

COMPARATIVE BIODECOLORIZATION AND DETOXIFICATION OF SYNTHETIC DYES BY INDIGENOUS BATIK WASTEWATER BACTERIA

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Abstract

The Malaysian batik industry, although economically important, generates dye-laden wastewater that poses significant environmental risks due to the lack of sufficient treatment facilities. Conventional remediation methods are often costly, produce secondary pollutants, or require advanced infrastructure, making them impractical for small-scale industries. Utilizing native bacterial strains offers a promising, eco-friendly alternative, however, this approach remains underexplored for batik wastewater. This study evaluated the decolorization potential of two indigenous isolates, KR1 and KR2, obtained from batik wastewater, against four commonly used dyes: Congo Red (CR), Crystal Violet (CV), Disperse Yellow (DY), and Methylene Blue (MB). Experiments were performed under static and shaking conditions at 37°C, unadjusted pH, 250 mg/L dye concentration, and 24 h incubation, with static conditions consistently outperforming shaking. Strain KR1 exhibited superior performance, removing 49% of CR, 90% of CV, 50% of DY, 51% of MB and 71% batik wastewater, whereas strain KR2 achieved only 23%, 35%, 20%, 23% and 23%, respectively. Ultraviolet-visible analysis confirmed CV degradation by strain KR1, indicated by the disappearance of peaks at 590, 330, and 260 nm and the emergence of new peaks at 220 and 360 nm, suggesting simpler metabolites. Phytotoxicity tests using *Vigna radiata* showed 100% germination and healthy seedling growth with strain KR1-treated CV, while untreated CV almost completely inhibited germination. These findings position strain KR1 as an efficient and sustainable bioremediation agent for batik wastewater, contributing to global sustainability goals through improved water quality management and resource recovery for agricultural reuse.

Keywords: Batik wastewater; Biodecolorization; Detoxification; Indigenous bacterial strains; Phytotoxicity study

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Introduction

Malaysia's batik industry is both culturally significant and economically valuable, contributing approximately RM158.3 million through job creation, tourism, and national revenue (Daud *et al.*, 2022). However, the sector's rapid expansion, coupled with its predominantly small- to medium-scale operations, has led to pressing environmental concerns, most notably the improper disposal of untreated or partially treated wastewater. Due to limited access to centralized treatment facilities, many batik producers discharge effluents directly into nearby water bodies (Syed *et al.*, 2021).

The environmental consequences of such practices are extensive. Visible discoloration of water bodies marks only the initial sign of pollution. Synthetic dyes in the effluent reduce light penetration, disrupting photosynthesis, and negatively impacting aquatic food webs and biodiversity. In addition, several dyes and associated chemicals are known to be mutagenic or carcinogenic, posing long-term health risks to humans and animals through bioaccumulation (Al-Tohamy *et al.*, 2022). Contaminated water can also impair drinking water supplies, irrigation systems, and fisheries, thereby threatening public health and food security.

Given these risks, dye removal from batik wastewater is a critical component of effective treatment strategies (Zakaria *et al.*, 2023). Dyes not only degrade water quality but also act as indicators of broader chemical contamination. Their complex aromatic structures, chemical stability, and resistance to biodegradation make them particularly difficult to eliminate using conventional treatment approaches. Several physical and chemical methods have been employed for dye removal, including coagulation-flocculation, membrane filtration, activated carbon adsorption, ion exchange, chemical precipitation, and advanced oxidation processes (AOPs). Although these methods can be effective, they have notable limitations (Setianingsih *et al.*, 2024). Physical techniques, such as membrane filtration and adsorption, are cost-prohibitive for small-scale industries and require ongoing maintenance. Chemical treatments often necessitate the use of additional reagents, leading to secondary pollution and sludge generation that require further handling. Moreover, these methods are typically non-specific to dyes and are less effective when applied to complex effluent compositions such as those found in batik wastewater.

Biological treatment, on the other hand, offers a sustainable and cost-effective alternative. Among

biological approaches, bacteria have shown particular promise due to their rapid growth, metabolic versatility, and ability to adapt to diverse environmental conditions. Specific bacterial strains are capable of enzymatically degrading or transforming dye molecules into less harmful compounds using enzymatic systems such as azoreductases, laccases, and peroxidases, which target complex dye structures under both aerobic and anaerobic conditions (Khan, 2025). Numerous studies have reported the success of individual bacterial strains and bacterial consortia in treating textile wastewater. Genera such as *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Rhodococcus* and *Alcaligenes* have demonstrated effective decolorization and detoxification of azo dyes at both laboratory and pilot scales (Bhayana *et al.*, 2023). These microbial treatments produce minimal secondary waste and can be integrated into existing wastewater management systems, offering economic viability and environmental sustainability.

Despite these advances, a significant research gap remains. Most existing studies rely on laboratory strains or bacteria isolated from non-batik industrial effluents, which may not be optimally adapted to the unique chemical composition of batik wastewater. It is likely that native bacterial populations thriving in batik wastewater possess specialized metabolic capabilities tailored to degrade the specific pollutants present in this effluent. However, these indigenous microbial communities remain largely unexplored. To address this gap, the present study aims to: (a) isolate and characterize indigenous bacterial strains from batik wastewater and evaluate their decolorization efficiency against four commonly used textile dyes namely Congo Red (CR), Crystal Violet (CV), Disperse Yellow (DY), and Methylene Blue (MB), as well as actual batik wastewater; (b) analyze the most efficiently decolorized dye using ultraviolet-visible (UV-Vis) spectroscopy to elucidate potential biodegradation metabolites; and (c) conduct a phytotoxicity assay to assess whether the treated dye solutions are detoxified sufficiently for potential reuse in agricultural irrigation. Together, these objectives support the United Nations Sustainable Development Goals, particularly SDG 6 (Clean Water and Sanitation), by contributing to the development of eco-friendly and locally adapted bioremediation strategies. By harnessing the potential of native bacteria, this research not only addresses an urgent environmental issue but also

enhances the resilience and sustainability of Malaysia's batik industry.

