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Biodecolorization and Treatment of Synthetic and Batik Wastewater by Indigenous Bacterial Isolates for Sustainable Bioremediation

Maegala Nallapan Maniyam[1,2,*], Hazeeq Hazwan Azman[3], Hasdianty Abdullah[1,3], Mouloud Kecha[4], Nor Suhaila Yaacob[1,3]

[1] Institute if Bio-IT Selangor, Universiti Selangor, 40000 Shah Alam, Selangor, Malaysia
[2] Faculty of Health Sciences, Universiti Selangor, 40000 Shah Alam, Selangor, Malaysia
[3] Faculty of Engineering and Life Sciences, Universiti Selangor, 45600 Bestari Jaya, Selangor, Malaysia
[4] Faculty of Nature Science and Life, University of Bejaia, 06000 Bejaia, Algeria

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ABSTRACT

The discharge of synthetic dyes from batik industries presents severe environmental risks due to their toxicity and persistence. Typical wastewater treatment processes are often costly and ineffective, necessitating eco-friendly alternatives. Microbial bioremediation offers a promising solution, yet the potential of indigenous bacterial strains remains underexplored. Therefore, this study evaluates the decolorization potential of two indigenous bacterial isolates, strain SK1 and strain SK2, under stationary and agitated conditions using Congo Red (CR) and Malachite Green (MG) as model dyes. Additionally, the strains were assessed for their effectiveness in treating actual batik wastewater and their impact on phytotoxicity. Under static conditions, strain SK1 and strain SK2 recorded growth values of 0.101 ± 0.027 and 0.201 ± 0.003 (optical density at 600 nm), with corresponding CR removal efficiencies of 3^{c} ± 1% and 14^{b} ± 2%, respectively after 3 days. Under shaking conditions, growth increased to 0.318 ± 0.001 for strain SK1 and 0.896 ± 0.018 for strain SK2, improving CR removal to 16^{b} ± 2% and 38^{a} ± 2%. For MG, both strains exhibited similar growth and performance under static conditions, with dye removal efficiencies of 12^{b} ± 2%. Under shaking conditions, growth increased to 0.568 ± 0.031 for strain SK1 and 0.816 ± 0.018 for strain SK2, though dye removal remained low at 21^{a} ± 2%. After 3 days, strain SK2 demonstrated strong bioremediation potential in actual batik wastewater, achieving reductions in biochemical oxygen demand (90%), chemical oxygen demand (94%), total suspended solids (91%) and total dissolved solids (51%), ensuring compliance with regulatory standards. Color removal reached 77% and pH was reduced from 10.5 to 7.7 In contrast, strain SK1 also reduced these parameters but failed to meet regulatory limits. Phytotoxicity tests showed that SK2-treated batik wastewater supported *Vigna radiata* germination with only 4% inhibition and a seedling length of 5.3 ± 2 cm, compared to untreated batik wastewater, which resulted in 71% inhibition and a seedling length of 0.9 ± 0 cm. These findings emphasize the potential of strain SK2 for eco-friendly dye decolorization and wastewater treatment. Future research will prioritize strain identification and process optimization to improve efficiency.

* *Corresponding author.*
E-mail address: maegala@unisel.edu.my

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1. Introduction

The batik industry is an essential part of Malaysia's cultural heritage and a significant contributor to the country's economy. Valued at millions of dollars annually, the industry plays a crucial role in supporting local artisans, small businesses and large-scale textile manufacturers [1]. However, despite its cultural and economic importance, batik production generates large volumes of wastewater that contain hazardous synthetic dyes and other pollutants. Without proper treatment, this wastewater poses serious environmental and health risks, making sustainable treatment solutions a pressing necessity.

Batik wastewater is a complex effluent containing high concentrations of synthetic dyes, waxes and other chemicals used in the dyeing and finishing processes [2]. If released untreated into the environment, these pollutants can significantly deteriorate water quality, endanger aquatic life and pose threats to human health. The improper disposal of batik wastewater into rivers and other water bodies has been linked to oxygen depletion, toxicity to aquatic organisms and bioaccumulation of hazardous substances in the food chain [3]. Due to its non-biodegradable nature, synthetic dye pollution is particularly concerning, as it persists in the environment for long periods and resists conventional degradation mechanisms [4].

