Malaysian Journal of Chemistry, 2025, Vol. 27(3), 1-19
(Special Issue: *2nd International Science and Technology Colloquium, i-COSTECH 2024*)

Discovering Decolourization Potential of Remazol Brilliant Blue R (RBBR) Dye by Fungal Monoculture and Consortium (*Penicillium* sp. and *Aspergillus* sp.)

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Remazol Brilliant Blue R (RBBR) dye is a prevalent and toxic anthraquinone dye used in the textile industry. This study investigates the decolourization potential of fungal monoculture and consortium (*Penicillium* sp. and *Aspergillus* sp.) obtained from the Universiti Teknologi MARA Negeri Sembilan culture collection centre for the remediation of RBBR dye. A 2 g of fresh fungal biomass was inoculated into 100 mgL^{-1} of RBBR dye solution and agitated at 100 rpm for 14 days, with decolourization activities analyzed via absorbance at 590 nm. The fungal monoculture and consortium demonstrated potential in decolourizing RBBR dye, with *Penicillium* sp. achieving 42.1%, the consortium (*Penicillium* sp. and *Aspergillus* sp.) at 29.9%, and *Aspergillus* sp. at 24.2%, respectively. Monoculture (*Penicillium* sp.) proved more effective, likely due to its higher enzyme production. The study revealed that decolourization activities were influenced by pH, initial dye concentration, agitation speed, biomass, and oxygen availability. Optimal decolourization was achieved at pH 7, 100 mgL^{-1} dye concentration, 50 rpm agitation speed, 2 g biomass, and in the absence of oxygen. The decolourization of RBBR dyes was accomplished through bioremediation, as evidenced by the noticeable changes in the ultraviolet-visible spectra between untreated and treated dyes.

Keywords: Monoculture; consortium; biodegradation; decolourization; Remazol Brilliant Blue R (RBBR) dye

Received: September 2024; Accepted: December 2024

In recent years, the utilization of synthetic dyes has surged due to the rapid growth of dye-based industries and increased demand for textiles [1]. Unfortunately, synthetic dyes are also recognized for their resilience, contributing to enduring environmental challenges, including reducing sunlight penetration, elevated biochemical oxygen demand (BOD) and chemical oxygen demand (COD), photosynthesis inhibition, and plant growth suppression. Due to their xenobiotic nature and some carcinogenic properties, synthetic dyes have resulted in water pollution, which poses severe threats to aquatic ecosystems [2, 3]. These pollutants can lead to detrimental effects on various organisms, such as fish, which may suffer from gill damage and impaired reproductive functions, and aquatic invertebrates, which face increased mortality and decreased mobility [4]. Moreover, they contribute to pipe corrosion, blockages, and bioaccumulation, producing hazardous sludge. The presence of dyes in effluents further impedes their reutilization, affecting subsequent dyeing processes [5].

The extensive array of synthetic dyes can be attributed to various chromophore groups, such as azo, anthraquinone, and triphenylmethane [6]. Azo dyes, constituting approximately 60% of all industrially important dye categories, are the most prevalent, followed by anthraquinones and indigoids [5]. Anthraquinone dyes, second to azo dyes in prevalence, are favoured for their affordability, availability, and excellent dyeing properties. Despite their advantages, anthraquinone dyes, exemplified by Remazol Brilliant Blue R (RBBR), are notoriously resistant to degradation in textile wastewater [3], posing significant environmental threats if discharged untreated.

Various techniques exist for degrading anthraquinone dyes, including Fenton oxidation, ozone oxidation, ultrasonic catalytic oxidation, photocatalytic oxidation, and microwave catalysis [7]. However, despite their firm and tedious nature, biological methods are preferred for their cost-effectiveness and minimal secondary pollution [8]. Microorganisms,

†Paper presented at the 2nd International Science and Technology Colloquium (i-COSTECH 2024)

such as algae, fungi, actinomycetes, and bacteria, have been employed for anthraquinone dye degradation, with microbial decolourization and degradation processes extensively studied [9].

Fungi play a pivotal role in bioremediation with their remarkable ability to adapt metabolism to varying environmental conditions. Intracellular and extracellular enzymes, including lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase, significantly contribute to dye degradation, rendering fungal cultures ideal candidates for this purpose [10]. However, a dearth of research explicitly focuses on the degradation of RBBR dye, specifically using fungal cultures. Previous studies have predominantly focused on the degradation of RBBR dye using various fungal monocultures, particularly those known for their laccase enzyme production.

The most studied species are *Pleurotus ostreatus*, *Trametes versicolor*, *Ganoderma lucidum*, *Mycoaciellabispora* sp., and *Coriolopsis caperata*, which has shown significant efficacy in RBBR decolourization [3, 11-13]. The laccase enzyme is particularly crucial, as it catalyzes the oxidation of phenolic compounds, which facilitates the breakdown of complex dye structures [14, 15]. However, the effectiveness of these monocultures can be constrained by factors such as high dye concentrations and the presence of toxic intermediates, which can inhibit fungal growth and enzyme activity [16].

In contrast, consortium approaches, where multiple fungal species are cultivated together, have been shown to enhance laccase production and overall dye degradation efficiency. For example, a study demonstrated that coculturing *Pleurotus* sp. and *Trametes* sp. led to significantly higher laccase activity than monocultures, resulting in better RBBR decolourization [17]. The enhanced degradation efficiency observed with consortium can be attributed to the synergistic effects of different fungi, which offer complementary metabolic pathways. This synergy can improve the breakdown of recalcitrant compounds in synthetic dyes and mitigate the toxic effects of high dye concentrations, thereby sustaining enzymatic activity and increasing degradation rates [18].

Furthermore, several studies have investigated specific fungi for RBBR decolourization. For instance, research has shown that the laccase produced by *Pleurotus florida* effectively decolourizes RBBR under optimized conditions, highlighting the enzyme's importance in the degradation process [13]. Similarly, other studies have indicated that the presence of phenolic compounds, such as dyes, can enhance laccase production in *Pleurotus ostreatus*, suggesting a synergistic relationship between dye presence and enzymatic activity [15]. Additionally, research examining the influence of pH on laccase activity and RBBR decolourization has underscored the role of environmental factors in optimizing fungal performance [19]. In summary, the optimization of RBBR dye degradation through fungal systems is increasingly supported by research highlighting the significant impact of environmental factors on enhancing fungal activity and overall degradation efficiency.

However, the potential of utilizing monocultures and consortiums for dye degradation in microorganisms, such as *Aspergillus* sp. and *Penicillium* sp., remains limited [20]. Exploring various fungal species for optimizing growth parameters in dye remediation, mainly through monoculture and consortium approaches, is a promising area of research in bioremediation and biodegradation technologies [21]. This research also presents an opportunity to identify fungal strains with significant potential for environmental applications, such as dye removal.

This study aims to assess the potential of fungal monoculture and consortium, i.e., *Penicillium* sp. and *Aspergillus* sp., in decolourizing RBBR dye, with decolourization efficiency (DE%) as a critical metric. By emphasizing the novel application of fungal monoculture and consortium in dye degradation, this research could highlight the practical significance of these fungal systems in wastewater treatment. Insights gained may contribute to optimizing bioremediation strategies, advancing dye removal technologies, and furthering environmental biotechnology. Additionally, the research could inform future studies on scaling these methods for industrial applications and suggest potential improvements in fungal consortium formulations for enhanced dye degradation.

EXPERIMENTAL

Culture Establishment and Biomass Generation

The *Penicillium* sp. and *Aspergillus* sp. fungal isolates obtained from the Universiti Teknologi MARA Cawangan Negeri Sembilan (UiTMCNS) culture collection centre were cultured on Potato Dextrose Agar (PDA) and sub-cultured periodically at room temperature (25 ± 2°C). Subsequently, five mycelial plugs were inoculated from each isolate into 100 ml of Potato Dextrose Broth (PDB). The isolates were then incubated for 7 days to generate biomass. The resulting fungi biomass was filtered, rinsed with sterile distilled water, and weighed. This freshly obtained biomass was then utilized for dye decolourization [22].

