



# Decolorization of Remazol Blue by Indigenous Bacteria Isolated from Textile Effluent

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**Abstract:** Reactive synthetic dyes, particularly Remazol Blue, are widely used in the textile industry and contribute to significant aquatic pollution when discharged untreated. This study aimed to isolate and evaluate indigenous bacteria from textile effluent for their ability to decolorize Remazol Blue. A total of 66 bacterial isolates were obtained and screened on Mineral Salt Medium supplemented with 200 ppm dye. Six isolates demonstrated decolorization efficiencies ranging from 30–41% at 200 ppm. These isolates were subsequently combined into a consortium and tested at increasing dye concentrations (200, 400, and 600 ppm). The consortium achieved maximum decolorization of 41.2% at 200 ppm after 96 hours, while lower efficiencies were observed at 400 ppm (18.3%) and 600 ppm (17.1%). The findings highlight the potential application of indigenous bacteria for the biological treatment of textile dye effluents and provide a basis for further optimization and scale-up studies.

**Keywords:** decolorization; indigenous bacteria; Remazol Blue; textile; dye waste.

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## 1. Introduction

The textile industry is a major contributor to Indonesia's economy through production, export value, and employment opportunities [1]. However, its rapid expansion has led to increased discharge of dye-containing effluents, which pose significant environmental challenges. During the dyeing process, up to 50% of synthetic dyes may remain unbound to fibers and be released into wastewater streams [2]. Among various dye classes, reactive dyes are widely used for their broad colour range, fabric versatility, and cost-effectiveness [3].

Remazol Blue, a widely applied reactive dye, belongs to the anthraquinone group and is known for its high solubility, chemical stability, and recalcitrance to degradation [4]. These properties, combined with its toxicity, make Remazol Blue a persistent pollutant that can disrupt aquatic ecosystems and pose risks to human and environmental health [5,6].

Conventional physicochemical treatment processes for dye removal are often costly, generate secondary pollution, and may be ineffective for complete mineralization of complex dye structures. Biological treatment has emerged as a promising alternative due to its cost efficiency, sustainability, and potential to completely degrade dye molecules. Indigenous bacteria from textile effluent environments are particularly advantageous, as they are naturally adapted to chemical stressors and may possess specialized metabolic pathways for dye transformation [7,8]. Several bacterial genera, including *Bacillus*, *Proteus*, *Pseudomonas*, and *Cyanobacteria* such as *Anabaena*, have demonstrated dye-decolourising and biodegrading capabilities through enzymatic mechanisms [9,10]. However, studies specifically examining indigenous bacterial communities capable of degrading anthraquinone-based dyes, such as Remazol Blue, remain limited.

Therefore, this study focuses on isolating and characterising native bacterial strains from textile factory effluent and on evaluating their ability to decolorize Remazol Blue. By identifying naturally adapted microbial candidates, this research aims to support the development of sustainable and environmentally compatible bioremediation strategies for textile wastewater treatment.

## 2. Materials and Methods

### 2.1. Sample collection and bacterial isolation.

Water and sediment samples were collected from three stages of a textile wastewater treatment system: the inlet tank, the physicochemical treatment tank, and the biological treatment tank. Each sample (1 mL or 1 g) was diluted to 9 mL with sterile 0.85% NaCl solution, homogenised, and then serially diluted to  $10^{-5}$ . Aliquots (100  $\mu$ L) of each dilution were plated in duplicate on Nutrient Agar (NA) using the spread-plate technique, and the plates were incubated at 37°C for 24 h. Distinct colonies were purified by morphology-based streaking on NA. Pure isolates were maintained on NA slants at 4°C for further analysis.

### 2.2. Screening of potential dye-decolorizing isolates.

Screening was performed following a modified method described by [11]. Single colonies from NA plates were inoculated into 5 mL Mineral Salt Medium (MSM) and incubated at 37°C and 150 rpm for 48 h until the culture reached an optical density ( $OD_{600}$ ) of 0.5. A 10% (v/v) inoculum was transferred into fresh MSM containing 200 ppm Remazol Blue. Cultures were incubated at 37°C, 150 rpm for 72 h. After incubation, 3 mL samples were centrifuged at 6,000 rpm for 10 min. Supernatants were analysed using a UV-Vis spectrophotometer (Shimadzu UV-1800) at  $\lambda_{max} = 600$  nm. A medium containing the dye without a bacterial inoculum served as an abiotic control, and blank-corrected initial absorbance values ( $A_0$ ) were recorded before incubation. Decolorization percentage was calculated as:

$$\text{Decolorization (\%)} = \frac{A_0 - A_i}{A_0} \times 100 \quad (1)$$

Where  $A_0$  = initial absorbance and  $A_i$  = absorbance after incubation. All experiments were conducted in triplicate.

