure of natural dyes like *Indigofera* and *Caesalpinia* affects degradation efficiency, unlike indigosol.

For synthetic dyes, the Kruskal-Wallis test indicated significant differences in degradation between blue and red dyes for methyl and remazol ( $p = 0.016$ ), but no significant difference for naphthol ( $p = 0.917$ ). These variations are likely linked to the interaction of enzymes such as azoreductase, laccase, and peroxidase with the dye molecules, as described by Mishra et al. (2022).

These findings align with Calderón-Tapia et al. (2024), emphasizing the importance of developing tailored bioremediation strategies for different dye types and colors, particularly in managing complex batik dye wastewater.

Prior research suggests that the efficacy of decolorization predominantly hinges on the specific bacterial strains utilized and environmental factors such as pH, dye concentration, and biomass dosage. For example, *Bacillus algicola* showcased the capacity to decolorize over 95% of azo dyes at pH 8.0 and

25°C (Chukowry et al. 2017). Additionally, *Pseudomonas* sp. and *Enterobacter cloacae* have demonstrated notable decolorization prowess across various dyes, encompassing red and blue variants (Ravi et al. 2024, Govindaraj et al. 2018).

### Identification of Batik Dye-Degrading Bacteria Using 16S rRNA Sequencing

The outcomes of bacterial identification through molecular analysis using the 16S rRNA sequencing technique can be evaluated based on the following criteria:

1. **Percentage Identity:** This metric signifies the level of resemblance between the provided DNA sequence and the target DNA sequence. Samples of microorganisms utilizing the 16S rRNA marker are deemed identical at the species level when the “percentage identity” surpasses 97.5%, and at the genus level when it exceeds 95% (Teng et al. 2014).
2. **Query Coverage:** This parameter indicates the degree to which the provided DNA sequence aligns with the target DNA sequence. When the provided DNA sequence completely matches the target DNA sequence in the NCBI database, the query coverage reaches 100%.
3. **E Value:** The E value, or expectation value, gauges the probability of the alignment occurring randomly. A favorable E value approaches zero, signifying that the alignment is highly improbable to have happened by chance.

The molecular identification results obtained from 16S rRNA sequencing by comparing the DNA sequences acquired with the GenBank nucleotide database from NCBI for bacterial identification are delineated in Table 7.

To precisely ascertain the predominant bacterial strains accountable for dye degradation, 16S rRNA gene sequencing was conducted. This molecular methodology yielded intricate

Table 7: Molecular Identification.

| Bacterial Isolate Code | Organism                            | Fragment | %Species identity | Query coverage [%] | E Value | Accession Number |
|------------------------|-------------------------------------|----------|-------------------|--------------------|---------|------------------|
| D                      | <i>Stenotrophomonas maltophilia</i> | 16S      | 99.93             | 100                | 0       | ON197508.1       |
| H                      | <i>Stutzerimonas stutzeri</i>       | 16S      | 99.93             | 100                | 0       | MN889324.1       |
| I                      | <i>Stutzerimonas stutzeri</i>       | 16S      | 99.93             | 100                | 0       | MN889324.1       |
| J                      | <i>Micrococcus</i> sp               | 16S      | 99.93             | 100                | 0       | JQ658425.1       |
| N                      | <i>Pseudomonas</i> sp               | 16S      | 100               | 100                | 0       | MK696212.1       |