Dye Decolourization Activity

A fresh weight of 2 g of fungal biomass (*Penicillium* sp. and *Aspergillus* sp.) was inoculated into a 100 mgL^{-1} Remazol Brilliant Blue R (RBBR) dye solution and incubated at 100 rpm for a period ranging from 14 days. The untreated dye solution was not inoculated with cells and was maintained as a negative control. 3 mL aliquots were collected daily and centrifuged for 10 minutes at 10,000 rpm. The supernatants were then analyzed for absorbance readings at a 590 nm wavelength designed to detect RBBR. The efficiency of decolourization was determined using the following Equation (1) [22].

$$\text{Decolourization efficiency (DE\%)} = \frac{\text{initial absorbance} - \text{observed absorbance}}{\text{initial absorbance}} \text{ x } 100 \quad (1)$$

Biosorption Isotherm Models

The Langmuir and Freundlich isotherm models were utilised to examine the biosorption of cell biomass with RBBR dyes at equilibrium circumstances [22]. The Langmuir isotherm, pertaining to monolayer adsorption of dyes on cellular biomass, is defined by Equation (2).

$$\frac{C_e}{q_e} = \left(\frac{1}{K_L q_m}\right) + \left(\frac{1}{q_m}\right) C_e \quad (2)$$

In this context, q_e represents the amount of dye absorbed (mg g^{-1}), C_e denotes the equilibrium dye concentration (mg L^{-1}), K_L signifies the Langmuir constant (L mg^{-1}), and q_m indicates the biosorption capacity (mg g^{-1}). The values of K_L and q_m were ascertained from the intercept and slope by graphing C_e/q_e against C_e. The Langmuir isotherm feature is represented by the separation factor, R_L, which is computed using Equation (3).

$$R_L = \left(\frac{1}{(1+K_L C_0)}\right) \quad (3)$$

C_0 denotes the initial dye concentration (mg L^{-1}). The R_L value was utilised to classify isotherm types as unfavourable (R_L>1), favourable (0<R_L<1), linear (R_L=1), or irreversible (R_L=0). The Freundlich isotherm, conversely, signifies the multilayer adsorption of dyes with cellular biomass and is expressed by Equation (4).

$$\ln q_e = \ln K_F + \left(\frac{1}{n}\right) \ln C_e \quad (4)$$

K_f represents the biosorption capacity (mg g^{-1}), whereas n denotes the biosorption intensity. The values of K_f and n were ascertained from the intercept and slope by graphing ln q_e against ln C_e.

Ultraviolet-visible Spectroscopy (UV-vis) Analysis

Biodegradation was assessed by analyzing the changes in the UV-visible spectra, specifically by comparing the peak shifts between the untreated dye (control) and treated dye. The treated samples were expected to show a reduction or complete disappearance of the absorbance peaks, indicating the occurrence of dye degradation following treatment with fungal cultures.

On day 14, untreated dye (control) and treated dye underwent centrifugation at 10,000 rpm for 10 minutes. Subsequently, the supernatants were collected, and their absorption spectra were measured using a UV-visible spectrophotometer across 400 nm to 800 nm wavelength. The spectral peaks of untreated and treated samples were compared [23].

Enumeration of Viable Cells

The resilience of fungal monoculture and consortium (*Penicillium* sp. and *Aspergillus* sp.) to RBBR dye was determined by counting the viable cells on the first and last day of dye treatment. Dye samples were collected on day 1 and day 14, followed by serial dilutions of the dye solution, which were spread on Potato Dextrose Agar (PDA) and incubated at 30°C for 7 days. The viable cells percentage was determined using the formula provided Equation (5) [24].

Optimization Study

The study examined the influence of various parameters on process optimization, such as pH, initial dye concentration (mgL^{-1}), agitation speed (rpm), biomass (g), and oxygen presence using the One-Factor-at-a-Time (OFAT) method. The OFAT approach simultaneously altered a single variable while maintaining the other variable at a constant level, enabling a focused analysis of the impact of each parameter on the optimization process [25].

Samples were collected every 24 hours over 14 days, with 3 ml aliquots of untreated dye (control) and treated dye being withdrawn and centrifuged for 10 minutes at 10,000 rpm. The absorbance of the resulting supernatant was then measured. The parameters were tested within the following ranges: pH values (5, 7, 9); initial dye concentrations (40, 60, 80, 100, 120 mgL^{-1}); agitation speeds (50, 100, 150 rpm); and biomass (1, 2, 4, 6, 8 g). The presence of oxygen for dye decolourization was investigated by introducing 2 g of fungal biomass (monoculture and consortium) into 100 mL of dye solution (100 mgL^{-1}) overlayed with 2 mL of paraffin oil to create anaerobic conditions. Meanwhile, aerobic conditions were simulated by omitting the paraffin oil in parallel experiments [25].

Statistical Analysis

The experiments were conducted in triplicate, and the resulting data were collected accordingly. Statistical analysis will be carried out using One-Way Analysis of Variance (ANOVA) with the Statistical Package for the Social Sciences (SPSS). Mean values were compared using Duncan's Multiple Range Test (DMRT) at a significance level of P<0.05 [26].

$$\text{Viable cells (\%)} = \frac{\text{live cell count on the last day of experiment}}{\text{live cell count on the first day of the experiment}} \text{ x } 100 \quad (5)$$

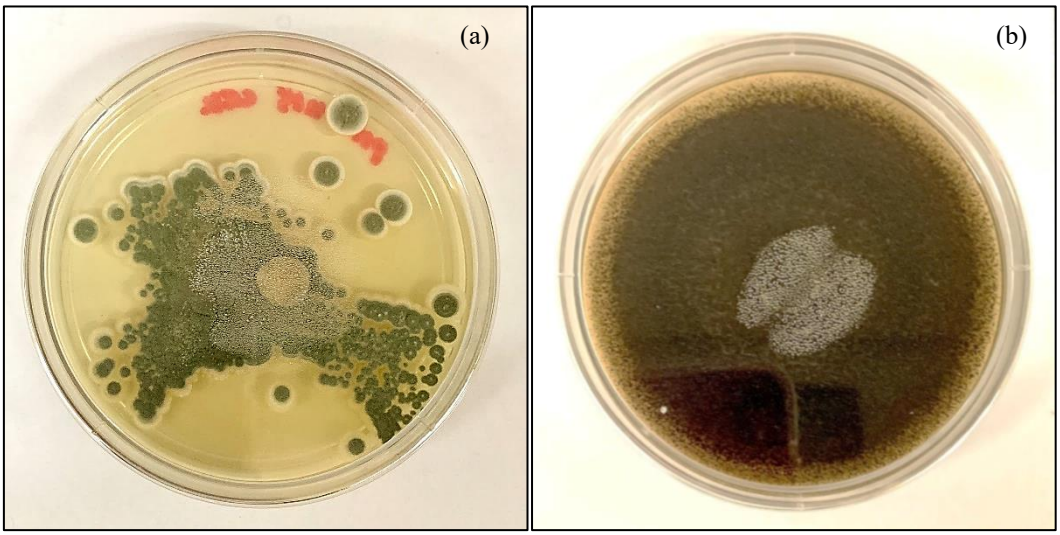

Figure 1. Top viewed of fungal culture on Potato Dextrose Agar; a) *Penicillium* sp.; b) *Aspergillus* sp.

RESULTS AND DISCUSSION

Establishment of Fungal Culture

Fungal isolates of *Penicillium* sp. and *Aspergillus* sp. sourced from the collection at the School of Biology, Universiti Teknologi MARA Cawangan Negeri Sembilan (UiTMCNS), underwent cultivation and periodic sub-culturing on Potato Dextrose Agar (PDA) under ambient conditions. Colonies of *Penicillium* sp. displayed distinct morphological attributes, characterized by vigorous sporulation and a predominantly bluish-green colouration at the centre with a white periphery and well-defined margins. The reverse side of the colony exhibited a yellowish-grey hue, accompanied by a velvety texture and an organized arrangement of phialoconidia spores (Figure 1a). These morphological characteristics are consistent with descriptions provided in existing literature for *Penicillium* sp. [27, 28].

Conversely, colonies of *Aspergillus* sp. initially appeared white on PDA, gradually darkening to black due to spore production over time. The reverse side of the colony presented a colourless to light yellow appearance, characterized by a cottony texture and a rough, irregular surface of conidia. (**Figure 1(b)**). These observations align with descriptions offered by prior studies for *Aspergillus* sp., a species commonly encountered in various research endeavours [29-31]. Subsequent transfer of mycelial plugs into Potato Dextrose Broth (PDB) for biomass generation revealed morphological characteristics consistent with those observed for *Penicillium* sp. and *Aspergillus* sp. These fungi are notable for their enzyme production, including laccases and peroxidases, which facilitate the degradation of complex dye structures. Consequently, they are promising candidates for the remediation of various pollutants [32]. The morphological observations corroborate existing literature and highlight the potential of these fungi for environmental remediation applications.