### 2.3. Dye decolorization by bacterial consortium.

Each selected isolate was cultured in MSM to an  $OD_{600} = 0.5$  under the same conditions as above. Equal volumes of each suspension were mixed to form the bacterial consortium. The consortium (10% v/v inoculum) was transferred into MSM containing Remazol Blue at 200, 400, and 600 ppm. Cultures were incubated at 37°C, 150 rpm for 5 days, and absorbance was measured every 24 h after centrifugation. Uninoculated dye-containing MSM served as abiotic controls; MSM without dye served as negative controls. Mineral Salt Medium contained (g/L):  $NH_4Cl$  1.0,  $MgSO_4 \cdot 7H_2O$  0.2,  $K_2HPO_4$  1.0,  $KH_2PO_4$  0.2,  $CaCl_2 \cdot 2H_2O$  0.02,  $NaCl$  5.0; pH was adjusted to 7.0 before sterilisation.

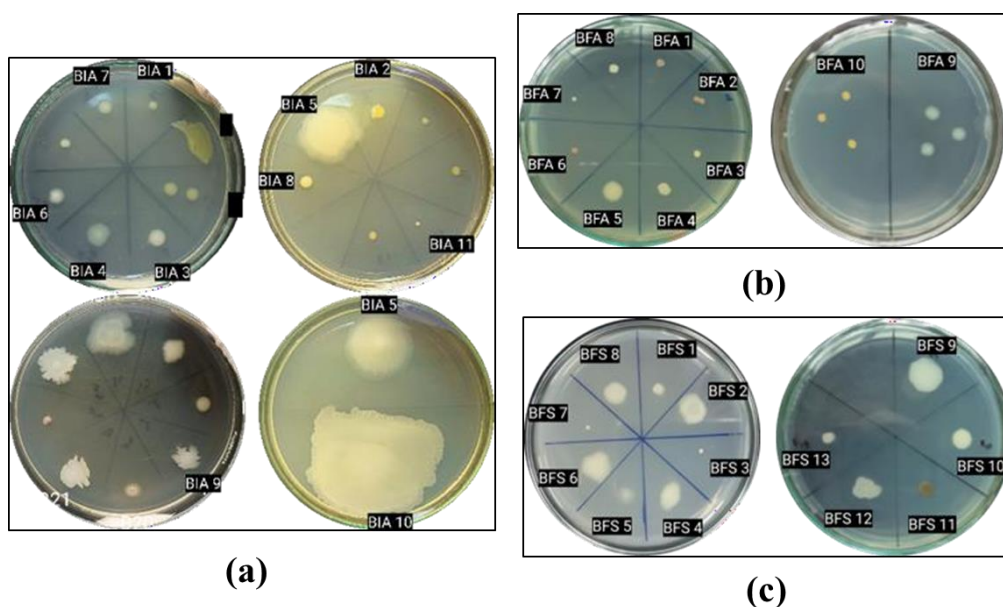
### 2.4. Data and statistical analysis.