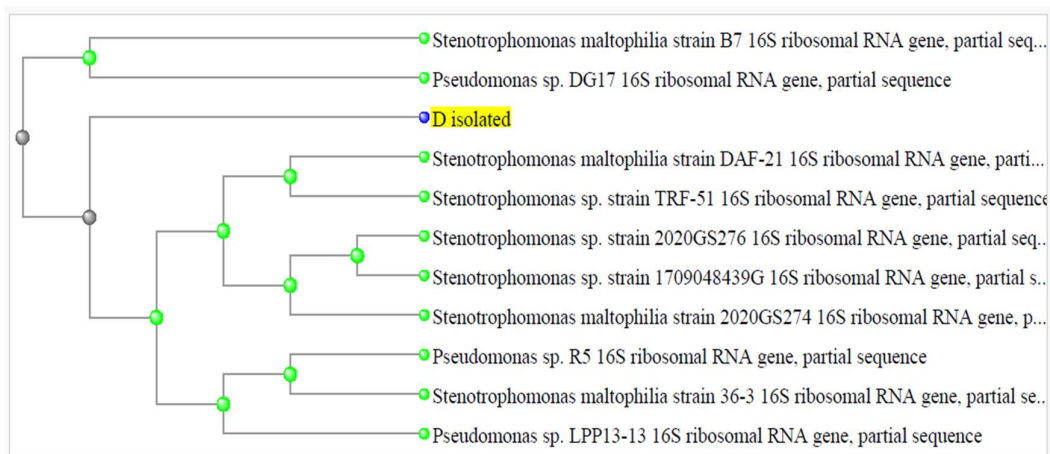


Fig. 3: Phylogenetic tree analysis for isolate D (NP-AT7).

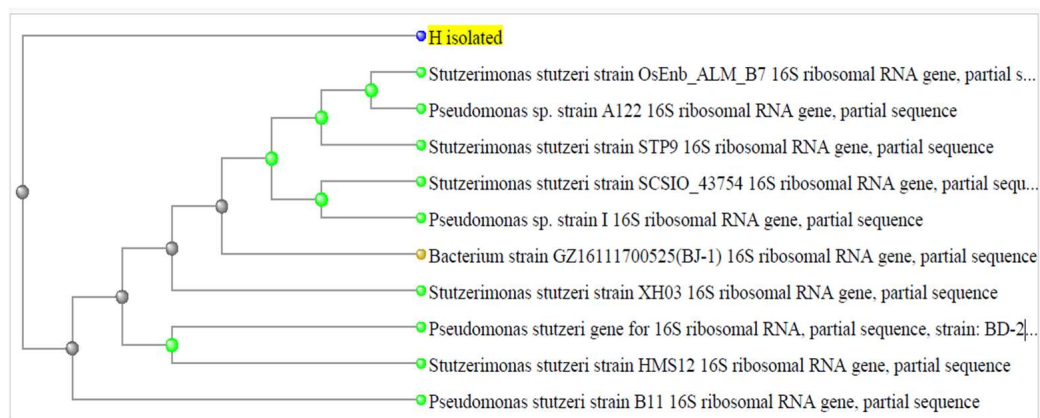


Fig. 4: Phylogenetic tree analysis for isolate H (RZ-ID4).

insights into the genetic affinities and potential functional capacities of the isolated bacteria. The outcomes of the phylogenetic analyses for isolates D, H, I, J, and N are depicted in Figs. 3, 4, 5, 6, and 7, respectively.

Fig. 3 depicts the phylogenetic tree of isolate D (NP-AT7), identified as *Stenotrophomonas maltophilia*, known for effectively degrading red naphthol and remazol dyes via laccase enzymes. These enzymes catalyze the oxidation of phenolic and non-phenolic compounds, including synthetic

dyes, by transferring electrons from dye molecules to oxygen, breaking down complex structures into simpler, less harmful forms (Vishani & Shrivastav 2022).

Fig. 4 shows the phylogenetic tree of isolate H (RZ-ID4), identified as *Stutzerimonas stutzeri*, which effectively degrades blue naphthol, red methyl, and blue remazol dyes. This is achieved through enzymes such as azoreductase, which initiates the breakdown of azo bonds in synthetic dyes, and laccase and peroxidase, which further