Dye Decolourization Activity

The decolourization efficiency (DE%) of Remazol Brilliant Blue R (RBBR) dye by monocultures of *Penicillium* sp. and *Aspergillus* sp., as well as a consortium combining both species revealed decolourization efficiency (DE%), yielded noteworthy results. *Penicillium* sp. exhibited the highest decolourization potential, followed by consortium (*Penicillium* sp. and *Aspergillus* sp.) and *Aspergillus* sp. Comparatively, *Penicillium* sp. (monoculture) exhibited the highest decolourization efficiency, achieving a DE% of 42.1%, while the consortium and *Aspergillus* sp. recorded DE% values of 29.9% and 24.2%, respectively (**Figure 2**). *Penicillium* sp. demonstrated the most rapid decolourization rates, reaching 28.3% and 31.1% on days 6 and 7, respectively. Meanwhile, the fungal consortium exhibited DE% values of 17.9% and 20.1%, and *Aspergillus* sp. achieved DE% values of 14.3% and 16.3% over the same period (**Figure 3**). The accelerated decolourization rates on days 6 and 7 may be attributed to factors such as the initial lag phase of cell growth, adaptation to the toxic effects of the dyes, and the time required for enzyme production necessary for degradation [33, 34].

These findings provide novel insights into the efficiency of different fungal treatments (monoculture and consortium) for dye decolourization. This study suggests that *Penicillium* sp. monocultures can surpass the decolourization activity of a consortium comprising species of *Penicillium* and *Aspergillus*. This aligns with previous research by [35], which highlighted the effectiveness of *Penicillium* sp. in decolourizing various textile dyes by producing

ligninolytic enzymes, including laccases and peroxidases, which play a role in dye degradation. This enhanced efficiency is likely due to the specialized enzymatic pathways employed by *Penicillium* sp., which may surpass those used by *Aspergillus* sp. [35]. Although this study did not delve into the specific enzymes involved, previous research has shown that producing various enzymes, such as laccase, azoreductase, and peroxidases, significantly contributes to breaking down dye molecules. These enzymes facilitate the degradation of complex dye structures, highlighting the efficiency of monocultures in such processes [36]. Future studies could further explore these enzymatic roles and assess the practical implications for industrial dye waste treatment [37].

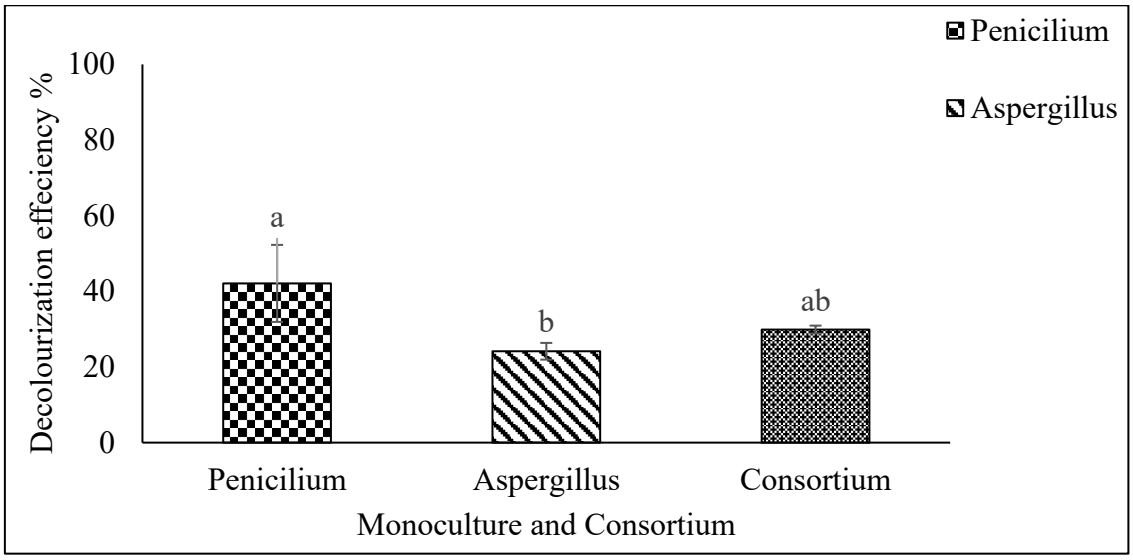

Figure 2. Comparison of maximum decolourization efficiency (DE%) by monoculture and consortium on RBBR dyes using non-optimized conditions (biomass 2 g, agitation speed 100 rpm, incubation at 30°C). Means with the same letter are not significantly different at HSD ($_{0.05}$), and the bars indicate the standard error of the mean.

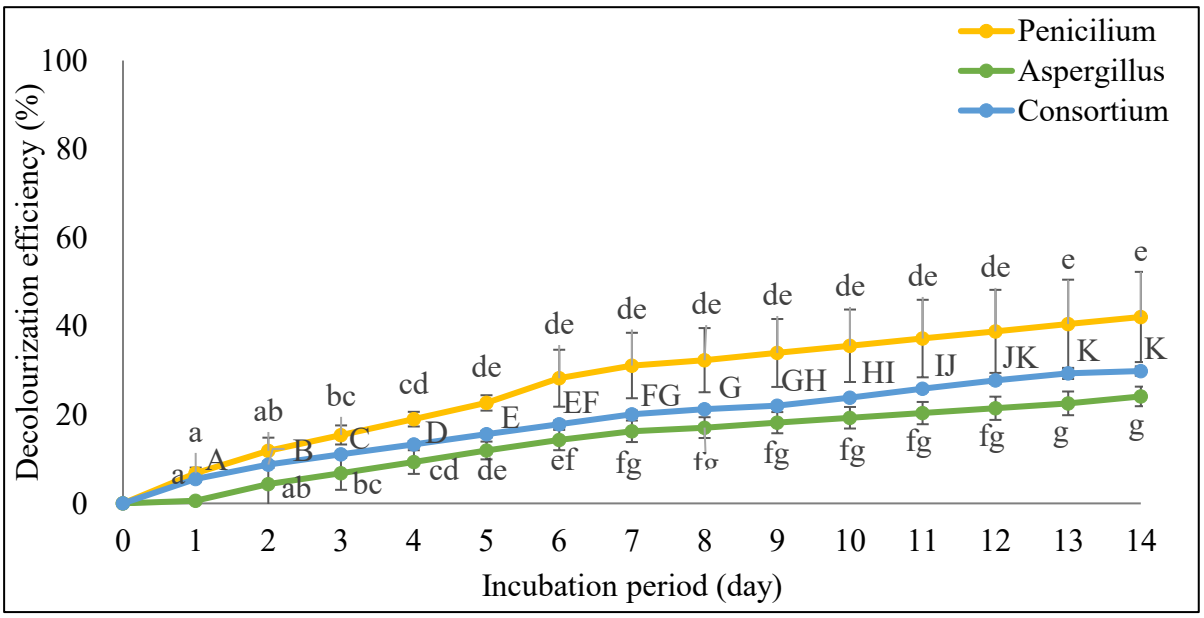

Figure 3. Decolourization efficiency (DE%) of RBBR dye by monoculture and consortium using non-optimized conditions (biomass 2 g, agitation speed 100 rpm, incubation at 30°C) throughout the 14 days of the incubation period. Means with the same letter are not significantly different at HSD ($_{0.05}$), and the bars indicate the standard error of the mean.

Conversely, lower decolourization achieved by consortium may be attributed to the antagonistic interactions among fungal isolates that may impede dye degradation. In addition, one of the other two fungi in the consortium may produce a metabolite that inhibits the dye biodegradation pathway leading to this effect. A similar finding was reported by [38] that documented the superior decolourization of Cibacron Brilliant Red achieved by the fungal consortium (*Aspergilus terrus* or *Aspergillus fumigatus*) as compared to fungal monoculture. Microbial consortiums may also experience challenges such as resource competition, leading to less efficient and less coordinated dye degradation processes [39]. Although various studies have highlighted the potential of *Penicillium* sp. in the bioremediation field [37, 40], this study revealed the need to explore synergistic interactions among microbial isolates used as biological agents. However, factors like nutrient availability and environmental stressors influence their coexistence, suggesting complex growth dynamics and competitive interactions rather than symbiotic relationships [41].