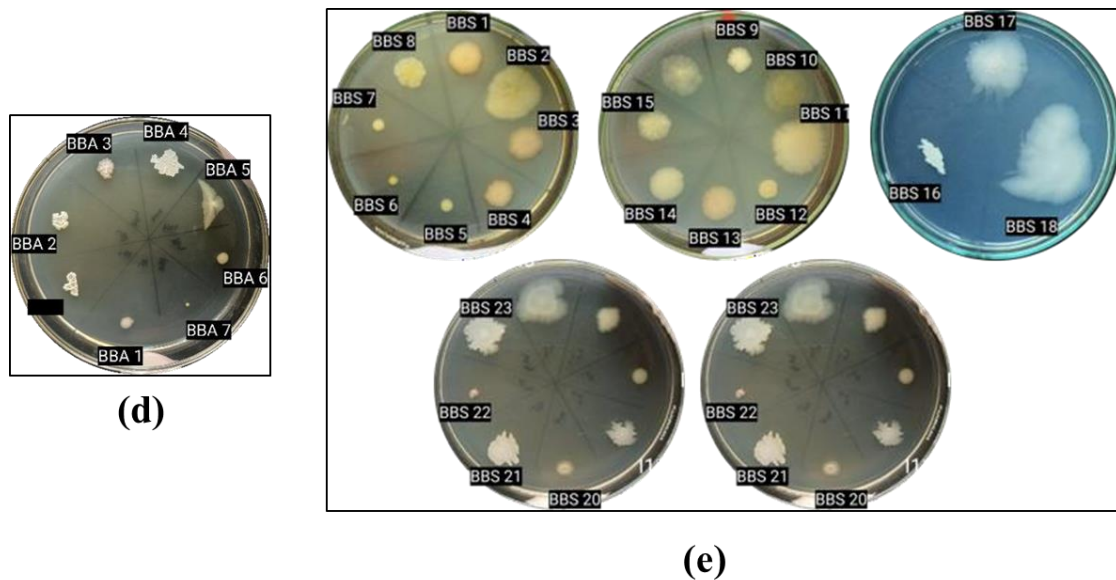
All experiments were performed in triplicate. Data were expressed as mean  $\pm$  standard deviation. Statistical significance among treatments was evaluated using one-way ANOVA ( $p < 0.05$ ) in IBM SPSS.

## 3. Results and Discussion

### 3.1. Isolation of bacteria from textile waste.

The results of bacterial isolation from water and sediment samples from the inlet, physicochemical, and biological tanks yielded 66 isolates with distinct morphological characters. Where in the inlet tank of water sample obtained 11 isolates (BIA 1-11), the physicochemical tank of water sample obtained 10 isolates (BFA 1-10), the physicochemical tank of sediment sample obtained 13 isolates (BFS 1-13), the biological tank of water sample were obtained 7 isolates (BBA 1-7), and) from the biological tank of sediment samples were obtained 25 isolates (BBS 1-25) (Figure 1). The number of isolates in the inlet and physicochemical tanks tended to be less than the number of isolates obtained from the biological tank. This can be caused by the fact that the incoming waste has not undergone processing in the inlet tank and still contains a large amount of pollutants.





**Figure 1.** Bacterial Isolates from (a) the Water Sample of Inlet Tank; (b) the Water Sample of Physico-Chemical Tank; (c) Sediment Sample of Physico-Chemical Tank; (d) Water Sample of Biological Tank; (e) Sediment Sample of Biological Tank.

In the physicochemical tank,  $\text{FeCl}_3$  coagulant is added, producing  $\text{Fe}^{2+}$  ions that can form free radicals and inhibit bacterial growth. This is in accordance with the research of [12], which states that the addition of  $\text{FeCl}_3$  to growth media at higher concentrations inhibited the growth of *Pseudomonas* sp. isolates. Kumar *et al.* [13] reported that the addition of  $\text{FeCl}_3$  in large quantities to the media can cause the formation of free radicals, which are toxic, and can degrade bacterial DNA and kill bacteria. Meanwhile, in the biological tank, the incoming waste has undergone processing, the pollutant content has been reduced compared to the inlet and physicochemical tanks, and aeration treatment ensures sufficient oxygen for bacterial growth. The concentration of dissolved oxygen is crucial, as it is required by bacteria for cell synthesis, which in turn influences their growth rate [14, 15].

### 3.2. Screening for potential isolates.

The results of the potential isolate screening showed that 6 isolates decolourised Remazol blue dye, with decolorization percentages ranging from 30.9% to 41.3% (Table 1). Of the 66 bacterial isolates obtained, only 6 were able to decolourise Remazol blue dye; this may be because not all isolates were specifically able to do so. This is consistent with the findings of [16], which showed that decolorization test results for a single isolate against textile dyes differed across the azo, anthraquinone, and triphenylmethane groups; no isolate yielded the same decolorization percentage across all dye types.