Materials and Methods

Chemicals

All chemicals used in this study were of analytical grade and readily available in the commercial market. They were supplied by reputable vendors, including Fisher Scientific (Singapore), Millipore (India), and HiMedia (India), and were used without further purification. All experimental procedures were carried out in a sterile environment following standard aseptic techniques.

Collection and analysis of sample

The sampling was conducted at Nur BB Batik Kelantan, Malaysia, which is an active batik manufacturing facility. Wastewater was collected from Stage 6 of the finishing and disposal process, the stage known to generate the highest pollutant load. Prior to storage in sterile high-density polyethylene (HDPE) bottles, the temperature of the batik wastewater was recorded at 37°C. This higher temperature is characteristic of the Stage 6 finishing/disposal area, where hot dyeing and steaming effluent is discharged before cooling downstream. Color analysis was conducted using the Biospectrophotometer Biomate 3 (Thermo Scientific, USA), while pH measurements were obtained using the Eutech Instruments pH 510 meter (Eutech Instruments Pte. Ltd., Singapore). The initial measurements indicated the alkaline nature of the wastewater with a pH of 10.5. Additionally, the wastewater exhibited a deep red coloration, with maximum absorbance observed at a wavelength of 600 nm. Following conventional procedures, the samples were kept in a refrigerator (CHILL-1050, Malaysia) at 4°C to avoid any physical-chemical changes in the batik wastewater.

Isolation of potential dye-decolorizing bacterial strains

A spread plate method using serial dilution was used to isolate bacterial strains. In particular, 9.9 mL of distilled water was used to serially dilute 0.1 mL of the batik wastewater until the concentration was 10^{-6} . From the diluted samples, 0.1 mL was spread onto nutrient agar prepared by dissolving 10 g of nutrient agar powder and 2 g of fine agar-agar powder in 500 mL of distilled water. After sterilization, the medium was poured into sterile Petri dishes at approximately 25-35 mL per plate and allowed to solidify. In the isolation step, pure nutrient agar was used without the addition of

any dye. This non-selective medium allowed recovery of indigenous heterotrophic bacteria from the batik wastewater prior to evaluating their dye-decolorizing abilities in subsequent experiments. The plates were then incubated at 37°C (MEMMERT 108L Incubator INB500, Germany) for 48 h. The incubation temperature of 37°C was selected because the batik wastewater sample measured 37°C at the time of collection. To preserve native physiological conditions and recover thermotolerant indigenous bacteria adapted to this environment, both isolation and subsequent regrowth were conducted at this temperature. Following incubation, individual colonies were repeatedly streaked on fresh nutrient agar to obtain pure bacterial cultures.

Characterization of potential dye-decolorizing bacterial strains

After reculturing from the initial nutrient agar plates, the pure isolates were re-streaked onto freshly prepared nutrient agar and incubated at 37°C for 48 h. Morphological characteristics such as colony shape, elevation, color, surface texture, and size were recorded (Bergey, 1994). The diameter of each colony was measured directly on the agar surface using a ruler. Microscopic features, including Gram staining and cell morphology, were also observed (Gunalan *et al.*, 2025). Biochemical characterization was conducted following the standard procedures described by Gunalan *et al.* (2025), including oxidase, indole, catalase, urease, and citrate tests, with reference to Bergey's Manual for bacterial identification.

Preparation of seed culture (inoculum)

Although six distinct colonies were initially isolated, only strains KR1 and KR2 were selected for further study because they were the most dominant and consistently occurring isolates and exhibited stable growth across repeated subcultures. The remaining four isolates showed poor viability and slow, inconsistent growth, making them unsuitable for standardized decolorization experiments. Individual colonies of strains KR1 and KR2 were carefully picked from freshly prepared nutrient agar plates using sterile inoculating loops. For inoculation, 50 mL of sterile nutrient broth (8 g/L) was dispensed into individual 100 mL Erlenmeyer flasks, and each strain was added separately. The flasks were incubated at 37°C in a shaking incubator set at 160 rpm (Lab Companion SI 600R, Korea). Strain KR1 was incubated for 15 h and strain KR2 for 27 h, to ensure both cultures reached the mid-exponential growth phase, indicating active bacterial proliferation.

Decolorization of selected dyes by strains KR1 and KR2

To assess the decolorization potential of strains KR1 and KR2, synthetic dyes namely Congo Red (CR), Crystal Violet (CV), Disperse Yellow (DY), and Methylene Blue (MB) were each prepared at a final concentration of 250 mg/L in a modified Minimal Salt Medium (10X M9). The medium consisted of 0.5 g/L KH_2PO_4 , 0.50 g/L K_2HPO_4 , and 0.05 g/L NaCl, and was sterilized prior to use. Fresh cultures of KR1 and KR2 in the mid-exponential phase were used to inoculate the dye-containing media at 10% (v/v). The inoculated samples were incubated at 37°C for 24 h under both shaking (160 rpm) and static (0 rpm) conditions in separate sets to compare performance under different aeration levels and the pH was left unadjusted.

Following incubation, a 1.5 mL aliquot was collected from each experimental setup and transferred into sterile Eppendorf tubes. The samples were centrifuged at $11\,000 \times g$ for 15 min at 4°C (Tomy MX-305 High-Speed Refrigerated Microcentrifuge, Japan) to remove bacterial cells and particulates. The supernatant was carefully collected and the absorbance of the clarified samples was measured across the 200 to 700 nm range using an ultraviolet-visible spectrophotometer. The specific maximum absorbance wavelengths (λ_{max}) used for quantitative analysis of each dye were as follows: CR: 498 nm, CV: 590 nm, DY: 357 nm, and MB: 668 nm.