While parameters such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolve solids (TDS) and total suspended solids (TSS) indicate the overall organic and particulate load of wastewater, the removal of synthetic dyes is significantly more difficult. Unlike organic pollutants that can be degraded through microbial action, synthetic dyes contain complex aromatic structures that resist natural breakdown [5]. Furthermore, their intense coloration reduces light penetration in aquatic ecosystems, disrupting photosynthetic activity and altering ecological balance [6]. Thus, effective treatment strategies must prioritize the removal of dyes alongside other pollutants to ensure comprehensive wastewater remediation.

Conventional treatment methods for batik wastewater include physical and chemical approaches. Physical approaches including adsorption, filtration and coagulation-flocculation are effective in removing suspended solids but have limited efficiency in degrading complex dye molecules [7-9]. Chemical treatments, including oxidation and precipitation, can break down dyes but often generate secondary pollutants and toxic by-products. These methods also require high operational costs and energy inputs, making them less sustainable for long-term wastewater management. Moreover, chemical treatments may not completely eliminate color and can result in incomplete mineralization of dyes, leading to persistent environmental contamination [10].

Biological treatment methods offer a more sustainable and eco-friendlier alternative for batik wastewater remediation. Algae, yeast and fungi have historically been the main microbial agents for dye wastewater remediation. However, their prolonged growth cycles and relatively low efficiency in dye decolorization have shifted research focus toward bacteria [11]. Bacteria offer several advantages, including shorter growth durations, ease of cultivation and rapid adaptation to wastewater conditions [12]. Additionally, their ability to degrade and mineralize dyes not only enhances decolorization efficiency but also reduces the toxicity and hazardous properties of the final by-products. Their genetic adaptability further increases their potential for optimized bioremediation applications.

Numerous studies have demonstrated the effectiveness of bacterial strains in the bioremediation of textile wastewater, highlighting their potential for efficient dye degradation and pollutant removal. For instance, a bacterial consortium achieved 73% color removal and 60-62% COD reduction under high pH and temperature conditions without the need for an external carbon source [13]. The treated effluent also exhibited a neutral pH, making it more environmentally suitable for discharge.

Another study utilizing the ligninolytic bacterial consortium WGC-D reported a gradual increase in COD removal over an incubation period of 4 days, reaching 89%, with a final COD concentration of 588 mg/L. Additionally, dye decolorization efficiency improved progressively, achieving 84% decolorization after 72 hours of incubation [14]. Furthermore, *Escherichia fergusonii* has been successfully employed in textile effluent remediation, demonstrating significant pollutant reductions, including 98% color removal, 75% reduction in TDS and notable decreases in sulphates (87%), bicarbonates (83%), chlorides (64%), calcium (84%) and COD (81%) [15]. These findings collectively emphasize the potential of bacterial strains in providing a sustainable and efficient substitute for textile wastewater treatment due to their adaptability to high dye concentrations and harsh wastewater conditions.

Despite the growing interest in biological wastewater treatment, research on the use of indigenous bacterial strains isolated directly from batik wastewater remains scarce. Most existing studies have focused on laboratory strains or bacteria sourced from other environments, which may not be optimally adapted to the complex and challenging conditions of batik effluents, particularly the presence of synthetic dyes and high chemical loads. This creates a critical gap in knowledge regarding the potential of native bacteria that have naturally adapted to such environments. To bridge this gap, the present study explores the isolation, characterization and dye decolorization capabilities of indigenous bacterial strains from batik wastewater. By harnessing these native microorganisms, the study aims to enhance treatment efficiency, improve dye removal performance and promote sustainable and cost-effective bioremediation solutions. Furthermore, the findings of this research are expected to contribute to the development of affordable, eco-friendly wastewater treatment systems tailored to local industrial conditions, thereby enriching the existing body of knowledge and supporting global efforts toward sustainable water resource management.