**Table 1.** Decolorization percentage from potential isolates.

Isolate	Absorbance		% Decolorization
	0 hours	72 hours	
K	1.325	1.288	2.8
BIA 7	1.325	0.778	41.3
BBS 11	1.325	0.796	39.9
BBS 9	1.325	0.815	38.5
BBS 5	1.325	0.869	34.4
BBS 15	1.325	0.907	31.5
BFS 5	1.325	0.915	30.9

Although microbial consortia often exhibit enhanced biodegradation efficiency through metabolic complementarity, our consortium did not demonstrate synergistic performance. Several ecological and metabolic factors may explain this outcome. First, competitive substrate uptake can occur when isolates share overlapping nutrient preferences or dye-utilisation pathways, leading to resource partitioning rather than cooperation. Such competition may limit the growth and metabolic activity of individual strains, thereby reducing overall degradation efficiency [13]. Second, antagonistic interactions, including secretion of inhibitory metabolites, quorum-sensing-mediated suppression, or local pH/oxygen shifts, may hinder partner cells and impair enzyme production [14]. Third, disruption of enzyme expression dynamics may arise in co-culture: strains that efficiently produce key oxidative or reductive enzymes under monoculture conditions may down-regulate these pathways due to altered regulatory signals, different redox balances, or competition for cofactors in mixed culture [15]. Finally, the absence of true metabolic complementarity could mean that the strains perform similar rather than sequential catabolic reactions, resulting in functional redundancy rather than cooperative enhancement [16]. Together, these factors suggest that microbial compatibility and ecological interactions are critical determinants of consortium performance and that rational strain pairing or metabolic engineering may be necessary to achieve synergistic dye degradation [17].

### 3.3. Decolorization test using a consortium of potentially isolates.

Follow-up tests used a consortium of potential isolates on MSM media containing Remazol blue 200, 400, and 600 ppm. The use of a consortium of potential bacterial isolates is expected to give a higher decolorization percentage than a single isolate. According to [17], the use of a consortium of bacterial isolates enables exploration of synergistic interactions. The results of decolorization by a consortium of potential isolates are shown in Table 2.

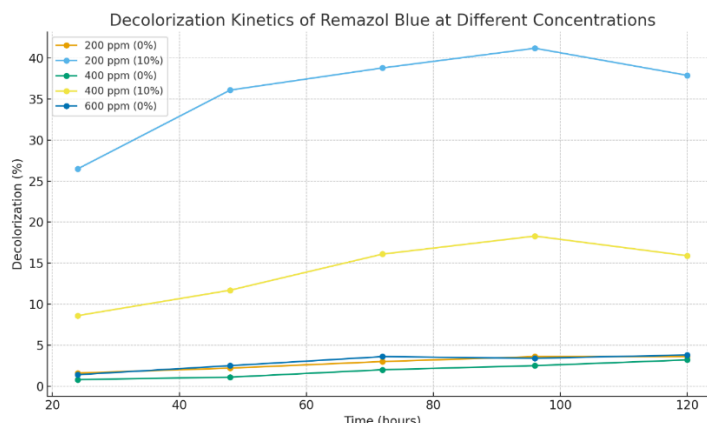
**Table 2.** Decolorization percentage of Remazol Blue by bacterial consortium.

Dye concentration (ppm)	Inoculum volume (%)	Decolorization percentage (%)				
		Observation time (hour)				
		24	48	72	96	120
200	0	1.6	2.2	3	3.6	3.6
	10	26.5	36.1	38.8	41.2	37.9
400	0	0.8	1.1	2	2.5	3.2
	10	8.6	11.7	16.1	18.3	15.9
600	0	1.4	2.5	3.6	3.4	3.8

Remazol blue dye decolorization by a consortium of potential isolates showed the highest percentage (41.2%) at 200 ppm for 96 hours of incubation, while those at 400 and 600 ppm were lower at 18.3% and 17.1%, respectively (Figure 2). The greater the dye concentration, the lower the percentage of decolorization appears to be. This can be caused by the toxic effects of dyes at high concentrations, which inhibit bacterial growth and their ability to decolourise dyes. According to Ahmed *et al.* [18], the decolorization percentage decreases as the dye concentration reaches 400 ppm. The decrease in decolorization percentage can be attributed to the dye's toxic effects, changes in enzyme metabolism, and the accumulation of by-products.

The percentage of decolorization increased from 24 hours to 96 hours of incubation, then decreased at 120 hours. This can occur because all the bacteria in the media are dead, leading to reduced decolorization. The same results were also reported in the study by [18], which found that the decolorization of textile dyes using isolate S8 increased up to 96 hours of incubation. After an incubation time of 96 hours, the percentage of decolorization appeared to

decrease. According to Afrin *et al.* [19], the percentage of decolorization of textile dyes decreased with increasing incubation time, due to the bacterial consortium entering a death phase, which reduced enzymatic activity in the media.