Biosorption Isotherm Models

The sorption mechanisms of RBBR dye by monoculture and consortium demonstrated a strong fit with the Langmuir isothermal model, displaying elevated R^2 values for both monoculture (R^2=0.999) and consortium (R^2=0.998) as illustrated in **Table 1** and **Figure 4**. The monoculture exhibited notably elevated values for maximum biosorption capacity (q_m=11.2) and Langmuir constant (K_L=0.012) as shown in **Table 1**. In the context of the consortium, the observed lower values of q_m (11.1) and K_L (0.010) suggest that monoculture is a more effective approach for the sorption of RBBR dyes, as indicated in **Table 1**. The separation factor values for the Langmuir model were observed to be within the range of 0 to 1, with monoculture at 0.350 and consortium at 0.402. This suggests that the dye sorption was advantageous, validating that the experimental data aligned with the Langmuir isotherm (**Table 1**). The analysis of the Freundlich model indicated that monoculture exhibited elevated K_F (1.78) and n (1.54) values, suggesting a more robust interaction with dye molecules in comparison to the consortium (K_F: 1.43; n: 1.67) (**Table 1**). However, the lower R^2 values for monoculture (R^2=0.985) and consortium (R^2=0.977) indicated that the Freundlich isotherm was not a suitable model. This suggested that RBBR dye sorption was uniform and did not include multilayer sorption. The alignment of experimental data with the Langmuir model indicated that the uptake of RBBR dye by *Penicillium* sp. was uniform and took place through monolayer sorption, ultimately resulting in the saturation of dye molecules on the binding sites of the cell surface [42]. A similar finding was reported by [43], who studied the kinetic behaviour of *Penicillium* species using various sorption models (Langmuir, Freundlich, Temkin, Dubinin-Radushkevich, and Sip isotherms) for the bioremediation of Congo red dye. In another study on the biosorption of Reactive Yellow 145 dye by *Penicillium* species, the results aligned well with the Langmuir and Freundlich isotherms models, indicating reliable sorption efficiency [44]. This study, therefore, highlights the potential of utilizing fungal monoculture for the sorption of RBBR dye. The occurrence of biosorption on the cell surface with dye molecules can be verified via Fourier-transform infrared spectroscopy (FTIR) analysis [22].

Enumeration of Viable Cells

Enumeration of viable cells provides insights into the metabolic activity and growth of microorganisms, which is essential for understanding their effectiveness in degrading synthetic dyes. Viable plate counts help correlate active cell numbers with decolourization efficiency, thereby revealing the relationship between fungal biomass and dye degradation. Following the evaluation of decolourization activity, *Penicillium* sp. demonstrated the highest efficiency in decolourization, followed by the consortium (*Penicillium* sp. and *Aspergillus* sp.) and *Aspergillus* sp. alone. Consequently, the decision was made to proceed with subsequent experimental methods utilizing only *Penicillium* sp. and the consortium (*Penicillium* sp. and *Aspergillus* sp.) based on their superior decolourization activity performance.

Viable cell enumeration showed that *Penicillium* sp. had a significantly higher number of live cells by day 14 compared to day 1, outperforming the consortium. Specifically, *Penicillium* sp. exhibited a remarkable cell recovery rate of 116.16% in the presence of Remazol Brilliant Blue R (RBBR) dye, while the consortium achieved only 0.33% recovery. This enhanced tolerance to RBBR dye suggests a superior decolourization efficiency of *Penicillium* sp., which aligns with previous studies reporting a higher cell recovery of 188% for *Penicillium simplicissimum* when exposed to cotton blue dye due to a more significant number of viable cells available for dye interaction [45, 46].

Table 1. Biosorption isotherm models for the sorption of RBBR dyes by monoculture and consortium cells.

Cells	Dyes	Langmuir			Freundlich		
		q_m	K_L	R^2	n	K_F	R^2
Monoculture	RBBR	11.21	0.012	0.999	1.54	1.78	0.985
Consortium		11.11	0.010	0.998	1.67	1.43	0.977

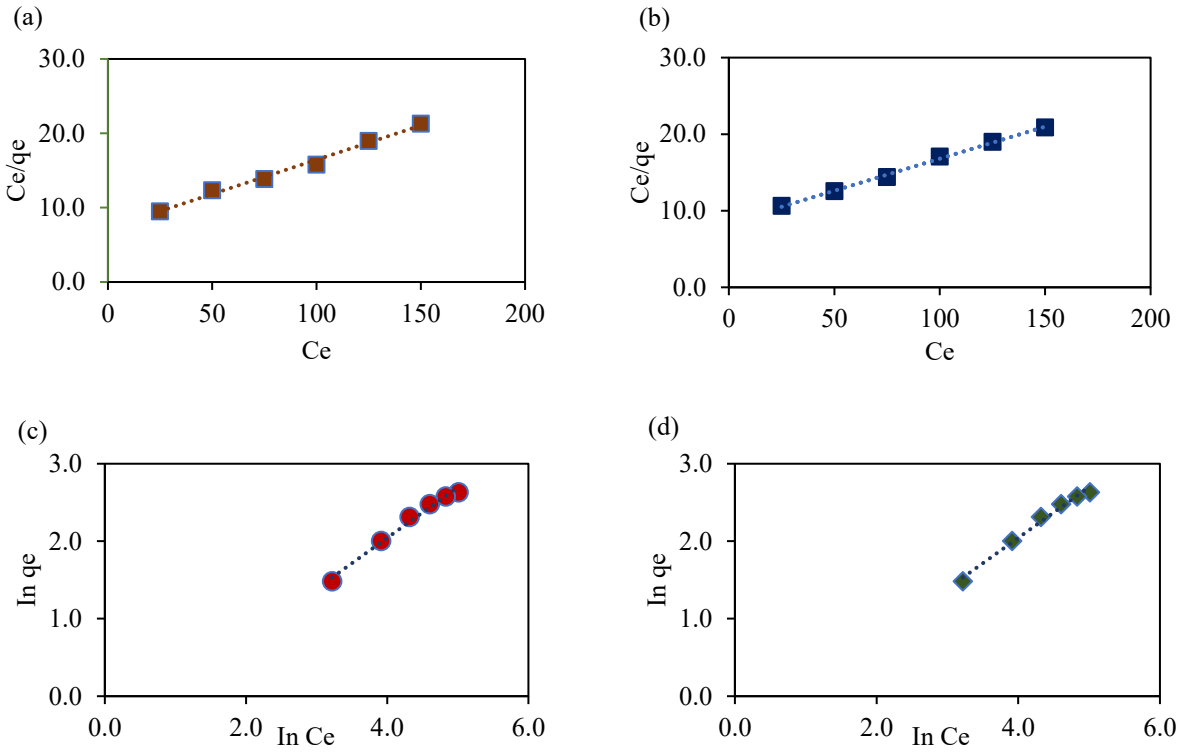

Figure 4. Isotherm model based on (a) Langmuir model for the sorption of RBBR dyes by monoculture and (b) consortium; (c) Freundlich model for the sorption of RBBR dyes by monoculture and (d) consortium.

In contrast, a reduced percentage of viable cells in the consortium indicates a decline in cell population, which reflects diminished dye decolourization efficiency. Supporting evidence from previous research shows that viable cell counts decreased over time, especially when exposed to high dye concentrations that are toxic to cells. This decline suggests that the metabolic activity of the cells may diminish under the stress of dye degradation, leading to a lower viable cell count on the final day of the experiment [47, 48]. Additionally, the prevalence of mycotoxins produced by fungi, including *Aspergillus* sp. and *Penicillium* sp., can inhibit the growth of competing fungi. This suggests that secondary metabolites produced by one genus may negatively affect the other, reinforcing the competitive dynamics between these fungi [49]. This interaction highlights the complex nature of microbial competition and its impact on dye decolourization processes.

Biodegradation of RBBR Dye by Fungal Monoculture and Consortium

The removal of RBBR by fungal monoculture and consortium indicated the occurrence of biodegradation and biosorption. In this study, ultraviolet-visible (UV-vis) analysis (400–800 nm) of treated dye indicated decolourization and reduction in dye concentration compared to untreated dye (control). Specifically, the disappearance of the absorption peak at 590 nm, which is characteristic of RBBR dye, suggests significant biodegradation of the dye. In contrast, the untreated dye control peak at 590 nm, suggesting that the dye chromophores remained intact and detectable, indicating the absence of biosorption and biodegradation [23].

The central peak for monoculture (*Penicillium* sp.) decolourized RBBR dye at 590 nm decreased in intensity with high decolourization efficiencies compared to the consortium (**Figure 5**). Similarly, a reduction in the absorption peak for RBBR dye was observed following treatment with both the monoculture and the consortium, signifying the breakdown of dye chromophores [50]. This indicates that the degradation of RBBR was due to chromophore breakdown [23]. The absence of peak is commonly used to indicate dye biodegradation, as similarly observed for the biodegradation of various dyes by *Aspergillus niger* [35], *Penicillium simplicissimum* [39], and *Lysinibacillus fusiformis* [45].