2. Methodology

2.1 Chemicals

The study utilized commercially available, analytical-grade chemicals supplied by Vivantis Technologies Sdn Bhd (Malaysia) and R&M Chemicals (UK). Further purification of these chemicals was not necessary before use.

2.2 Microorganism

The bacterial strains SK1 and SK2 were isolated from wastewater originating from the batik industry in Malaysia, specifically from a manufacturing facility, Jadi Batek, near Kuala Lumpur. These strains were kept at -80°C in the Institute of Bio-IT's Culture Collection Unit at Universiti Selangor in Selangor, Malaysia. For experimental use, a single bead was separated and directly inoculated onto a nutrient agar plate. These strains are undergoing molecular analysis by a different team and will be added to GenBank® (NCBI) upon identification.

2.3 Decolorization Study

The medium used in the experiment was a modified 10X M9 salts solution that contained 5 g/L NaCl, 50 g/L KH_2PO_4, 50 g/L K_2HPO_4 and 0.5 g/L glucose. The broths were cultured at 37°C with constant shaking at 160 rpm (Jeio Tech SI-600R, Korea) at pH 7 after being individually inoculated with a loopful of strains SK1 and SK2. It took 6 hours for strain SK1 and 24 hours for strain SK2 to reach the exponential phase. The exponentially growing cultures were then inoculated (10% v/v) into

a new medium containing 150 mg/L of either Malachite Green (MG) or Congo Red (CR), and they were incubated separately for 4 days in triplicate under the same conditions (pH 7, 160 rpm and 37°C).

Both continuous agitation and static conditions were used to measure growth at 600 nm and the remaining dye concentrations in the culture broth daily for 4 days. A 1 mL sample was centrifuged at 11 000 ×g for 10 minutes at 4°C (Tomy MX-305 High-Speed Refrigerated Microcentrifuge, Japan) and the supernatant was collected in order to calculate the percentage of decolorization. By measuring the decrease in absorbance (Biospectrophotometer Bio-Mate 3, Thermo Scientific, USA) at the corresponding λ_{max} values of 598 nm for CR and 618 nm for MG, as indicated by Eq. (1), decolorization activity was computed as a percentage.

Decolorization activity (%) was calculated as $\frac{[A_{initial} - A_{observed}]}{A_{initial}} \times 100$ (1)

$A_{initial}$ = initial absorbance
$A_{observed}$ = observed absorbance

2.4 Bioremediation of Batik Wastewater

Wastewater was obtained from a nearby batik plant, with an initial pH of 10.5 and a temperature of 37°C. The wastewater exhibited a deep blue hue with maximum absorption at a wavelength of 300 nm. All research parameters, including temperature, pH, TDS, TSS, BOD, COD and color, were measured before and after a 3-day treatment at 160 rpm with 30% (v/v) strains SK1 and SK2, respectively, following the APHA Standard Methods for the Examination of Water and Wastewater. Triplicate absorbance readings were taken at the beginning and end of the incubation period. A control set without strains SK1 and SK2 was also included for comparison.

2.5 Phytotoxicity Study

Mung bean (*Vigna radiata*) seeds were obtained from a local market and selected based on uniform size, shape and viability. To eliminate surface contaminants, seeds were sterilized with 0.1% (w/v) hydrogen peroxide for 2 minutes, followed by thorough rinsing with distilled water. Germination experiments were conducted using Petri dishes lined with filter paper, with three experimental groups: (i) control (distilled water), (ii) untreated wastewater and (iii) treated wastewater. Each Petri dish contained ten seeds, which were moistened with 5 mL of the respective test solutions. The dishes were incubated under controlled conditions at 25 ± 2°C with a 16:8-hour light-dark cycle for seven days. After seven days, seedlings were carefully removed to measure root and shoot lengths using a digital caliper along with seed germination rate (%).