**Figure 2.** Decolorization kinetics of Remazol Blue by bacterial consortium at different initial dye concentrations.

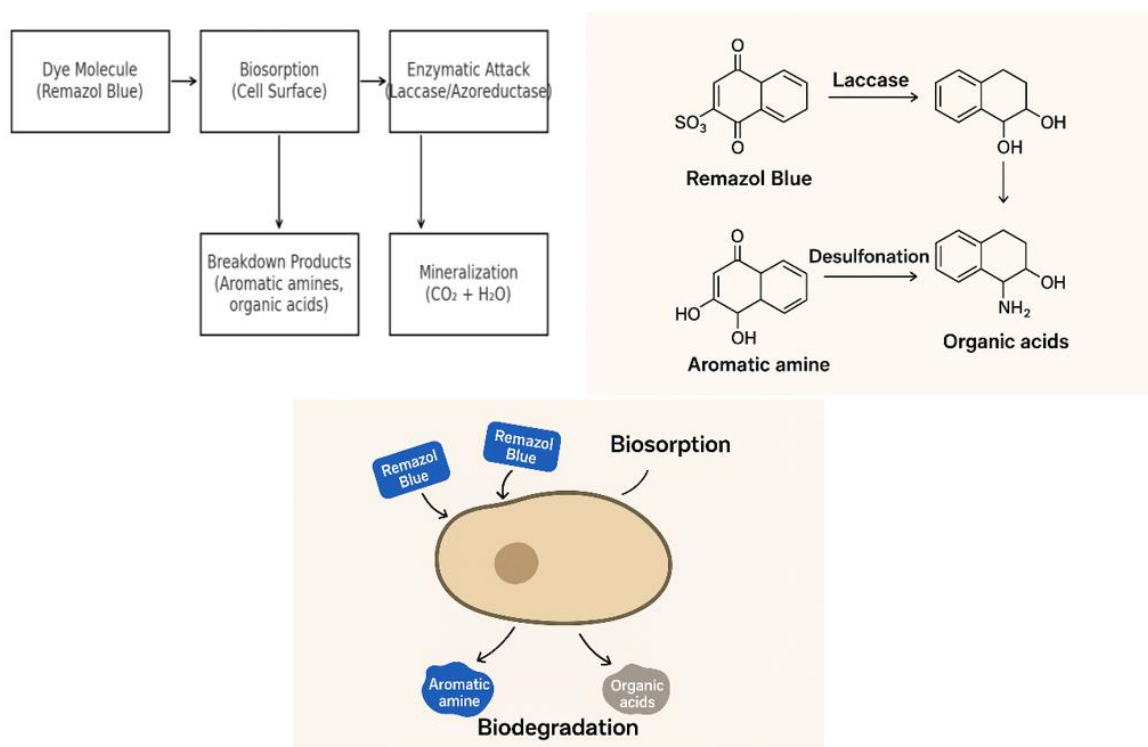
The decolorization value in the abiotic control ranged from 0.8 to 3.6%. This could be due to the Remazol blue dye being decolourised abiotically via a chemical reaction. Based on Boonyakamol *et al.* [20], the abiotic decolorization of RB4 dye is through the process of dye precipitation. The same was also reported in the study by Khalid *et al.* [21], which conducted decolorization tests of Acid Red-88, Reactive Black-5, Direct Red-81, and Disperse Orange-3 dyes in abiotic controls, showing decolorization values of 5.4%-8.5%. This value is smaller than the biotic decolourization value obtained with bacterial isolates so that it can be disregarded.

Based on follow-up test results, the consortium of potential isolates at 200 ppm did not achieve a higher decolorization rate than a single isolate. This could be due to a lack of synergistic activity among the isolates in the bacterial consortium. The same was also reported in the study by Abd El-Rahim *et al.* [22]. In the Ponceau S and Evans Blue dye decolorization test, the decolorization value of the bacterial consortium was lower than that of a single isolate. This could be due to antagonism between isolates in the consortium.

The mechanism of dye decolorization by bacterial isolates can involve biosorption and biodegradation. In the biosorption process, the dye is trapped in the bacterial cell matrix, and its chemical structure remains unchanged. Meanwhile, the dye biodegradation process occurs enzymatically, altering its chemical structure. This is in accordance with Madhushika *et al.* [23], who reported that biosorption occurs by trapping the dye within the bacterial cell. This occurs because electrons are transferred between the adsorbate and the adsorbent, leading to a high correlation coefficient.