Even though the study did not identify the specific enzymes responsible for dye biodegradation, it is suggested that peroxidase and laccases may play a role in breaking down dye molecules into less toxic compounds [23]. Furthermore, previous studies have also demonstrated that microbial monocultures exhibit superior decolourization efficiency (DE%) compared to the consortium for various dyes (azo and reactive dyes). The ability of individual strains to produce specific enzymes and adapt to the presence of dyes often leads to enhanced performance in decolourization capability [51-54]. The occurrence of

dye biodegradation can be further confirmed via liquid chromatography-mass spectrometry (LC-MS), high-performance liquid chromatography (HPLC), and high-resolution mass spectrometry (HRMS), which commonly lead to the production of diverse simpler compounds [55].

Although the toxicity of the treated dye was not determined in this study but can be confirmed via a phytotoxicity test using seed germination such as *Vigna radiata*, as conducted by [23] on the phytotoxicity of triphenylmethane dyes by non-white rot fungus *Penicillium simplicissimum*. Additionally, an analysis of the elemental composition and surface morphology of the fungus before and after dye exposure could be conducted using SEM-EDX to indicate morphological changes in fungal cells, highlighting its tolerance and adaptability to RBBR dye [22]. Research has shown that dye toxicity can have serious effects on microbial cells, leading to issues such as cell distortion, elongation, or rupturing [56]. However, it is expected that no significant difference for *Penicillium* sp., as the number of viable cells achieved on the last incubation day was higher compared to the initial day.

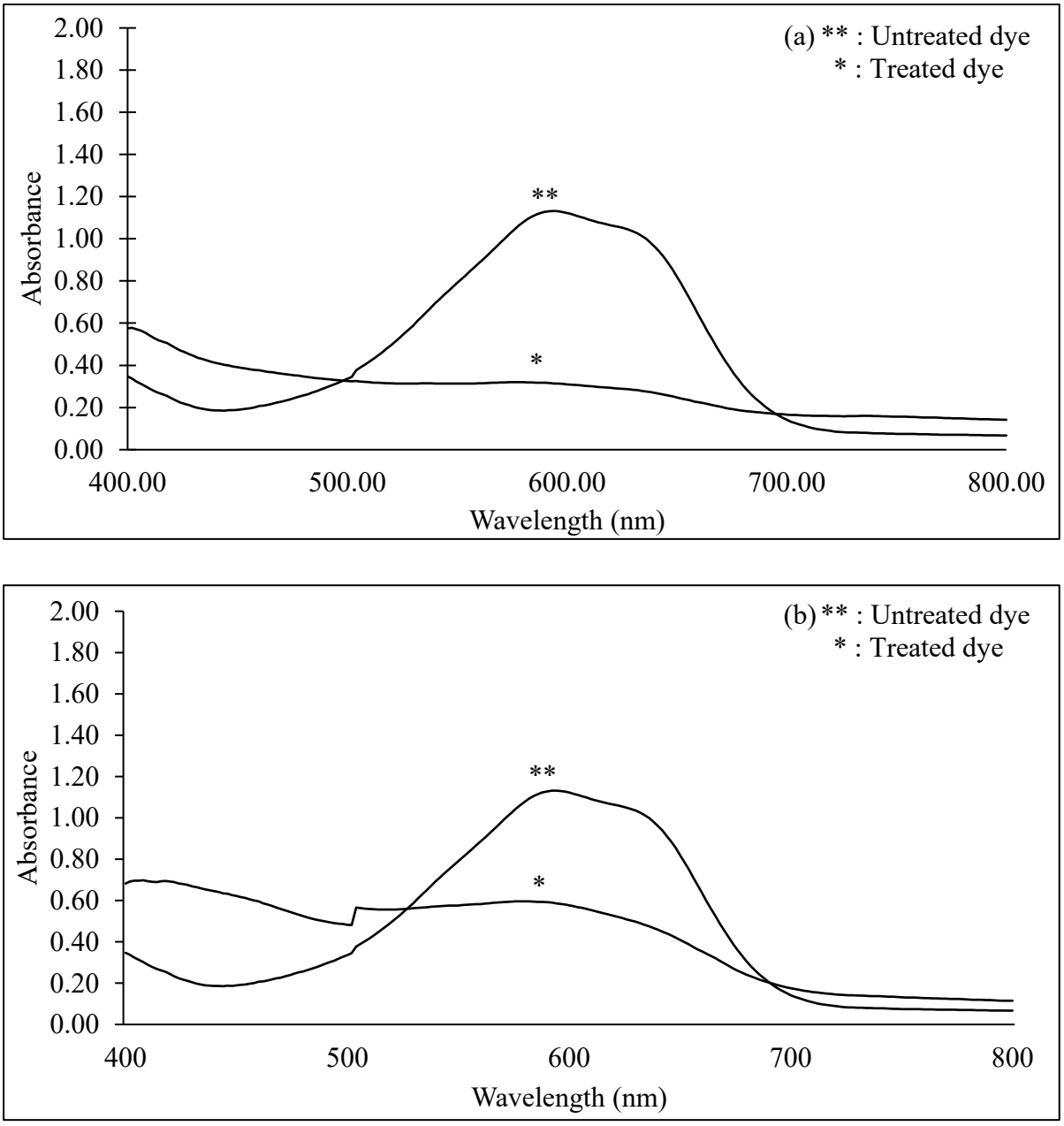

Figure 5. UV-vis absorption spectra (400-800 nm) of RBBR dyes before (untreated) and after treatment (treated) by (a) monoculture and (b) consortium.

Optimization Study

Effect of Different pH on Decolourization of RBBR Dyes by Monoculture and Consortium

The decolourization of RBBR dyes by both the monoculture (*Penicillium* sp.) and the consortium *(Penicillium* sp. and *Aspergillus* sp.) was significantly influenced by pH levels. Decolourization efficiency was optimal at a neutral pH of 7, with efficiencies of 46.6% for the monoculture and 46.3% for the consortium, respectively (**Figure 6**). At pH 5, the decolourization efficiencies were notably diminished, with the monoculture reaching 36.3% and the consortium at 33.6%. Conversely, at pH 9, higher decolourization efficiencies were achieved, reaching 79.3% for the monoculture and 78.3% for the consortium. These results demonstrate that while both the monoculture and consortium showed the best dye degradation at a neutral pH of 7, it could also effectively degrade a significant amount of dye at pH 9. This highlights the fungal ability to degrade dyes effectively, with a preference for a neutral pH. Tolerance to varying pH by dye-decolourizing microbes is essential, making them suitable for the practical biotreatment of dyeing mill effluents [57].

The alignment of optimal pH observed in this study is consistent with prior findings on the efficacy of fungi in decolourizing azo dyes. For instance, *Aspergillus fumigatus* demonstrated a 90% decolourization rate of Basic Yellow 3 at a neutral pH of 7. On the other hand, the study of *Penicillium simplicissimum* for decolourization rate of Methyl Violet (98%), Crystal Violet (95%), and Cotton Blue (82%) was higher at pH 7 [45]. In the case of Acid Violet 49, a broader pH ranges from 4.0 to 9.0 was found effective across different fungal isolates. Similarly, for Acid Orange 7, *Aspergillus fumigatus* achieved a maximum decolourization of 90% at pH 7. In comparison, Aspergillus *niger* and *Fusarium moniliforme* exhibited decolourization rates of 79% and 78%, while *Penicillium purpurogenum* showed similarly high efficiency at pH 9, respectively. The results of this study align with previous findings, which also identified the most suitable pH range for colour removal to be between 5.5 and 9.0 under anoxic conditions [58].

On the other hand, studies found that the decolourization of Congo red dye by *Penicillium crustosum* PWWS-6 and malachite green by *Penicillium ochrochloron* underscores the importance of pH regulation in dye degradation processes [59, 60]. The consistency observed in the impact of ambient pH on intracellular pH, protein expression, and regulatory mechanisms, as elucidated in related literature [24], may underlie the observed trends. A study conducted by [61] found that the degradation of Red RR dye, Green dye, and Safranine dye was highest at pH 7, followed by pH 10 and pH 5. *Rhizopus stolonifer* was identified as the most effective fungus for decolourizing Red RR dye and Green dye across all 3 pH levels tested, followed by *Penicillium chrysogenum*, and the highest decolourization recorded was 67% for Green dye at pH 7 by *Rhizopus stolonifer*.