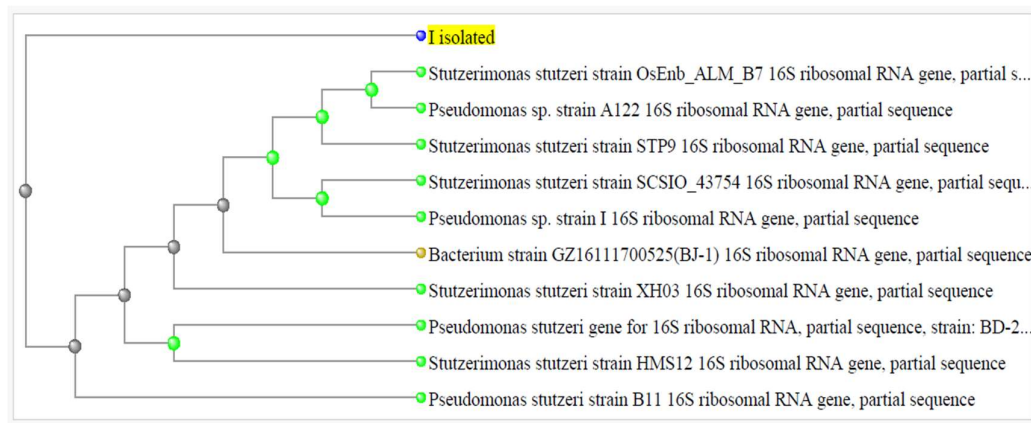


Fig. 5: Phylogenetic tree analysis for isolate I (NP-ID4).

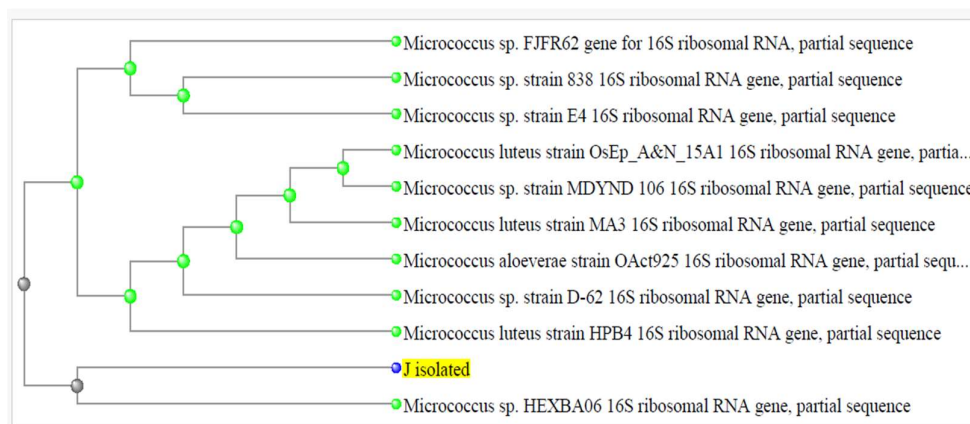


Fig. 6: Phylogenetic tree analysis for isolate J (RZ-NP6).

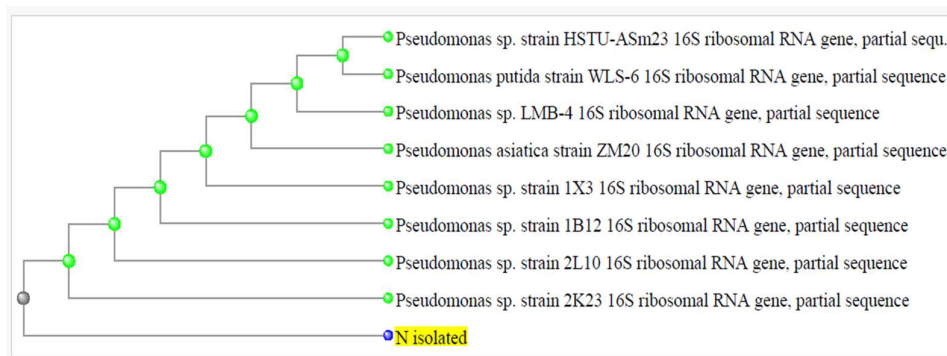


Fig. 7: Phylogenetic tree analysis for isolate N (RZ-AT7).

oxidize dye molecules into less harmful compounds (Joshi et al. 2020).

Fig. 5 depicts the phylogenetic tree of isolate I (NP-ID4), identified as *Stutzerimonas stutzeri*, which efficiently degrades natural *Indigofera*, red remazol, and blue remazol dyes. This ability is attributed to its enzymatic repertoire,

including azoreductase, laccase, and peroxidase, which collaboratively break down complex dye molecules into simpler compounds, accelerating decolorization and reducing environmental impacts (Mishra et al. 2022).

Fig. 6 illustrates the phylogenetic tree of isolate J (RZ-NP6), identified as *Micrococcus* sp., which exhibits versatility

in degrading dyes such as natural *Indigofera*, *Caesalpinia*, indigosol, remazol, and naphthol, attributed to its robust enzymatic systems. These capabilities make it a strong candidate for bioremediating diverse dye pollutants (Ikram et al. 2023). Similarly, studies by Calderón-Tapia et al. (2024) highlight the degradation potential of *Micrococcus aloeverae* for CN and Hg, achieving efficiencies up to 98.80%.