Biosorption is a passive physicochemical process in which dye molecules bind to the surfaces of bacterial cells. This process does not require metabolic activity and can occur in both living and dead bacterial biomass. Bacterial cell walls contain various functional groups, such as carboxyl, hydroxyl, amino, and phosphate groups, which interact with dye molecules via mechanisms including ion exchange, hydrogen bonding, van der Waals forces, and electrostatic interactions (Figure 3). Biosorption is influenced by factors such as pH, temperature, dye concentration, and the dye's nature (anionic or cationic). For example, at lower pH, positively charged dye molecules may more readily bind to negatively charged sites

on the bacterial surface. The process is generally rapid and reversible, making it suitable for initial dye removal and concentration from wastewater [23].



**Figure 3.** Proposed biosorption–biodegradation pathway for Remazol Blue by microbial cultures. Dye molecules first adsorb onto the microbial cell surface, followed by enzymatic attack mainly via laccases and azoreductases, generating intermediate aromatic amines and organic acids, which are subsequently mineralized to CO<sub>2</sub> and H<sub>2</sub>O.

In contrast to biosorption, biodegradation is an active, enzymatic process in which bacteria metabolise dye molecules, breaking them down into simpler, non-toxic compounds. This process depends on the metabolic capacity of specific bacterial strains, which produce enzymes such as azoreductases, laccases, and peroxidases (Figure 3). These enzymes cleave complex dye structures, including azo bonds (–N=N–), phenolic rings, and other chromophores responsible for colour. Biodegradation often results in complete mineralization of the dye into CO<sub>2</sub>, water, and inorganic salts, thereby offering a permanent solution to dye pollution. This process typically requires optimal conditions such as suitable temperature, pH, oxygen levels, and a carbon source for co-metabolism. Facultative or anaerobic conditions are often required for the initial breakdown of azo dyes, followed by aerobic conditions for the degradation of resulting aromatic amines [24].

Based on the research of Andleeb *et al.* [24], the mechanism of biodegradation of anthraquinone dyes occurs first through the separation of small molecule groups around the anthraquinone ring from the parent compound aerobically. According to Li *et al.* [25], the anthraquinone ring gradually breaks to form compounds with smaller molecules through oxidation and hydrolysis. Further cleavage of the anthraquinone metabolites forms benzoic acid. According to Thakur *et al.* [26], the biodegradation of dyes by bacteria involves enzymatic processes, including laccase, lignin peroxidase, tyrosinase, oxidoreductase, and azoreductase.

In many studies, the primary enzymatic actors in Remazol Brilliant Blue R (RBBR) decolourization are extracellular oxidative enzymes produced by ligninolytic fungi, particularly laccases (multicopper oxidases) and peroxidases (including H<sub>2</sub>O<sub>2</sub>-dependent lignin

peroxidase-type activities). For example, a crude laccase preparation from *Trametes versicolor* achieved substantial decolourization of RBBR [26]. Another study using soybean peroxidase reported the effective decolourization of RBBR, indicating that peroxidase-based oxidation also contributes significantly [27]. These enzyme systems begin by oxidising the anthraquinone chromophore or its substituents, generating radical species that destabilise the conjugated aromatic system and initiate breakdown.

Once the initial oxidation occurs, published pathways indicate that subsequent chemical transformations include hydroxylation, demethylation, desulfonation (loss of  $-\text{SO}_3^-$  groups), and, eventually, aryl-ring cleavage, producing lower-molecular-weight aromatic acids. For instance, in a mechanistic diagram for RBBR laccase degradation, reduction of the dye is followed by hydroxylation of aromatic rings and deamination, then oxidation and ring opening [5]. In fungal culture studies (e.g., *Aspergillus fumigatus* and *Aspergillus terreus* degrading RBBR), HPLC profiles documented the disappearance of principal dye peaks and the appearance of new minor peaks after treatment, consistent with the formation of transformation products [28]. Importantly, because RBBR is anthraquinone-based and not azo-based, the mechanism is dominated by these oxidative transformations rather than simple azo-bond reductive cleavage (which is more common for azo dyes).