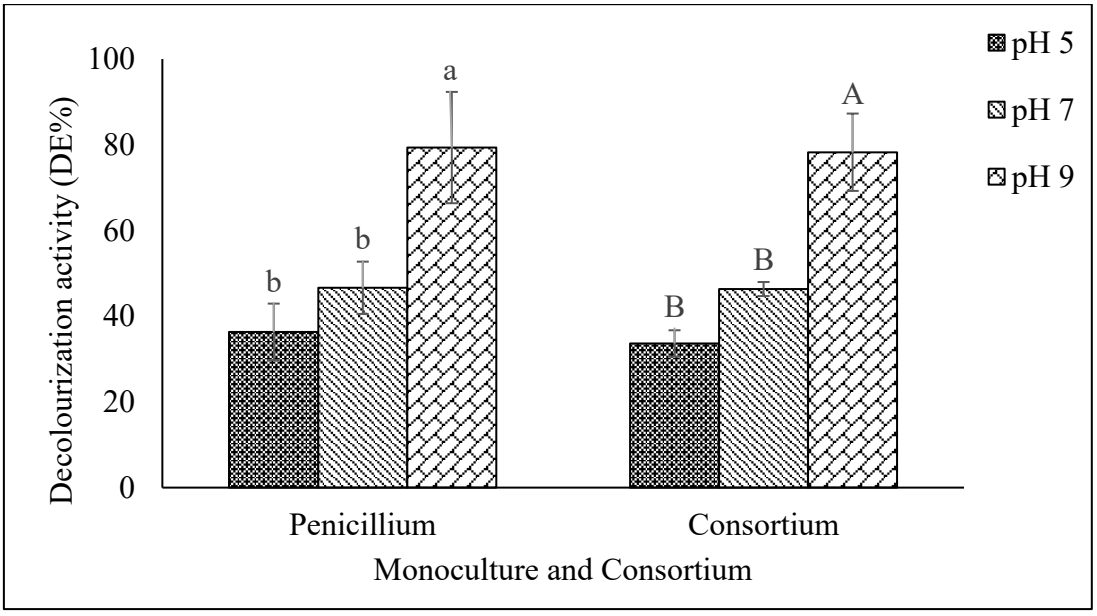

Figure 6. Effect of pH of RBBR dye by monoculture and consortium throughout the 14 days of the incubation period. Means with the same letter are not significantly different at HSD ($_{0.05}$), and the bars indicate the standard error of the mean.

Further research by [62] demonstrated that environmental pH variations significantly affect permease enzyme activity and metabolite export, suggesting that optimizing dye degradation may be most effective at neutral pH levels. A study by [63] found that dye degradation efficiencies at both acidic (pH 4) and alkaline (pH 9) conditions were comparable to those at neutral pH (pH 7), indicating that extreme pH conditions can adversely impact microbial growth. Similarly, [64] observed reduced decolourization efficiency with increasing pH and dye concentration. [65] further noted that lower pH conditions hinder decolourization due to competition between dye cations and H^+ ions.

Notably, the electrostatic interaction between negatively charged biomass and positively charged dye cations is more vital at higher pH levels. [63] emphasized that pH critically influences the transport of dye molecules across cell membranes, a critical factor in the decolourization process. These findings underscore the intricate relationship between pH, microbial physiology, and dye degradation, providing important insights for optimizing bioremediation strategies.

Effect of Different Dye Concentrations on Decolourization of RBBR Dyes by Monoculture and Consortium

The study demonstrated that the initial dye concentration significantly influenced the decolourization of RBBR dyes. The optimum dye concentrations for removing RBBR dyes by monoculture (*Penicillium* sp.) and the consortium (*Penicillium* sp. and *Aspergillus* sp.) were determined to be 100 mgL^{-1}. Decolourization achieved by both monoculture and consortium at lower dye concentrations (40, 60, and 80 mgL^{-1} for monoculture and consortium) did not exhibit significant differences from the decolourization efficiency at the optimal concentration. However, decolourization efficiency decreased considerably for both monoculture and consortium at higher dye concentrations, particularly at 120 mgL^{-1}. The monoculture achieved decolourization efficiencies of 74.8%, 66.1%, 65.6%, 43.8%, and 39.3% for dye concentrations of 40, 60, 80, 100, and 120 mgL^{-1}, respectively. In contrast, for the consortium, decolourization efficiencies were 37.6%, 39.1%, 36.2%, 23.8%, and 20.8% for the corresponding dye concentrations (**Figure 7**). The decrease in decolourization efficiency observed at higher dye concentrations may be attributed to the potential toxicity exerted on live cells, leading to compromised viability and enzyme activities, consequently impacting decolourization efficacy [60].

For instance, *Aspergillus niger* showed a decolourization efficiency of approximately 48% for Remazol Brilliant Blue R (RBBR) at 200 mgL^{-1}, with efficiency decreasing as dye concentrations increased, indicating inhibitory effects on fungal activity [66]. Similar findings are observed in *Penicillium* sp., where elevated dye concentrations negatively impact decolourization due to the toxic effects on fungal metabolism. Studies by [67] and [68] highlight that the efficiency of dye decolourization by laccase enzymes is also compromised at high dye concentrations. [67] reported that high dye concentrations lead to toxic byproducts that inhibit further decolourization. At the same time, [68] found that laccase from *Pichia pastoris* was effective at lower dye concentrations but showed reduced efficiency and increased reaction times at higher concentrations.

A study by [69] emphasized that dye concentration is a critical factor influencing decolourization efficiency. The study indicated that increased RBBR concentrations resulted in decreased decolourization percentages, highlighting the inhibitory effects of high dye concentrations on microbial activity. This trend is further supported by findings from [50], who observed decreased decolourization efficiency from 90% to 50% for Methyl Orange as the concentration increased from 100 mgL^{-1} to 500 mgL^{-1}. Similarly, [70] found that *Aspergillus flavus* decolourized Crystal Violet by 85% at 50 mgL^{-1} but only 40% at 200 mgL^{-1}. Further research by [71] on *Penicillium* sp. and Reactive Black 5 revealed a decline in decolourization efficiency from 92% to 60% as the dye concentration increased from 100 mgL^{-1} to 500 mgL^{-1}. Additionally, [72] reported that *Aspergillus oryzae* achieved 78% decolourization of an azo dye at 200 mgL^{-1}, which decreased to 30% at 1000 mgL^{-1}. Collectively, these studies indicate that higher dye concentrations adversely affect the decolourization efficiency of fungi. This reduction in efficiency is primarily due to the toxic effects of the dyes on the microorganisms, impairing their viability and enzymatic activities essential for effective dye degradation.

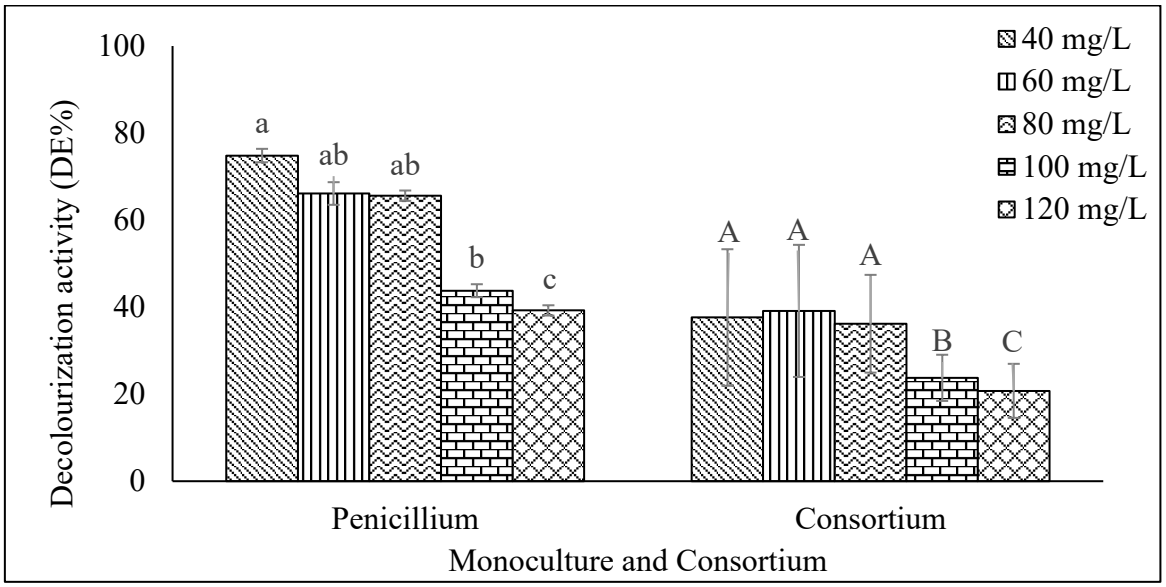

Figure 7. Effect of concentration of RBBR dye by monoculture and consortium throughout the 14 days of the incubation period. Means with the same letter are not significantly different at HSD ($_{0.05}$), and the bars indicate the standard error of the mean.