Fig. 7 illustrates the phylogenetic tree of isolate N (RZ-AT7), identified as *Pseudomonas* sp., which effectively degrades natural *Indigofera* and red remazol dyes through azoreductase and dioxygenase enzymes. These enzymes disintegrate dye molecules into simpler, harmless forms, enabling efficient decolorization and detoxification of dye-laden wastewater (Ihsanullah et al. 2020). Decolorization assessments revealed that Isolate N achieved a 94.72% degradation rate for red remazol, while Isolate J excelled with 99.62% for natural *Indigofera* blue dye, highlighting their potential for eco-friendly bioremediation.

### Testing a Combination of Bacterial Isolates or Consortium Bacteria as Batik Dye Waste Degraders

The efficacy of a combination of bacterial isolates, or a consortium of bacteria, in the degradation of batik dye waste surpasses that of individual isolates. The superior performance of these indigenous bacterial consortia in the decomposition of batik dye waste is evident in the illustrations depicted in Figs. 8 and 9 below.

Fig. 8 illustrates that numerous bacterial consortia exhibited remarkable efficacy in degrading blue batik dyes. Particularly noteworthy is the INJ consortium, consisting of *Stutzerimonas stutzeri*, *Pseudomonas* sp., and *Micrococcus* sp., which demonstrated the highest degradation efficiency for indigosol blue at an impressive rate of 90.29%. In a related study, Sarwan et al. (2024) demonstrated that cellulolytic bacterial isolates not only achieved high efficiency in breaking down cellulosic wastes but also effectively deinked colored paper within 24 hours. These findings emphasize the potential of integrating cellulolytic bacteria into bioremediation consortia to target complex pollutants, such as batik dyes, for enhanced degradation and eco-friendly waste management. This exceptional performance can be attributed to the synergistic enzymatic activities of the three isolates, likely encompassing azoreductase, laccase, and peroxidase, well-known for their pivotal roles in dye degradation (Joshi et al. 2020).

The HIN consortia also displayed significant effectiveness, achieving degradation efficiencies of 79.94% for indigosol blue. This underscores the ability of these consortia to leverage the diverse metabolic pathways of their constituent isolates to effectively dismantle intricate dye molecules (Barathi et al. 2022).

When it comes to natural indigofera blue dye, the INJ consortium once again excelled with a degradation efficiency of 61.22%, demonstrating its versatility across various dye types. Additionally, consortia such as HNJ and DHJ