A control experiment was conducted for each dye under identical conditions but without bacterial inoculation, to account for abiotic dye degradation or adsorption. The extent of decolorization was calculated by comparing the absorbance values of the experimental and control samples at the respective λ_{max} for each dye following Equation 1 below:

Decolorization activity (%) was calculated

$$\text{as } \frac{[A_{\text{initial}} - A_{\text{observed}}]}{A_{\text{initial}}} \times 100 \quad (1)$$

A_{initial} = initial absorbance at λ_{max}

A_{observed} = observed absorbance λ_{max}

Evaluation of Crystal Violet toxicity using seed germination assay

A phytotoxicity study was conducted to evaluate the suitability of decolorized dye solutions for irrigation. Uniform seeds of *Vigna radiata* were surface-sterilized with 0.1% (v/v) hydrogen peroxide, rinsed with sterile distilled water and

placed in sterile Petri dishes lined with filter paper. Treatments included distilled water (control), untreated dye solutions (250 mg/L) and dye solutions decolorized using strain KR1 (250 mg/L). Each dish, containing 40 seeds, received 5 mL of the respective solution and was incubated at 27°C for 7 d. Treatments were performed in triplicate and filter papers were kept moist with the corresponding solutions. After seven days, the seedlings were carefully removed to measure the germination rate (%) and the lengths of roots and shoots using a digital caliper. The total seedling length was calculated by summing the root and shoot lengths.

Statistical analysis

All experiments were performed in triplicate and results were expressed as mean $\pm$ standard deviation. Statistical comparisons were conducted using one-way ANOVA followed by appropriate post-hoc tests namely Duncan analysis to assess significant differences between treatments. A p-value of less than 0.05 was considered statistically significant.

Table 1. Colony morphology and biochemical characterization of strains KR1 and KR2

Morphological Characteristic	Strain	
	KR1	KR2
Size	2-5 mm	2-3 mm
Shape	Circular	Irregular
Color	White	Pale yellow
Margin	Even	Uneven
Elevation	Convex	Low convex
Opaque	Opaque	Opaque
Surface	Shiny	Moist and smooth
Biochemical Characteristic	Strain	
	KR1	KR2
Citrate	+	-
Urease	+	-
Oxidase	-	-
Catalase	+	-
Gram	+	-

Results and Discussion

Characterization of potential dye-decolorizing bacterial strains

Two indigenous bacterial strains were successfully isolated from batik wastewater and characterized based on their colony morphology on nutrient agar, as illustrated in Figure 1. The first strain, designated as KR1 (Figure 1a), formed white, circular colonies ranging in size from 2 to 5 mm as presented in Table 1. These colonies exhibited a smooth and shiny surface, even margins, and a convex elevation. The consistent appearance and structure of strain KR1 colonies suggest a well-

adapted and stable strain, possibly indicating the dominance of a single bacterial population.

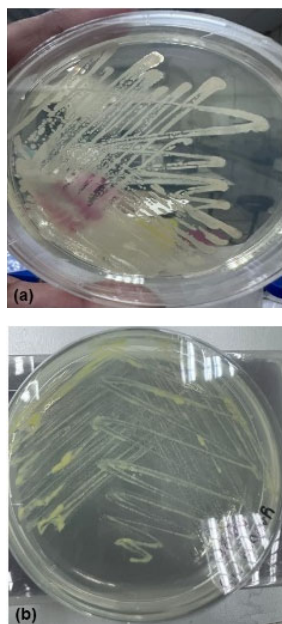


Figure 1. Pure isolates KR1 and KR2 obtained after repeated subculturing. Images represent purified single-colony growth used for morphological characterization: (a) strain KR1 (b) strain KR2

The second strain, referred to as KR2, displayed distinctly different characteristics. It formed pale yellow colonies measuring between 2 and 3 mm in diameter (Figure 1b). These colonies were irregular in shape, with uneven margins, and a low convex elevation. The surface appeared moist and smooth, in contrast to the shinier surface of strain KR1. The yellow pigmentation may suggest the production of compounds associated with stress response or environmental adaptation. Both strains appeared opaque, indicating a dense cellular structure within the colonies. The observed differences in size, shape, color, and surface texture highlight the morphological diversity among bacterial strains isolated from batik wastewater.

Standard biochemical assays were conducted to evaluate the metabolic characteristics of both strains and to aid in their preliminary identification. Strain KR1 tested positive for citrate utilization, urease activity, and catalase production but negative for oxidase as tabulated in Table 1. Gram staining revealed that strain KR1 was Gram-positive, which is consistent with the presence of a thick peptidoglycan cell wall. The ability to utilize citrate as a sole carbon source and produce urease and

catalase suggests that strain KR1 is a metabolically versatile organism, capable of withstanding nutrient limitation and oxidative stress. Based on this biochemical profile and Gram staining result, the strain is likely to belong to the genus *Bacillus*. This genus is well known for its environmental resilience and its role in biodegradation processes.

In contrast, strain KR2 tested negative for all biochemical assays, including citrate, urease, oxidase, and catalase. It was identified as Gram-negative, indicating the presence of a thinner peptidoglycan layer and an outer membrane. The absence of enzymatic activity in these standard tests suggests that strain KR2 may be a more specialized or slower-growing organism, as indicated by its extended mid-exponential phase (27 h) with limited metabolic versatility. Based on its Gram-negative reaction, lack of oxidase and catalase activity, and yellow pigmentation, the strain could tentatively be classified as a species member of the genus *Flavobacterium* in accordance with the traits described in Bergey's Manual. This genus is commonly found in aquatic environments, including wastewater, and is known for its yellow colony pigmentation, which aligns with the observed traits.