Effect of Different Agitation Speeds on Decolourization of RBBR Dyes by Monoculture and Consortium

The decolourization of Remazol Brilliant Blue R (RBBR) dyes is influenced by agitation speed, with discernible impacts observed across varying speeds. The optimal decolourization efficacy was attained at an agitation speed of 50 revolutions per minute (rpm) for both monoculture (*Penicillium* sp.) and the consortium (*Penicillium* sp. and *Aspergillus* sp.). Conversely, agitation speeds of 100 rpm and 150 rpm resulted in less favourable outcomes compared to the optimal speed of 50 rpm. Specifically, at 50 rpm, monoculture and consortium configurations exhibited respective decolourization efficiencies of 51.2% and 32.6%, while at 100 rpm, these efficiencies were recorded at 54.1% and 33.4%, and at 150 rpm, at 68.2%, and 33.5% (**Figure 8**). This phenomenon at 50 rpm is attributed to the heightened distribution of nutrients and oxygen transfer facilitated by agitation [73]. Moreover, agitation at 50 rpm promotes enhanced decolourization efficacy by facilitating increased surface contact and distribution of dye molecules, thus augmenting dye absorption [74].

In related studies, *Aspergillus nidulans* have the ability to survive and utilize anaerobic metabolic pathways under low oxygen conditions for using azo dyes [75]. On the other hand, A study by [51] demonstrated higher decolourization efficiency (73–82%) for triphenylmethane dye (Malachite Green, Methyl Violet, and Crystal Violet) under static conditions after treatment with *Penicillium simplicissimum*. Similarly, a study conducted by [76] shows that the decolourization percentages on 22 dyes across various chromophore groups, anthraquinone, azo and triphenylmethane, were notably lower under agitation compared to static conditions at the same pH (3.2) and temperatures (30°C and 35°C) over 24 hours.

Furthermore, [24] observed that while the difference in decolourization efficiency between 50 rpm and 150 rpm was not statistically significant, reducing agitation speed from higher to lower speeds markedly improved performance. This suggests lower speeds better facilitate interactions between the dye and microbial. This finding is supported by [77], who demonstrated that lower agitation speeds enhance the degradation and decolourization of lignin derivatives, indicating that excessive agitation might hinder treatment efficiency.

As a concluding remark, the findings from these studies underscore the importance of understanding and applying the role of optimal agitation speeds in enhancing dye decolourization. These speeds improve oxygen transfer, nutrient distribution, and cell-dye interactions. However, it is also evident that some agitation is crucial to facilitate the contact between the dye and the decolourizing agents, thereby ensuring effective treatment.

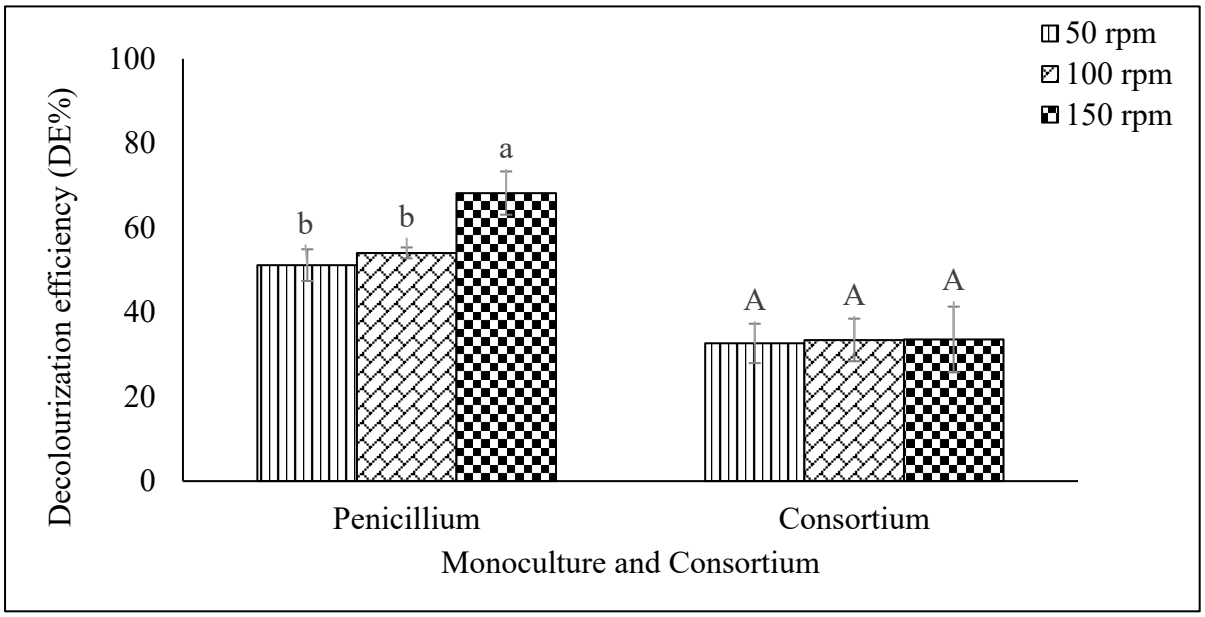

Figure 8. Effect of agitation speed of RBBR dye by monoculture and consortium throughout the 14 days of the incubation period. Means with the same letter are not significantly different at HSD ($_{0.05}$), and the bars indicate the standard error of the mean.

Effect of Different Biomass on Decolourization of RBBR Dyes by Monoculture and Consortium

The findings indicated that the optimal biomass quantity for effectively removing Remazol Brilliant Blue R (RBBR) dye among the various biomass is 2 g. **Figure 9** demonstrates that decolourization efficiency escalates with increasing biomass quantity. Using fungal monoculture (*Penicillium* sp.) and the consortium (*Penicillium* sp. and *Aspergillus* sp.), a minimum biomass threshold of 2 g was identified, resulting in a significant enhancement in decolourization efficiency compared to the utilization of 1g. Notably, monoculture achieved a maximum decolourization efficiency of 57.3% with 2 g of cell biomass, with marginal increases observed at 4 g, 6g, and 8g biomass quantities. Conversely, the consortium exhibited no significant difference between 1g (22.95%) and 2 g (23%) biomass quantities as the minimum requirement for decolourization efficiency. However, the consortium with 1g of biomass exhibited a delayed onset of rapid decolourization efficiency, commencing on day 12, in contrast to the observed increase in decolourization efficiency with 2 g biomass as the incubation period progressed.

The influence of biomass on the decolourization efficiency of dyes is a critical factor in optimizing bioremediation processes. Research indicates that a biomass concentration of around 2 g is often optimal for significantly improving dye decolourization. For instance, studies have shown that using 2 g of biomass can lead to a decolourization efficiency of approximately 97% for the dye Alizarin Yellow R, utilizing the basidiomycete *Mycena purpureofusca*, which highlights the effectiveness of this concentration in achieving high decolourization rates [78]. This enhanced efficiency is attributed to the increased secretion of enzymes by the fungi, which are essential for the biodegradation of dyes [79].

Moreover, the relationship between biomass quantity and decolourization efficiency is well-established, where higher biomass yields better results. For example, a study demonstrated that the decolourization of Reactive Black 5 reached up to 76% efficiency using the bacterium *Aeromonas hydrophila* under optimized conditions, emphasizing the importance of biomass in the process [80]. Similarly, another study reported significant degradation of Methyl Orange at a concentration of 750 mgL^{-1}, utilizing the bacterium *Pseudomonas aeruginosa*, further supporting the notion that higher biomass levels correlate with improved decolourization efficiency [81]. Conversely, lower biomass levels have been associated with reduced enzyme production and, consequently, lower decolourization efficiency [82].

However, balancing the biomass concentration with practical considerations is crucial, as more than 2 g may incur additional costs and time, making it less feasible for large-scale applications [51]. Therefore, while increasing biomass can enhance decolourization rates, the optimal concentration of 2 g is a practical and effective choice for maximizing efficiency in dye remediation processes [74].