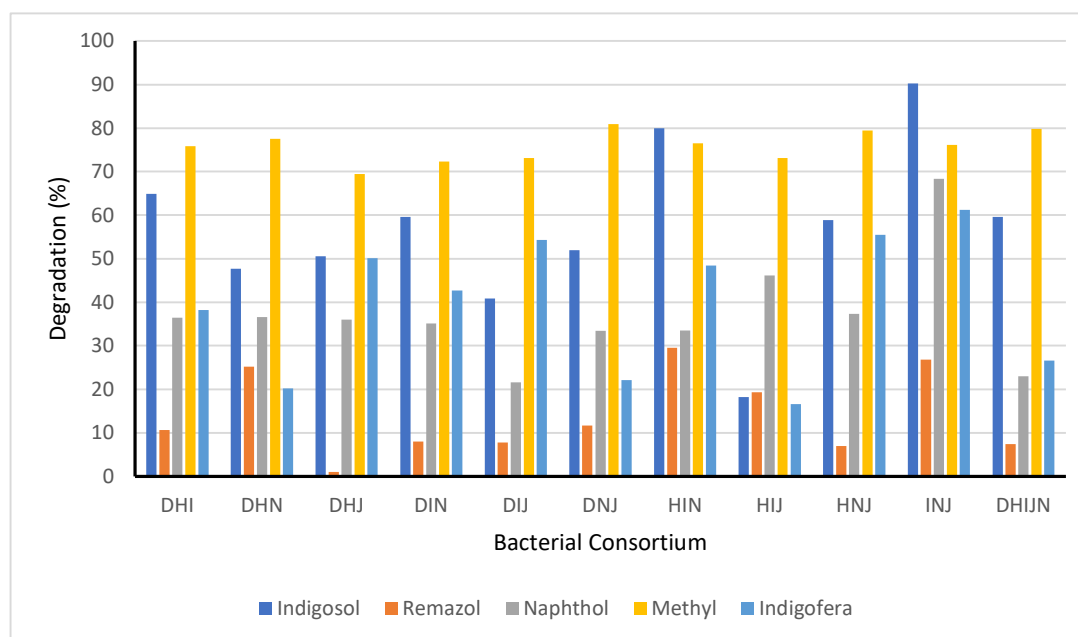


Fig. 8: Bar diagram of the ability of a combination of bacterial isolates/consortium bacteria to degrade blue batik dye waste.

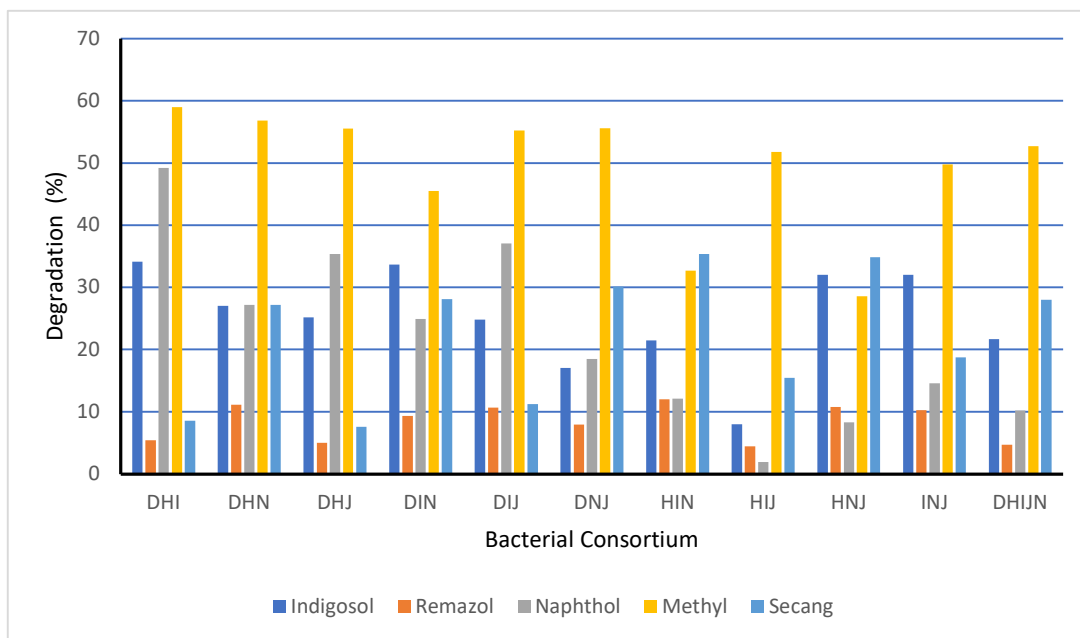


Fig. 9: Bar diagram of the ability of a combination of bacterial isolates/consortium bacteria to degrade red batik dye waste.

exhibited commendable performance, achieving degradation efficiencies of 55.52% and 50.16%, respectively.

In the case of methyl blue dye, the DNJ consortium attained the highest efficiency at 80.92%, closely trailed by DHN with 77.54% and HNJ with 79.45%. These findings underscore the significance of selecting consortia with complementary enzymatic capabilities to optimize degradation efficiency (Mutiara et al. 2023).

Fig. 9 illustrates the degradation efficiencies of various bacterial consortia for red batik dye waste. The findings indicate that the degradation capacity for red dyes was generally lower than that of blue dyes. Concerning secang red dye, the consortium HIN demonstrated the highest degradation efficiency at 35.34%, followed by DNJ at 30.14%. These outcomes suggest that specific combinations of bacteria are more adept at breaking down natural red dyes due to their distinct enzymatic profiles.