However, strain KR2 lacked the fruity or sweet odor that is typically associated with *Flavobacterium*, casting some uncertainty on this classification. Other potential candidates based on its phenotypic characteristics include *Chryseobacterium*, *Sphingobacterium*, and *Myroides*. Therefore, due to the inherent limitations of phenotypic characteristics, 16S rRNA gene sequencing will be performed in future work to confirm the taxonomic identity of these bacterial strains.

Decolorization of selected dyes by strains KR 1 and KR 2

Congo Red (CR), Crystal Violet (CV), Disperse Yellow (DY), and Methylene Blue (MB) were selected for decolorization analysis due to their widespread application in the textile industry, particularly in batik production and synthetic fabric dyeing. These respective dyes represent different chemical classes, namely azo, triphenylmethane, disperse, and thiazine, each with distinct structural complexity and stability in aqueous environments (Nachiyar *et al.*, 2023). Their persistent nature, vivid coloration, and resistance to degradation make them suitable models for evaluating the effectiveness of biological treatment systems using indigenous strains KR1 and KR2. Furthermore, these dyes are known for their potential toxicity and environmental impact when discharged without treatment, highlighting the importance of assessing

microbial decolorization as a means of reducing pollutant levels in textile effluents (Slama *et al.*, 2021).

Strains KR1 and KR2 were cultivated in modified minimal salt medium at 37°C, a temperature selected to reflect the environmental conditions of the batik wastewater site from which the isolates were originally sourced. In control treatments lacking bacterial inoculation, the concentrations of CR, CV, MB, DY, and batik wastewater remained largely unchanged over the experimental period, indicating minimal dye loss due to abiotic degradation. Additionally, heat-killed cultures exhibited only a slight reduction in dye concentration, with removal efficiencies limited to approximately 2 to 4%. This minimal decline suggests that passive processes such as biosorption played a negligible role in dye removal. Instead, the observed decolorization is attributed primarily to the active metabolic functions of the bacterial strains. Measurements taken after 24 h of incubation revealed that extending the incubation period to 48 h did not yield significant additional decolorization for any of the dyes under either static or shaking conditions, supporting the use of a 24-h time point for comparative analysis.

The decolorization of CR, CV, MB, and DY by strains KR1 and KR2 was notably more effective under static conditions compared to shaking cultures. In static cultures, the limited aeration likely promotes a microaerophilic or low-oxygen environment, which may enhance the expression or activity of reductive enzymes such as azo reductases, peroxidases, and laccases. These enzymes are known to catalyze the breakdown of complex dye structures, particularly those containing azo and aromatic groups, by facilitating electron transfer under reduced oxygen tension (Srivastava *et al.*, 2022). In the case of azo dyes,

decolorization often begins with the cleavage of the azo bond by the enzymatic action of azo reductases, a process that is generally favored in anaerobic or low-oxygen conditions (Srivastava *et al.*, 2022). Findings from the present study are supported by previous reports in which tropical strains of *Rhodococcus* efficiently decolorized MB and CR under static conditions (Maniyam and Hari, 2021; Sundarajoo *et al.*, 2022). In contrast, shaking conditions increase oxygen availability, which may shift bacterial metabolism toward aerobic respiration and reduce the expression or activity of these enzymes.

The decolorization performance of strains KR1 and KR2 against CR was assessed under static and shaking conditions after 24 h of incubation (Table 2). Strain KR1 showed a significantly higher decolorization rate under both conditions compared to strain KR2 ($p < 0.05$). Under static conditions, strain KR1 achieved $49 \pm 0\%$ decolorization, while strain KR2 reached only $23 \pm 0\%$. In shaking conditions, a similar trend was observed, with strain KR1 decolorizing $23 \pm 2\%$ and strain KR2 $3 \pm 1\%$ of the dye. These results indicated that strain KR1 possesses a stronger capacity to degrade CR, possibly due to more active metabolic pathways or greater enzyme expression involved in dye breakdown.

Similar findings were reported by Tripathi *et al.* (2024), who observed that *Pseudomonas aeruginosa* MT-2 achieved 98% decolorization of CR under static conditions with an initial dye concentration of 50 mg/L at pH 7 and 35°C over 48 h, compared to only 74% under shaking conditions. Interestingly, the current study demonstrated superior performance, achieving substantial CR decolorization at a much higher dye concentration (250 mg/L compared to 50 mg/L) in a shorter duration. This represents a fivefold

Table 2. Effect of static and shaking conditions on the removal of Congo Red, Crystal Violet, Disperse Yellow, Methylene Blue, and batik wastewater by strains KR1 and KR2

Dyes	Condition	Decolorization efficiency of strain KR1 (%)	Decolorization efficiency of strain KR2 (%)
Congo red (498 nm)	Static	$49^a \pm 0$	$23^b \pm 2$
	Shaking	$23^b \pm 0$	$3^c \pm 1$
Crystal violet (590 nm)	Static	$90^a \pm 1$	$35^b \pm 2$
	Shaking	$37^b \pm 2$	$21^c \pm 2$
Disperse yellow (357 nm)	Static	$50^a \pm 3$	$20^b \pm 2$
	Shaking	$12^c \pm 2$	$3^d \pm 0$
Methylene blue (668 nm)	Static	$51^a \pm 0$	$23^c \pm 1$
	Shaking	$35^b \pm 0$	$12^d \pm 2$
Batik wastewater (600 nm)	Static	$71^a \pm 0$	$23^b \pm 1$
	Shaking	$25^b \pm 0$	$12^c \pm 2$