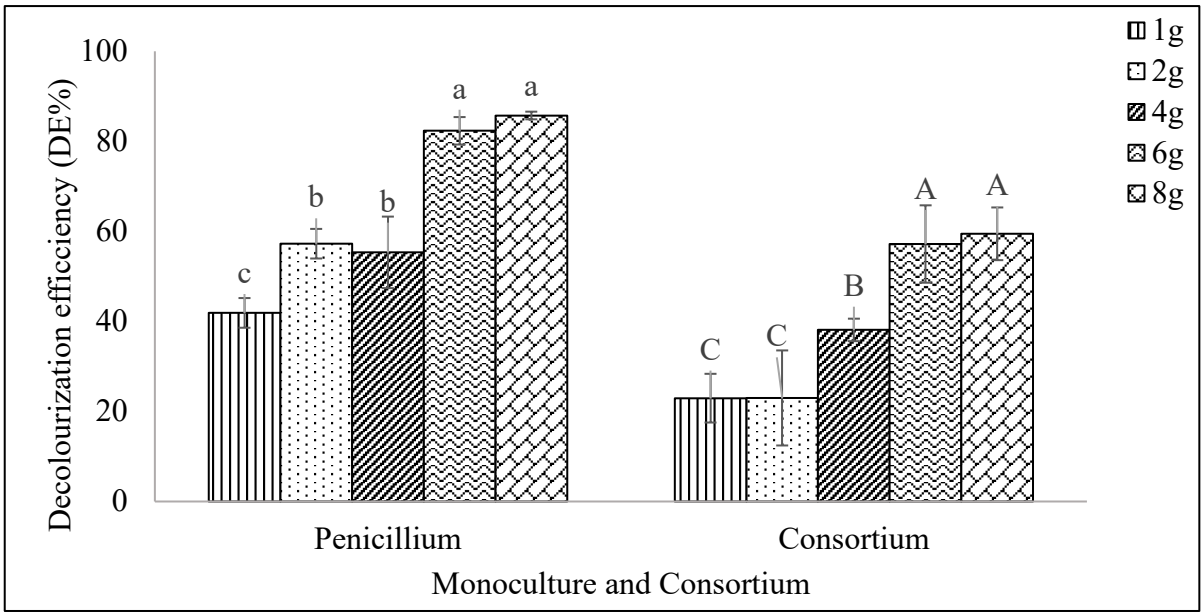

Figure 9. Effect of biomass of RBBR dye by monoculture and consortium throughout the 14 days of the incubation period. Means with the same letter are not significantly different at HSD ($_{0.05}$), and the bars indicate the standard error of the mean.

Effect of Oxygen on Decolourization of RBBR Dyes by Monoculture and Consortium

The decolourization efficiency of Remazol Brilliant Blue R (RBBR) dye was significantly influenced by oxygen availability. The findings indicate that the decolourization efficiency of RBBR dyes is notably higher in anaerobic environments. Specifically, monoculture and consortium systems achieved 68.6% and 42% decolourization efficiencies in the absence of oxygen, respectively. In contrast, the presence of oxygen led to inferior decolourization activity, with significantly lower efficiencies observed for monoculture (50.5%) and consortium (33.7%) (**Figure 10**). To our knowledge, this study represents the first documentation of fungal monoculture (*Penicillium* sp.) and consortium (*Penicillium* sp. and *Aspergillus* sp.) exhibiting heightened decolourization activity under anaerobic conditions compared to aerobic conditions.

Although research on RBBR dye decolourization under anaerobic conditions is limited, similar trends have been observed with azo dyes, which often show increased decolourization under anaerobic conditions [83]. This difference in decolourization efficiency is mainly because azo reduction enzymes are susceptible to the presence of oxygen, which impairs their activity in aerobic settings [84]. Additionally, the electron-withdrawing nature of the azo bond renders dye molecules less susceptible to oxidative reactions, thereby conferring resistance to aerobic biodegradation [85]. Given that azo dyes are also commonly used in the textile industry, insights gained from their decolourization under anaerobic conditions can help understand the behaviour of other dye types, including RBBR.

Reports from previous studies align with the current findings, demonstrating that oxygen presence or absence significantly impacts dye decolourization efficiency by various fungal species. While studies on RBBR dye decolourization under anaerobic conditions are limited, there is notable evidence from studies on azo dyes indicating that anaerobic environments can enhance decolourization. For instance, [86] reported that the decolourization of azo dyes was markedly more efficient under anaerobic conditions compared to aerobic ones, with anaerobic conditions facilitating the breakdown of the azo bond and resulting in higher decolourization rates. Similarly, [87] found that various azo dyes achieved greater decolourization efficiency when treated under anaerobic conditions with mixed microbial cultures. The absence of oxygen boosted enzymatic activity critical for degrading complex dye structures [87]. Previous study by [88] also noted that aerobic conditions led to lower decolourization rates due to reduced azoreductase enzyme activity in bacterial strains. This suggests that anaerobic conditions might be more favourable for higher decolourization efficiencies. These studies highlight the importance of oxygen levels in optimizing dye decolourization processes.

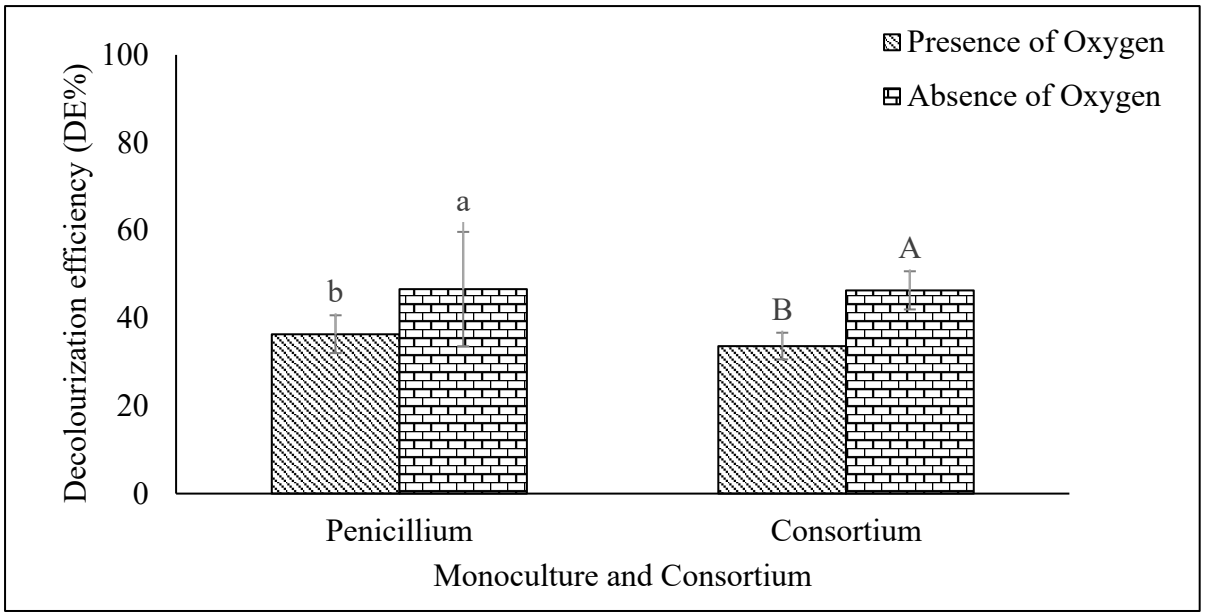

Figure 10. Effect of oxygen of RBBR dye by monoculture and consortium throughout the 14 days of the incubation period. Means with the same letter are not significantly different at HSD ($_{0.05}$), and the bars indicate the standard error of the mean.

Even though fungi generally thrive in aerobic conditions, there is some evidence that aerobic microorganisms can perform anaerobic degradation. For example, [89] demonstrated that *Bacillus cereus* DC11 could decolourize over 55% of Acid Blue 25 under anaerobic conditions, compared to less than 5% under aerobic conditions. This suggests that anaerobic environments offer alternative electron acceptors, such as the oxygen in carbonyl groups of anthraquinone dyes. This implies that aerobic microorganisms may be able to secrete enzymes that support anaerobic functions [90]. Additionally, obligately aerobic fungi like *Aspergillus nidulans* have shown the ability to survive and utilize anaerobic metabolic pathways, including alcoholic fermentation, under low oxygen conditions. Thus, these fungi may also be capable of degrading azo dyes under anaerobic conditions [75]. This finding underscores the importance of oxygen conditions in optimizing dye decolourization processes.

CONCLUSION

This study elucidated the decolourization potential of both monoculture (*Penicillium* sp.) and consortium (*Penicillium* sp. and *Aspergillus* sp.) on RBBR dye. This comprehensive finding revealed the biosorption and biodegradation ability of both fungal monoculture and consortium, highlighting their potential for environmentally sustainable dye remediation treatments. Nonetheless, the predominance of either mechanism was not discerned, as both mechanisms typically coexist. The application of monoculture, particularly *Penicillium* sp., yielded more pronounced results compared to the consortium, highlighting their potential as biological agents for the degradation of RBBR dye. They can be further investigated for their mechanisms and optimized for applications in wastewater treatment offering valuable insights for enhancing bioremediation techniques and advancing dye removal technologies.

ACKNOWLEDGEMENTS

Authors acknowledge the Universiti Teknologi MARA for funding under the Geran Penyelidikan MyRA [600-RMC/GPM LPHD 5/3 (092/2022)] and Geran Inisiatif Penyeliaan (GIP) [600-RMC/GIP 5/3 (099/2023)].

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