In the realm of synthetic naphthol red dye, the DHI consortium exhibited the highest degradation efficiency at 49.23%. This achievement is remarkable, especially considering the intricate chemical structure of naphthol dyes, which typically pose challenges for degradation (Mahmood et al. 2015). As for indigosol red dye, the DHI consortium showcased a relatively high degradation efficiency of 34.13%, closely followed by DIN at 33.63%. These results imply that certain bacterial combinations are proficient in targeting and disintegrating indigosol dye molecules (Abu Talha et al. 2018).

Isolate J (*Micrococcus* sp.) demonstrates superior performance both individually and within consortia, which can be attributed to its high enzymatic activity. This isolate is capable of producing key enzymes such as oxidase and reductase, which play a critical role in breaking down complex dye molecules. The study highlights that isolate J achieved the highest degradation rates for natural *Indigofera* dye of 99.62% (Fig. 1), remazol red (85.74%), and naphthol blue (83.05%).

At the consortium level, isolate J significantly contributes due to its complementary enzymatic activity when paired with other isolates, such as *Pseudomonas* sp. and *Stutzerimonas stutzeri*, in the INJ consortium. This consortium achieved a decolorization efficiency of 90.29% (Fig. 8) for indigosol blue dye, demonstrating synergy supported by the presence of enzymes such as azoreductase, laccase, and peroxidase. These results are consistent with analogous studies, such as (Elkema et al. 2023), where the singular isolate, *Enterobacter* sp. ASH decomposed 80% of crude oil. Conversely, another study by Calderón-Tapia et al. (2024) suggested that individual strains generally exhibit lower decolorization capabilities in comparison to consortia.

The superiority of isolate J over others may also be linked to its metabolic flexibility, enabling it to degrade a wide range of natural and synthetic dye compounds under various environmental conditions. Its high enzymatic activity and effectiveness within consortia make isolate J a promising candidate for large-scale bioremediation applications.

The capacity of bacteria to degrade batik dyes is significantly impacted by various factors, including: a) culture characteristics and ideal conditions, such as the presence of sodium chloride, initial dye concentration, inoculum size, and pH (Palanivelan et al. 2024), b) the utilization of specific carbon and nitrogen sources, such as mannitol and ammonium sulfate, which are pivotal for achieving optimal removal efficiency (Ramaswamy et al. 2022).

Drawing from these explanations, the benefits of employing bacterial consortia in the degradation of batik dye waste encompass: a) bacterial consortia offer accelerated degradation rates and heightened efficiency, rendering them efficacious for bioremediation (Kurade et al. 2023), b) consortia exhibit resilience to substantial environmental fluctuations, rendering them suitable for practical-scale operations and less reliant on sterile conditions (Ramaswamy et al. 2022), c) the synergistic effects of microbial consortia furnish significant advantages over singular cultures in mineralizing complex dye compounds, leading to enhanced decolorization (Pinheiro et al. 2022), d) the utilization of bacterial consortia in bioremediation is cost-effective and environmentally sustainable, presenting a promising technology for treating batik dye waste (Barathi et al. 2022).

The results of the ANOVA analysis for the consortium of bacterial isolates in decolorizing batik dye waste, in both blue and red colors, are delineated in the subsequent Table 8.

The ANOVA results in Table 8 show no significant differences in the ability of bacterial consortia to degrade blue ( $F = 0.573$ ,  $p = 0.811$ ) and red batik dye waste ( $F = 0.294$ ,  $p = 0.972$ ). ANOVA was performed because the data met the assumptions of normality and homogeneity, and separate analyses were conducted for blue and red dye degradation rates. These findings indicate that all tested consortia have comparable degradation capabilities for both dye types, consistent with previous studies highlighting the efficiency

of bacterial consortia in degrading complex dye structures due to their synergistic enzymatic activities (Barathi et al. 2022, Mishra et al. 2022).

The consistent performance of the consortia suggests they are well-suited for degrading batik dye wastewater, likely due to their ability to adapt to the chemical composition of the waste and produce diverse enzymes. These results support the feasibility of using any tested consortia for bioremediation purposes. Future research should focus on optimizing environmental conditions and scaling up these methods for industrial applications (Ramaswamy et al. 2022).