*The control group did not receive any bacterial inoculum. The initial pH was maintained without adjustment and each dye namely Congo Red, Crystal Violet, Disperse Yellow, and Methylene Blue was applied at a concentration of 250 mg/L. Samples were incubated for 24 h at 37°C. Dye removal percentages are presented as the mean values of three replicates along with their corresponding standard errors. Statistically significant differences were observed between the experimental groups ($p < 0.05$).

increase in dye load and a 100% reduction in treatment time, highlighting the efficiency of strain KR1. When comparing static conditions to shaking environments, three strains of *Bacillus* demonstrated higher efficiency in decolorizing CR, achieving decolorization rates between 50% and 60%, whereas shaking conditions resulted in lower performance, with decolorization levels remaining below 45% (Elmelegy *et al.*, 2024). In contrast to static incubation, the *Shewanella xiamenensis* G5-03 strain exhibited enhanced growth under agitated conditions. However, static incubation resulted in significantly higher CR decolorization, achieving 90.2% within 10 h, whereas agitation markedly reduced decolorization efficiency to just 14.1% ($p < 0.00001$), confirming that elevated oxygen levels during agitation had an inhibitory effect on the decolorization process (Silva *et al.*, 2021).

When strains KR1 and KR2 were evaluated for CV removal, a similar pattern was found, showing notable gains in decolorization efficiency under static settings. After 24 h, strain KR1 achieved the highest decolorization under static conditions at $90 \pm 1\%$, while strain KR2 recorded $35 \pm 2\%$. Under shaking conditions, however, the efficiency declined, with strains KR2 and KR1 showing $21 \pm 2\%$ and $37 \pm 2\%$ decolorization, respectively. This trend is consistent with the CR results, where both strains also showed reduced performance under shaking conditions.

Comparable results were reported by Upadhyay *et al.* (2023), who achieved nearly complete decolorization of 300 mg/L CV using immobilized *Trametes versicolor* in sponge and dishwasher systems, although 96 h were required to reach this level of efficiency. Immobilization enhanced cell stability, sustained enzymatic activity, and improved tolerance to high dye concentrations, contributing to the effective removal of the dye. In contrast, strain KR1 achieved rapid decolorization of 250 mg/L within 24 h without the need for immobilization, indicating strong intrinsic enzymatic activity. Owing to its efficiency, robustness, and ability to function effectively under static conditions without additional support systems, strain KR1 demonstrates promising potential for scale-up in wastewater treatment applications, where reliability and reduced processing time are critical. A previous study using *Aspergillus flavus* demonstrated a similar trend, as reported by Ghanaim *et al.* (2025), where 94% of 50 mg/L CV was decolorized under static conditions within 8 d following optimization of experimental parameters. In contrast, under shaking conditions at 120 rpm, the decolorization rate significantly declined to 72% over the same period.

After 24 h of incubation at 37°C, decolorization of DY under static conditions reached $50 \pm 3\%$, while only $12 \pm 2\%$ was observed under shaking conditions when strain KR1 was utilized as the biological agent. This significant difference indicates that static incubation substantially enhanced decolorization efficiency, resulting in a 317% improvement compared to shaking conditions ($p < 0.05$). A similar pattern was observed with strain KR2, although the overall decolorization was markedly lower, recording only $20 \pm 2\%$ under static conditions and $3 \pm 0\%$ under shaking conditions. Significant differences were observed in the decolorization of 50 mg/L Yellow EXF dye by *Proteus mirabilis* under static and shaking conditions which is identical to the current findings. After 24 h, nearly 80% decolorization was achieved under static conditions, while only 2% was observed under shaking. This supports the conclusion that agitation adversely affects dye removal efficiency (Madhushika *et al.*, 2022).

In addition, the present findings demonstrated superior performance, achieving 50% enhanced decolorization of 250 mg/L DY within 24 h, compared to the decolorization of a lower concentration of DY (200 mg/L) reported for *Trametes versicolor*, which required an extended incubation period of 7 d (Fayyaz *et al.*, 2025).

During a 24-h incubation at 160 rpm, MB decolorization under shaking conditions reached $23 \pm 1\%$ for strain KR1 and $12 \pm 1\%$ for strain KR2. In contrast, under static conditions, significantly higher decolorization was observed, with strain KR1 achieving $51 \pm 0\%$ and strain KR2 reaching $35 \pm 0\%$ ($p < 0.05$), as shown in Table 2. Similarly, Ariffin and Anuar (2022) reported that *Pseudomonas aeruginosa* strain UMTFA2 (EP) and *Klebsiella pneumoniae* strain UMTFA1 (EK) achieved 10.52% and 11.65% MB decolorization under static conditions over an 8-day period. In contrast, strain KR1 achieved 338% greater decolorization in an incubation time that was 700% shorter under comparable dye concentrations. Additionally, Maniyam and Hari (2021) highlighted the influence of agitation, reporting 20% MB decolorization under shaking conditions after 24 h, compared to 86% under static conditions. Static conditions enabled strain KR1 to achieve the highest decolorization of batik wastewater at $71 \pm 0\%$, significantly outperforming strain KR2, whose maximum removal reached only $23 \pm 1\%$. These findings demonstrate that strain KR1 possesses superior metabolic versatility and is well-adapted to the complex chemical composition of batik wastewater, making it the most promising candidate for biological wastewater treatment applications. All things considered, strain KR1 demonstrated

significantly superior decolorization efficiency for CR, CV, DY, MB and batik wastewater, with the highest removal observed for CV. These findings suggest that strain KR1 is a promising candidate for further optimization in CV decolorization studies under static conditions.