2.6 Statistical Analysis

The groups were compared using the one-way analysis of variance (ANOVA) feature of IBM SPSS version 23. The Duncan test was selected for post hoc analysis and is represented by different letters. Statistical significance was indicated at a p-value of less than 0.05.

3. Results and discussion

3.1 Decolorization Study

Concentrations of 150 mg/L CR and MG in control samples (lacking strains SK1 and SK2, respectively) were comparatively constant throughout the experiment, indicating that abiotic factors were not the cause of dye loss. In addition, only 2-3% percent of the color was removed from the heat-inactivated cultures. This finding suggests that active metabolic activities of these strains were probably the cause of dye decolorization and that biosorption was not a significant contributing component. Since additional incubation until day 4 did not considerably enhance decolorization under any setting for all tested strains and dyes, the incubation duration was reported on day 3.

The decolorization capacity of strains SK1 and SK2 for CR and MG was evaluated under both static and agitated conditions. As shown in Table 1, both strains exhibited higher decolorization efficiency under agitation. This enhanced performance may be attributed to improved nutrient and oxygen dispersion, facilitating more efficient dye removal [16]. Additionally, strains SK1 and SK2 demonstrated greater cell growth under agitated conditions compared to static conditions, likely contributing to enhanced decolorization through increased enzyme secretion.

Table 1
Comparison of Congo Red and Malachite Green removal performance by strains SK1 and SK2 under static and shaking conditions

Indigenous isolate	Strain SK1	Strain SK2
Congo Red		
Growth (600 nm) at static condition	0.101 ± 0.027	0.201 ± 0.003
Growth (600 nm) at shaking condition	0.318 ± 0.001	0.896 ± 0.018
Dye removal (%) at static condition	3^{c} ± 1	14^{b} ± 2
Dye removal (%) at shaking condition	16^{b} ± 2	38^{a} ± 2
Malachite Green		
Growth (600 nm) at static condition	0.229 ± 0.017	0.218 ± 0.013
Growth (600 nm) at shaking condition	0.568 ± 0.031	0.816 ± 0.018
Dye removal (%) at static condition	12^{b} ± 2	12^{b} ± 2
Dye removal (%) at shaking condition	21^{a} ± 2	21^{a} ± 2

The mean values of triplicate samples ± standard errors are presented for both growth and dye removal percentages. Statistically significant differences ($p < 0.05$) between the tested groups were noted.

Previous studies have reported that tropical strains of *Rhodococcus* effectively decolorized CR, Methyl Red, Methylene Blue and Methyl Orange under static conditions [12, 17-19]. However, our findings contrast with these reports. The higher decolorization efficiency observed under static conditions in previous studies has been largely attributed to limited oxygen availability, which favors anaerobic or microaerophilic metabolic pathways. Under such conditions, bacteria tend to release key reductive enzymes, particularly azoreductase, which catalyze the cleavage of azo bonds (–N=N) present in synthetic dyes, leading to the breakdown of complex dye molecules into simpler aromatic amines. These intermediates can then be further degraded under subsequent aerobic conditions [20]. In contrast, agitation enhances oxygen transfer, stimulating the activity of oxidative metabolic pathways and promoting the production of oxidative enzymes such as laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP). These enzymes are known to attack the chromophoric structures of dyes through oxidative reactions, leading to the breakdown of dye molecules into smaller, less toxic components [20]. Laccases, for instance, can catalyze the oxidation of phenolic and non-phenolic substrates, while MnP and LiP participate in the oxidative cleavage of complex aromatic

structures. The combined action of these reductive and oxidative enzyme systems, depending on environmental conditions, is crucial in the complete biodegradation of synthetic dyes. These variations suggest that decolorization efficiency under static or agitated conditions is likely influenced by the specific microorganism and the types of enzymes it produces.