The results of this study can be applied on a field scale by developing bioreactors based on bacterial consortia to treat industrial wastewater. Pilot-scale testing is necessary to determine optimal conditions, such as retention time and nutrient requirements. These consortia can also be integrated into existing wastewater treatment systems to reduce COD and color intensity. Regular monitoring and safety evaluations of degradation products are essential to ensure that the process is safe and sustainable.

## CONCLUSIONS

This study demonstrates that indigenous bacterial isolates, both individually and in consortia, possess significant abilities to decolorize batik dye wastewater, including both natural and synthetic dyes. The findings and implications of this research are as follows:

**Identification and characterization of indigenous bacteria:** The study isolated 15 bacterial strains from batik dye wastewater, five of which displayed substantial dye degradation abilities. These isolates were identified as *Stenotrophomonas maltophilia*, *Stutzerimonas stutzeri* (H and I), *Micrococcus* sp., and *Pseudomonas* sp. Each of these bacteria showcased the capacity to degrade specific dyes such as remazol, naphthol, and indigosol. The utilization of 16S rRNA gene sequencing was pivotal in precisely identifying these bacteria, emphasizing the genetic and functional diversity within the microbial community of the wastewater.

**Effectiveness in dye decolorization:** The biodegradation assays verified that the identified bacterial strains could efficiently decolorize batik dye wastewater. Isolate J (*Micrococcus* sp.) and isolate N (*Pseudomonas* sp.) demonstrated the highest overall degradation efficiency, underscoring their resilience and adaptability. Particularly, isolate J achieved a 99.62% degradation of natural dye *Indigofera*, 85.74% for red remazol, and 83.05% for blue naphthol, while isolate N excelled in degrading red remazol dye (94.72%). Moreover, bacterial consortia like INJ (comprising *Stutzerimonas stutzeri*, *Pseudomonas* sp., and

Table 8: ANOVA results on the ability of the bacterial consortium to degrade blue and red batik dye waste.

| Degradation rate of blue batik dye waste |                |    |             |       |       |
|------------------------------------------|----------------|----|-------------|-------|-------|
|                                          | Sum of Squares | df | Mean Square | F     | Sig.  |
| Between Groups                           | 3257.344       | 9  | 361.927     | 0.573 | 0.811 |
| Within Groups                            | 25280.041      | 40 | 632.001     |       |       |
| Total                                    | 28537.385      | 49 |             |       |       |
| Degradation rate of red batik dye waste  |                |    |             |       |       |
| Between Groups                           | 820.905        | 9  | 91.212      | 0.294 | 0.972 |
| Within Groups                            | 12389.037      | 40 | 309.726     |       |       |
| Total                                    | 13209.943      | 49 |             |       |       |

*Micrococcus* sp.) exhibited remarkable efficiency, achieving a 90.29% decolorization of indigosol blue dye.

This study supports SDG 6 (Clean Water and Sanitation) by providing an eco-friendly bioremediation solution that effectively reduces the toxicity of batik dye wastewater, thereby helping to preserve water resources. Additionally, the study contributes to SDG 12 (Responsible Consumption and Production) by promoting a sustainable approach using local bacteria for industrial waste treatment. This solution is not only cost-effective but also encourages more responsible production practices in creative industries such as batik.

There were no significant differences in the ability of bacterial isolates or consortia to degrade blue and red dyes, or between natural dyes (*Indigofera*, *Caesalpinia*) and synthetic dyes (methyl, naphthol, remazol, indigosol). This demonstrates that the bacteria exhibited consistent efficiency across various types and colors of dyes.

These findings underscore the robust enzymatic frameworks of these bacteria, enabling them to degrade multiple dye compounds under diverse environmental conditions. This study concludes that these bacterial isolates and consortia hold substantial potential for practical implementation in the bioremediation of batik dye waste, providing a cost-effective and environmentally friendly solution to tackle the challenges associated with batik dye waste.

Further research can focus on testing bacterial consortia on complex industrial effluents, such as those from textiles or manufacturing, to assess their effectiveness under real-world conditions. This can include optimizing factors like pH, temperature, and salt concentration to enhance performance. Additionally, developing bacteria with specific capabilities through microbiological engineering could help degrade more complex toxic compounds. Evaluating the environmental impact and safety of degradation by-products is also crucial to ensure the method is safe and sustainable. This approach offers a practical solution for environmentally friendly waste management.

## ACKNOWLEDGMENTS

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