Profiling of metabolic intermediates using Ultraviolet-visible (UV-Vis) analysis

The ultraviolet-visible (UV-Vis) spectrum of CV was analyzed between 200 and 700 nm for both untreated control samples and those treated with strain KR1 under static and shaking conditions. Initially, the control sample exhibited prominent absorption peaks at 590, 330, and 260 nm, which are associated with the dye's aromatic structure and conjugated double bonds (Maniyam *et al.*, 2022). After 24 h of incubation under static conditions, the peak at 590 nm was completely eliminated, with no observable shift in its wavelength (Figure 2). This decline suggests that the chromophoric structure responsible for the dye's color, particularly the extended conjugated system and electron-donating groups was disrupted, leading to decolorization. Similar findings were reported by Ayed *et al.* (2009), who observed that the intensity of the absorption peaks at 592 nm and 330 nm decreased during CV decolorization by *Bacillus* sp. No spectral changes were seen in the control sample after the same period, confirming that the transformations resulted from microbial action.

Under static conditions, a shift of the 260 nm peak to 265 nm and changes in intensity at 330 nm were recorded, indicating degradation of the aromatic and naphthalene ring structures (Cao *et al.*,

2019). These spectral alterations also imply the formation of simpler intermediate compounds, possibly containing isolated aromatic units, as part of the dye degradation pathway (Ali *et al.*, 2016). These spectral changes indicate the breakdown of the dye's core molecular structure, particularly the triphenylmethane ring, which is primarily responsible for its intense coloration. (Cao *et al.*, 2019). In addition, the emergence of additional peak at 220 nm indicating the presence of single benzene derivatives (Cao *et al.*, 2019) and 360 nm suggesting the possible formation of new metabolites. In contrast, only minimal spectral changes were observed for the characteristic peaks at 590, 330, and 260 nm when CV decolorization was performed under shaking conditions at 160 rpm for 24 h, indicating a low decolorization efficiency.

A comparison of the microbial cell color before and after CV decolorization revealed no visible color change in strain KR1. In addition, Srinivasan and Sadasivam (2021) reported that bacteria decolorize dyes either by biodegradation or surface adsorption. Biodegradation is marked by the disappearance or shift of the main absorbance peak, while adsorption results in a uniform decrease in UV-Vis peaks without new peak formation. Therefore, these findings demonstrated that strain KR1 was capable of removing CV through biodecolorization and biodegradation. Further investigations will focus on using advanced analytical techniques namely Fourier-transform infrared spectroscopy and mass spectroscopy to identify and predict the breakdown products and specific pathways involved in the dye biodegradation process.

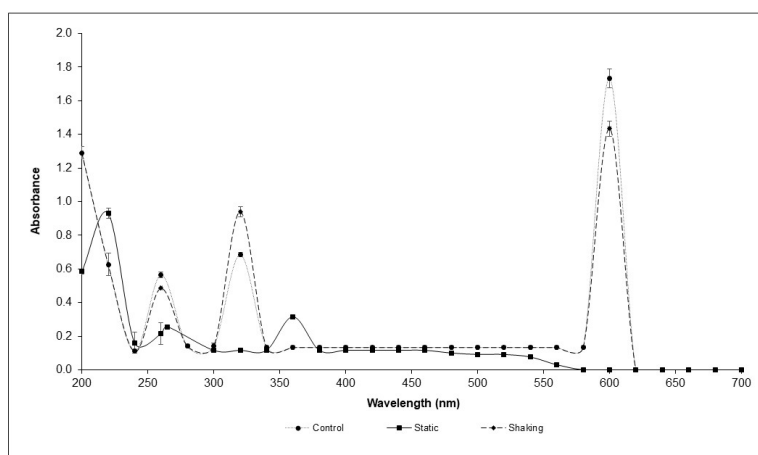


Figure 2. Ultraviolet-Visible (UV-Vis) spectral profiles of Crystal Violet during decolorization by strain KR1 under static and shaking conditions. Incubation was conducted at 37°C for 24 h with 250 mg/L of Crystal Violet and the pH was not adjusted. Error bars indicate the standard error of three replicates

Evaluation of Crystal Violet toxicity using seed germination assay

Table 3 presents the findings on the toxicity of CV and its metabolites generated during decolorization by strain KR1 under static conditions. CV is known to exhibit phytotoxic, cytotoxic, and genotoxic effects, inhibiting seed germination and seedling growth (Mandal *et al.*, 2024). The results of the phytotoxicity assessment indicated that untreated CV significantly inhibited the germination of *Vigna radiata* seeds, reducing the germination rate by 96%, as shown in Table 3. In contrast, treatment with strain KR1 effectively mitigated this toxic effect, with seed germination fully preserved and reaching 100%, comparable to the control group.

Table 3. Germination and growth response of *Vigna radiata* to Crystal Violet before and after treatment by strain KR1

Parameter	Distilled Water (Control)	250 mg/L Crystal Violet (untreated)	250 mg/L Crystal Violet metabolites (treated)
Germination (%)	100 ± 0	4 ± 1	100 ± 0
Shoot Length (cm)	8 ± 1	1 ± 0	8 ± 0
Root Length (cm)	7 ± 0	1 ± 0	8 ± 0

* Error bars depict the average ± standard error

Additionally, seedlings grown in the solution containing metabolites from the treated CV sample were approximately eight times longer than those exposed to the untreated CV solution, indicating significantly improved growth and reduced phytotoxicity ($p < 0.05$). Tian *et al.* (2024) supported these findings by demonstrating that co-cultivation of *Aspergillus fumigatus* and *Pseudomonas fluorescens* effectively decolorized CV, producing degradation products that exhibited reduced toxicity toward *Vigna radiata* and *Triticum aestivum*. This was evidenced by improved germination rates in comparison to seeds exposed to untreated CV. These findings indicate that the toxicity of CV was substantially reduced following treatment with strain KR1, suggesting that the biological process not only removed the dye but also detoxified harmful components. This outcome highlights the potential for the treated CV solution to be safely repurposed for agricultural applications, promoting more sustainable and eco-friendly water reuse practices.