When strains SK1 and SK2 were tested for CR removal, significant improvements in both growth and decolorization performance under shaking conditions were demonstrated. Strain SK2 showed a notable increase in optical density to 0.896 ± 0.018, followed by strain SK1 with an optical density of 0.318 ± 0.001 after 3 days of incubation (Table 1). This enhanced growth was accompanied by improved CR removal, with strain SK2 achieving the highest decolorization rate of 38^{a} ± 2%, followed by strain SK1 at 16^{c} ± 2%. Under static conditions, limited growth correlated with relatively low CR removal rates. Strain SK2 outperformed strain SK1 with a removal efficiency of 14^{b} ± 2%, significantly higher than strain SK1, which achieved removal rate of 3^{c} ± 1%.

These findings are noteworthy compared to a study by Hanis *et al.,* [21], in which *Bacillus* sp. achieved approximately 82% decolorization on day 3 at a maximum dye concentration of 25 mg/L. Similarly, Kaushik and Seth [22] reported a decolorization rate of 94% for 100 mg/L CR using *Bacillus subtilis* after 3 days of incubation under shaking conditions. Despite achieving a slightly lower decolorization percentage, strain SK2 demonstrated remarkable effectiveness in handling significantly higher concentrations of CR. Strain SK2's ability to efficiently decolorize more concentrated dye solutions emphasizes its potential for industrial wastewater treatment, where dye concentrations are generally higher.

Interestingly, no significant differences ($p > 0.05$) were observed in the MG removal performance of strains SK1 and SK2 under either condition, with both achieving less than 25% decolorization under agitated conditions. Agitation resulted in a 43% higher removal of MG compared to static conditions. Similarly, Barapatre *et al.,* [23] reported that agitation at 120 rpm increased MG decolorization by 133% compared to static conditions. Rani and Singh [24] also demonstrated the impact of agitation on decolorization, achieving 93% MG removal under agitated conditions after 120 hours of incubation, compared to 44% under static conditions. Notably, strain SK2 demonstrated remarkable CR removal at high concentrations, suggesting its potential for effective dye decolorization in industrial wastewater treatment, although both strains showed limited MG decolorization, highlighting the influence of dye structure and microbial enzymatic activity on decolorization efficiency.

3.2 Bioremediation of Batik Wastewater

The effectiveness of locally isolated bacterial strains SK1 and SK2 in treating batik wastewater was assessed by evaluating key water quality parameters before and after treatment as shown in Table 2. The untreated wastewater had a high pH of 10.5 ± 0.3, indicating strong alkalinity. After treatment, strain SK2 reduced the pH to 7.7 ± 0.2, bringing it closer to neutral, which is more suitable for safe discharge and potential reuse. In contrast, strain SK1 lowered the pH to 8.9 ± 0.1, which, while an improvement, remained more alkaline than the wastewater treated with SK2. The greater pH adjustment by strain SK2 suggests its enhanced capability for pH neutralization in wastewater treatment. Temperature fluctuations were minimal during treatment, with wastewater temperatures changing from 37.0 ± 0.0°C to 36.0 ± 0.1°C for strain SK2 and remaining at 37.0 ± 0.0°C for strain SK1, indicating that neither strain significantly altered the thermal properties of the wastewater.

The key indicators of organic pollution are BOD and COD. Before treatment, the wastewater exhibited a BOD of 512.5 ± 2.1 mg/L and a COD of 4518.0 ± 2.6 mg/L, reflecting a high organic load.

Treatment with strain SK2 led to a 90% reduction in BOD (49.0 ± 1.8 mg/L) and a 94% reduction in COD (251.8 ± 1.3 mg/L), demonstrating its high efficiency in degrading organic pollutants. In contrast, strain SK1 was significantly less effective, reducing BOD by only 37% (322.4 ± 2.7 mg/L) and COD by 15% (3847.5 ± 0.3 mg/L).

The important indicators of water clarity and pollution control are TSS and TDS. Initially, the wastewater had a TSS concentration of 1147 ± 3.1 mg/L and a TDS concentration of 40.6 ± 0.8 mg/L. Strain SK2 significantly reduced TSS to 98 mg/L (91% removal) and TDS to 19.8 mg/L (51% removal), highlighting its strong ability to remove suspended and dissolved pollutants. Conversely, strain SK1 showed lower efficiency, reducing TSS to 603 mg/L (47% removal) and TDS to 37.5 mg/L (8% removal). These findings emphasize the superior biodegradation and pollutant removal capacity of strain SK2 compared to strain SK1.