Finally, several studies emphasise that reaction conditions, enzyme cofactors, and mediators affect pathway efficiency and the depth of degradation. For example, the presence of redox mediators such as N-hydroxybenzotriazole (HBT) enhanced laccase-mediated RBBR decolourization. Also, a study on the fungal pellet treatment of RBBR noted significant combined contributions of biosorption (substrate pre-concentration on biomass) and enzymatic oxidation. Additionally, optimal pH, temperature,  $\text{H}_2\text{O}_2$  concentration (for peroxidase) or oxygen supply (for laccase) are often critical parameters that determine whether the pathway stops at chromophore disruption (decolourisation) or proceeds to full breakdown/mineralisation [29].

In many cases, bacterial dye decolorization involves a synergistic effect of biosorption and biodegradation. Initially, dyes may be adsorbed onto the cell surface, concentrating them near bacterial enzymes and facilitating subsequent enzymatic breakdown. This combination enhances the efficiency and speed of the decolorization process. Bacterial dye decolorization through biosorption and biodegradation offers an effective, sustainable, and environmentally friendly approach to treating dye-laden wastewater. While biosorption provides a quick means of removing dyes from solution, biodegradation ensures complete detoxification and mineralization. Future research focused on isolating highly efficient bacterial strains, optimizing environmental conditions, and applying genetic and metabolic engineering can further enhance the potential of bacterial systems in industrial wastewater treatment [30].

Several factors, including pH, temperature, dye concentration, and incubation time, influenced the effectiveness of decolorization. Higher dye concentrations generally led to reduced decolorization efficiency, possibly due to toxic effects or saturation of bacterial degradation pathways. The mechanism of decolorization is likely enzymatic, involving reductive cleavage of the azo bond and the further breakdown of the dye structure into simpler, potentially less toxic metabolites. Although this study did not involve detailed metabolite analysis, the observed colour loss and changes in absorbance spectra suggest biodegradation rather than mere adsorption. Compared to physical and chemical methods, using indigenous bacteria offers several advantages, including lower operational costs, minimal sludge

generation, and greater environmental sustainability. Moreover, using bacteria already present in textile effluents eliminates the need for external inoculants and enhances the feasibility of in situ bioremediation strategies. However, it is important to note that this study did not assess the complete mineralisation of the dye compounds or the fate of their breakdown products. Future work should include advanced analytical techniques such as GC-MS or HPLC to identify intermediate and final degradation products and assess their toxicity. In addition, scaling up the process in bioreactors and integrating it with existing treatment systems will be essential to evaluate its practical applicability. The indigenous bacterial isolate(s) investigated in this study offer a promising solution for the biological treatment of dye-laden effluents, supporting the growing emphasis on green and sustainable technologies in industrial wastewater management.

#### **4. Conclusions**

This study successfully isolated 66 bacterial strains from textile effluent treatment units, exhibiting diverse morphological characteristics. Screening on MSM medium supplemented with 200 ppm Remazol Blue identified six indigenous isolates capable of achieving  $\geq 30\%$  decolorization, indicating their inherent adaptation to dye-polluted environments. Subsequent consortium testing demonstrated a maximum decolorization efficiency of 41.2% at 200 ppm; however, no synergistic enhancement was observed compared to the most effective single isolate. Moreover, decolorization efficiency declined at higher dye concentrations, suggesting inhibitory or toxic effects at elevated pollutant loads. While the findings highlight the promising potential of indigenous bacteria for reactive dye removal, the scope of this work remains preliminary. Key limitations include the absence of molecular identification of isolates, the lack of enzymatic or metabolite-level confirmation of biodegradation pathways, and a relatively short incubation period. Therefore, further investigation is needed to verify whether color loss resulted primarily from biodegradation or biosorption. Future research should focus on molecular characterization of the isolates, enzyme profiling to elucidate dye-degrading mechanisms, optimization of environmental and nutritional parameters to enhance performance, and validation in pilot-scale wastewater treatment systems. Such efforts will contribute to the development of more robust, sustainable bioremediation strategies for textile dye effluents.

#### **Author Contributions**

Conceptualization, A.B. and D.W.; formal analysis, D.R.; investigation, A.A.K.P.; writing—original draft preparation, A.B., D.W., R.H.B.S., and M.P.; supervision, A.B. and A.S. All authors have read and agreed to the published version of the manuscript.

#### **Institutional Review Board Statement**

Not applicable.

#### **Informed Consent Statement**

Not applicable.

## Data Availability Statement

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

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Declared none.

## Conflicts of Interest

The authors declare no conflict of interest.

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