Conclusions

This study demonstrated that the indigenous bacterial isolates KR1 and KR2 obtained from batik wastewater are capable of decolorizing four commonly used synthetic dyes namely CR, CV, DY, and MB, as well as actual batik wastewater, with static conditions providing the highest removal efficiency. Strain KR1 showed superior performance, particularly in the decolorization of CV, which was supported by UV-Vis spectral changes indicating dye transformation. Phytotoxicity assessment further confirmed that the treated dye solution exhibited markedly reduced toxicity, achieving full seed germination compared to the untreated control. These findings highlight the potential application of native bacterial strains for environmentally friendly batik wastewater treatment and support future development of sustainable, locally adapted bioremediation strategies. Further studies involving molecular identification and pilot-scale validation are recommended.

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Author Contributions

Conceptualization: M.N.M.; Methodology: M.N.M., K.R., N.S.; Software: H.H.A., H.A., N.S.Y.; Validation: M.N.M.; Formal Analysis: M.N.M., K.R., N.S., H.H.A., H.A., N.S.Y.; Investigation: M.N.M., K.R., N.S.; Resources: M.N.M.; Data Curation: M.N.M.; Writing – Original Draft: M.N.M.; Writing – Review & Editing: M.N.M.; Visualization: M.N.M.; Supervision: M.N.M.; Project Administration: M.N.M.; Funding Acquisition: M.N.M. All authors have read and agreed to the published version of the manuscript.

Ethics Statement

This study did not require ethical approval because it involved no human or animal participants, personally identifiable information, or any materials subject to institutional ethical review.

Declaration of Generative AI Use

During the preparation of this manuscript, the authors used ChatGPT to enhance the language, clarity, and readability of the text. Following its use, the authors carefully reviewed and revised the content where necessary and assume full responsibility for the final published work.

Conflict of Interest

The authors declare that they have no conflicts of interest related to this work.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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References

- Ali, H. M., Shehata, S. F., & Ramadan, K. M. A. (2016). Microbial decolorization and degradation of crystal violet dye by *Aspergillus niger*. *International Journal of Environmental Science and Technology*, 13(12), 2917-2926. <https://doi.org/10.1007/s13762-016-1117-x>
- Al-Tohamy, R., Ali, S. S., Li, F., Okasha, K. M., Mahmoud, Y. A. G., Elsamahy, T., & Sun, J. (2022). A critical review on the treatment of dye-containing wastewater: Ecotoxicological and health concerns of textile dyes and possible remediation approaches for environmental safety. *Ecotoxicology and Environmental Safety*, 231, 113160. <https://doi.org/10.1016/j.ecoenv.2021.113160>
- Ariffin, F., & Anuar, N. E. M. C. (2022). Biodegradation of methylene blue by bacteria strains isolated from contaminated soil. *Malaysian Applied Biology*, 51(3), 25-35. <https://doi.org/10.55230/mabjournal.v51i3.2190>
- Ayed, L., Cheriaa, J., Laadhari, N., Cheref, A., & Bakhrouf, A. (2009). Biodegradation of crystal violet by an isolated *Bacillus* sp. *Annals of Microbiology*, 59(2), 267-272. <https://doi.org/10.1007/BF03178327>
- Bergey, D. H. (1994). *Bergey's manual of determinative bacteriology* (9th ed.). Lippincott Williams & Wilkins.
- Bhayana, T., Saxena, A., Gupta, S., & Dubey, A. K. (2023). Enhanced decolourisation and degradation of azo dyes using wild versus mutagenic improved bacterial strain: A review. *Vegetos*, 36(1), 28-37. <https://doi.org/10.1007/s42535-022-00496-y>
- Cao, D. J., Wang, J. J., Zhang, Q., Wen, Y. Z., Dong, B., Liu, R. J., & Geng, G. (2019). Biodegradation of triphenylmethane dye crystal violet by *Cedecea davisae*. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 210, 9-13. <https://doi.org/10.1016/j.saa.2018.11.004>
- Daud, N. M., Abdullah, S. R. S., Hasan, H. A., Ismail, N. I., & Dhokhikah, Y. (2022). Integrated physical-biological treatment system for batik industry wastewater: A review on process selection. *Science of the Total Environment*, 819, 152931. <https://doi.org/10.1016/j.scitotenv.2022.152931>
- Elmelegy, S. A., Khalil, M. S., & Saleh, M. (2024). Efficient biodegradation and detoxification of Congo red via newly isolated *Bacillus* strains. *Egyptian Journal of Chemistry*, 67(1), 67-76. <https://doi.org/10.21608/ejchem.2023.199509.7719>
- Fayyaz, I., Saddick, S., Mahmood, R. T., Asad, M. J., Hussain, M. A., Hu, J., & Saydaxmetova, S. (2025). Biodegradation of azo and disperse dyes by *Trametes versicolor*: Process optimization and MnP enzyme dynamics. *Results in Engineering*, 25, 103980. <https://doi.org/10.1016/j.rineng.2025.103980>
- Ghanaim, A. M., Mahdy, O. M. E., & Mohamed, H. I. (2025). Biodegradation of azo dyes by *Aspergillus flavus* and its bioremediation potential using seed germination efficiency. *BMC Microbiology*, 25(1), 7. <https://doi.org/10.1186/s12866-024-03703-9>
- Gunalan, P., Maniyam, M. N., Azman, H. H., Abdullah, H., & Yaacob, N. S. (2025). Decolourization of methylene blue using indigenous microbes. *AIP Conference Proceedings*, 3271(1), 050012. <https://doi.org/10.1063/5.0258593>
- Khan, M. F. (2025). Recent advances in microbial enzyme applications for sustainable textile processing and waste management. *Sci*, 7(2), 46. <https://doi.org/10.3390/sci7020046>
- Madhushika, H. G., Ariyadasa, T. U., & Gunawardena, S. H. P. (2022). Biodegradation of reactive yellow EXF dye: Optimization of physiochemical parameters and analysis of degradation products. *International Journal of Environmental Science and Technology*, 19(3), 1683-1694. <https://doi.org/10.1007/s13762-021-03220-6>
- Mandal, K., Das, D., Bose, S. K., Chaudhuri, A., Chakraborty, A., Mandal, S., & Roy, S. (2024). Spectroscopic approach to optimize the biogenic silver nanoparticles for photocatalytic removal of ternary dye mixture and ecotoxicological impact of treated wastewater. *Scientific Reports*, 14(1), 31174. <https://doi.org/10.1038/s41598-024-82341-7>
- Maniyam, M. N., Azman, H. H., Abdullah, H., & Yaacob, N. S. (2022). Process optimization for efficacious biodecolorization of crystal violet by Malaysian *Rhodococcus pyridinivorans* using monothetic analysis. *Journal of Applied Biology and Biotechnology*, 10(2), 107-113. <https://doi.org/10.7324/JABB.2022.10s212>
- Maniyam, M. N., & Hari, M. (2021). Optimization of culture conditions for improved green biodecolorization of methylene blue by *Rhodococcus pyridinivorans* strain UCC 0003. *BioTechnologia*, 102(2), 125-140. <https://doi.org/10.5114/bta.2021.106519>
- Nachiyar, C. V., Rakshi, A. D., Sandhya, S., Jebasta, N. B. D., & Nellore, J. (2023). Developments in treatment technologies of dye-containing effluent: A review. *Case Studies in Chemical and Environmental Engineering*, 7, 100339. <https://doi.org/10.1016/j.csee.2023.100339>
- Setianingsih, N. I., Budihardjo, M. A., Yuliasni, R., Malik, R. A., & Warsito, B. (2024). Potential application and strategies of aerobic granular sludge (AGS) technology for wastewater treatment in Indonesia: A review.