Table 2
Changes in wastewater parameters before and after treatment with strains SK1 and SK2 compared to the Malaysian Environmental Quality Act 1974 standards

Parameter	Before treatment	After treatment	Malaysian Environmental Quality Act 1974	
			Standard A	**Standard B**
Strain SK1				
pH	10.5 ± 0.3	8.9 ± 0.1	6.0 – 9.0	5.5 – 9.0
Temperature (°C)	37.0 ± 0.0	37.0 ± 0.0	40	40
BOD (mg/L)	512.5 ± 2.1	322.4 ± 2.7	20	50
COD (mg/L)	4518.0 ± 2.6	3847.5 ± 0.3	80	250
TSS (mg/L)	1147.0 ± 3.0	603.0 ± 2.4	50	100
TDS (mg/L)	40.6 ± 0.8	37.5 ± 0.2	Not stated	Not stated
Strain SK2				
pH	10.5 ± 0.3	7.7 ± 0.2	6.0 – 9.0	5.5 – 9.0
Temperature (°C)	37.0 ± 0.0	36.0 ± 0.1	40	40
BOD (mg/L)	512.5 ± 2.1	49.0 ± 1.8	20	50
COD (mg/L)	4518.0 ± 2.6	251.8 ± 1.3	80	250
TSS (mg/L)	1147.0 ± 3.0	98.0 ± 2.0	50	100
TDS (mg/L)	40.6 ± 0.8	19.8 ± 1.2	Not stated	Not stated

Standard A: applicable to discharges into any inland waters within catchment areas.
Standard B: applicable to discharges into any other inland water or Malaysian waters.

Color removal is a critical aspect of batik wastewater treatment due to the presence of synthetic dyes. After 3 days of incubation at 160 rpm and 37°C, strain SK2 achieved a 77 ± 1% color removal efficiency at 300 nm (blue hue), indicating its strong dye-decolorizing ability. In contrast, strain SK1 removed only 26% of the color, demonstrating its limited capacity for dye degradation. The significantly higher decolorization efficiency of strain SK2 suggests its strong potential for treating dye-laden wastewater effectively.

Importantly, the final BOD (49 mg/L), COD (251 mg/L), and TSS (98 mg/L) values in wastewater treated with strain SK2 comply with Standard B regulations under the Malaysian Environmental Quality Act 1974, making the effluent suitable for discharge into inland waters. With its 77% color removal efficiency and substantial reductions in organic and solid pollutants, strain SK2 presents a promising eco-friendly solution for sustainable wastewater management and potential water reuse applications.

3.2 Phytotoxicity Study

The phytotoxicity assessment revealed that exposing *Vigna radiata* seeds to untreated batik wastewater (without strain SK2 treatment) significantly inhibited germination, reducing it by nearly 71% as illustrate in Figure 1. In contrast, treatment with strain SK2 resulted in only a slight 4% decline in germination compared to the control. Additionally, seedlings grown in untreated batik wastewater were markedly stunted, measuring approximately 5.9 times shorter than those cultivated in the decolorization metabolites ($p < 0.05$).

Prior research has consistently shown that, in comparison to untreated samples, treated textile wastewater improves seedling development and germination rates. For instance, Selim *et al.,* [25] found that *Vicia faba* seedlings germinated in textile effluent treated with an *Aspergillus flavus* and *Fusarium oxysporum* consortium exhibited growth comparable to those in a distilled water control. Similarly, a study assessing the toxicity of different treated textile wastewaters processed through a bioaugmented packed bed column bioreactor—incorporating *Citrobacter* sp. M41 with granulated corncob and its biochar—showed a significant reduction in phytotoxicity when tested with *Vigna radiata* seeds [26]. These findings suggest that batik wastewater, once effectively detoxified using strain SK2, has the potential for safe irrigation use, supporting sustainable water management and resource conservation.

While the current study provides promising evidence of reduced toxicity after treatment with strain SK2, phytotoxicity testing was limited to a single plant species under controlled laboratory conditions. Future studies should incorporate a wider range of plant species with varying sensitivities, including both monocots and dicots, to better assess the broader ecological safety of treated effluent. Additionally, field-based assessments under different soil types, irrigation regimes and environmental conditions are essential to validate the practical application of treated batik wastewater in agriculture.

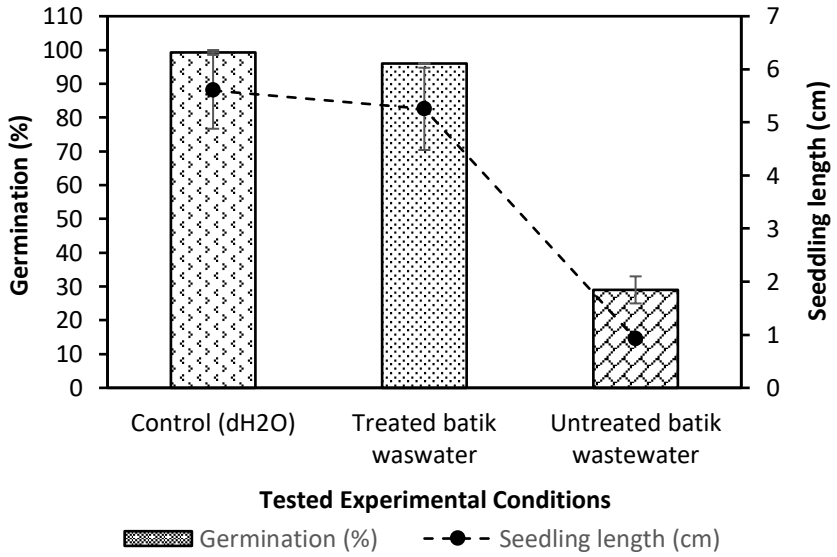

Fig. 1. Effects of treated and untreated batik wastewater on germination percentage and seedling length of *Vigna radiata*. Seedling length was measured as the sum of root and shoot lengths. Error bars represent the mean ± standard error

4. Conclusions

This study investigated the decolorization efficiency of strains SK1 and SK2 against CR and MG, as well as their bioremediation potential in treating batik wastewater. The results demonstrated that both strains actively contributed to dye decolorization through metabolic processes rather than biosorption. Strain SK2 exhibited significantly higher decolorization efficiency, particularly for CR under agitated conditions, achieving a removal rate of 38% compared to 16% by strain SK1. While MG removal was relatively low for both strains, agitation improved the decolorization efficiency by 43%, suggesting the role of enhanced oxidative enzyme activity in dye decolorization. The bioremediation study further highlighted the potential of strain SK2 in improving wastewater quality. After treatment, strain SK2 successfully reduced all tested parameters namely color, pH, temperature, BOD, COD, TSS and TDS, complying with Malaysian Environmental Quality Act 1974 Standard B for effluent discharge. The phytotoxicity study further validated the environmental safety of treated wastewater. Seeds of *Vigna radiata* exposed to untreated wastewater showed a 71% reduction in germination, whereas wastewater treated with strain SK2 resulted in only a 4% decline, indicating significant detoxification. These findings suggest that bioremediated batik wastewater could potentially be reused for irrigation, supporting sustainable water resource management (Sustainable Development Goal 6: Clean Water and Sanitation). While this study highlights the effectiveness of strain SK2 as a promising eco-friendly solution for industrial wastewater management, future research should focus on exploring a wider diversity of indigenous bacterial strains. This approach could provide valuable insights into the natural microbial diversity present in batik wastewater and help identify novel strains with even greater efficiency in dye degradation and pollutant removal. Additionally, further work is encouraged to optimize bioremediation conditions, elucidate detailed enzyme mechanisms and evaluate large-scale applications to enhance wastewater treatment performance and long-term environmental safety.

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