- Desalination and Water Treatment*, 320, 100756. <https://doi.org/10.1016/j.dwt.2024.100756>
- Silva, E. R., Dall'Oglio, E. L., Vasconcelos, L. G., & Morais, E. B. (2021). Decolorization of the benzidine-based azo dye Congo red by the new strain *Shewanella xiamenensis* G5-03. *Brazilian Journal of Biology*, 82, e237386. <https://doi.org/10.1590/1519-6984.237386>
- Slama, H. B., Chenari Bouket, A., Pourhassan, Z., Alenezi, F. N., Silini, A., Cherif-Silini, H., & Belbahri, L. (2021). Diversity of synthetic dyes from textile industries, discharge impacts and treatment methods. *Applied Sciences*, 11(14), 6255. <https://doi.org/10.3390/app11146255>
- Srinivasan, S., & Sadasivam, S. K. (2021). Biodegradation of textile azo dyes by textile effluent non-adapted and adapted *Aeromonas hydrophila*. *Environmental Research*, 194, 110643. <https://doi.org/10.1016/j.envres.2020.110643>
- Srivastava, A., Dangi, L. K., Kumar, S., & Rani, R. (2022). Microbial decolorization of Reactive Black 5 dye by *Bacillus albus* DD1 isolated from textile water effluent: Kinetic, thermodynamics & decolorization mechanism. *Heliyon*, 8(2), e08834. <https://doi.org/10.1016/j.heliyon.2022.e08834>
- Sundarajoo, A., Maniyam, M. N., Azman, H. H., Abdullah, H., & Yaacob, N. S. (2022). *Rhodococcus* strain UCC 0010 as green biocatalyst for enhanced biodecolourization of Congo red through response surface methodology. *International Journal of Environmental Science and Technology*, 19(4), 3305-3322. <https://doi.org/10.1007/s13762-021-03400-4>
- Syed Shaharuddin, S. I., Shamsuddin, M. S., Drahman, M. H., Hasan, Z., Mohd Asri, N. A., Nordin, A. A., & Shaffiar, N. M. (2021). A review on the Malaysian and Indonesian batik production, challenges, and innovations in the 21st century. *SAGE Open*, 11(3). <https://doi.org/10.1177/21582440211040128>
- Tian, Y., Wu, K., Lin, S., Shi, M., Liu, Y., Su, X., & Islam, R. (2024). Biodegradation and decolorization of crystal violet dye by cocultivation with fungi and bacteria. *ACS Omega*, 9(7), 7668-7678. <https://doi.org/10.1021/acsomega.3c06978>
- Tripathi, M., Shukla, S., Singh, R., Singh, S., Singh, P., Singh, P. K., & Manimekalai, R. (2024). Physicochemical investigations of textile wastewater and process parameter optimization for biodecolorization of Congo red dye by *Pseudomonas aeruginosa* MT-2 strain. *Journal of Pure and Applied Microbiology*, 18(4), 29. <https://doi.org/10.22207/JPAM.18.4.29>
- Upadhyay, R., Khan, H. I. U. H., & Przysaś, W. (2023). An evaluation of decolorization mechanism of synthetic dyes belonging to the azo, anthraquinone, and triphenylmethane group, as a sustainable approach, by immobilized CB8 strain (*Trametes versicolor*). *Desalination and Water Treatment*, 284, 268-277. <https://doi.org/10.5004/dwt.2023.29270>
- Zakaria, N., Rohani, R., Wan Mohtar, W. H. M., Purwadi, R., Sumampouw, G. A., & Indarto, A. (2023). Batik effluent treatment and decolorization—A review. *Water*, 15(7), 1339. <https://doi.org/10.3390/w15071339>