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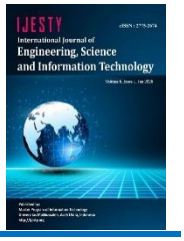

Biodecolorization of Azo Dye using Trametes sp. UM 12 Isolated from East Kalimantan

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Abstract

The textile industry releases substantial amounts of azo dyes, including Reactive Black 5 (RB5), which persist in the environment due to their stable chemical structures and toxic properties. White-rot fungi-based biological treatment is an environmentally friendly and affordable method for removing dyes from water by producing ligninolytic enzymes, including laccases. The research assesses *Trametes* sp. UM 12's decolourisation ability compared to Phanerochaete chrysosporium for RB 5 decolourisation efficiency. The research examined the effects of pH, temperature, agitation speed, and initial RB5 concentration on fungal decolourisation. The optimal removal of *Trametes* sp. was achieved at pH 5 and 30–35°C with agitation at 150 rpm, resulting in more than 95% colour removal within 48–72 hours, and it reached 99.7% decolourisation after 96 hours at 100 mg/L RB5. The process removed 98.7% and 96.8% of the dye at 100 mg/L and 250 mg/L after 120 hours, but only 52.1% and 31.8% at 500 mg/L and 1000 mg/L, respectively. The process shows substrate inhibition and enzyme saturation at high pollutant concentrations. The research shows *Trametes* sp. UM 12 outstanding performance, making it suitable for treating wastewater in tropical environments.

Keywords: *Decolourisation, Dye, Laccase, RB5, White Rot Fungi.*

1. Introduction

The amount of wastewater pollution from synthetic dyestuff contamination has increased significantly. The textile industry releases 2.0 × 105 tons of dyes into wastewater streams annually [1]. The textile industry uses azo dyes as its primary synthetic dyestuff category, accounting for 70% of all dyes employed [2][3]. The textile industry uses Reactive Black 5 (RB 5) as a primary dye for cotton, cellulosic fibres, wool, and nylon due to its excellent water solubility [4]. The chemical substance causes significant environmental damage. The azo bond-containing dyes break down into aromatic amines, which have powerful carcinogenic potential [3][5]. Discharging wastewater into water bodies requires specific wastewater treatment methods [6]. Biological treatment methods use microorganisms to decompose dyes because these organisms perform better than physical and chemical methods, thanks to their ability to adapt and operate, and their sustainability and affordability [1][2][4].

White-rot fungus-based biological treatment methods have become popular alternatives due to their affordable operations, modest management requirements, and environmentally friendly characteristics compared to conventional physiochemical methods, including ozonation, ion exchange, and coagulation-flocculation and adsorption 7]. Fungi possess multiple enzymatic activities through their extracellular ligninolytic enzymes, including lignin peroxidases, manganese peroxidases, and laccases[8]. Recent studies recognise laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) as a vital enzyme for environmental biotechnology operations [9]. Recent studies on the genus Trametes demonstrate its broad adaptability across different environments while producing enzymes [10][11][12]. The Trametes species exhibit a strong ability to degrade RB 5 and other synthetic dyes through effective degradation processes [13]. Numerous studies have focused on identifying high-performing strains among temperate species, yet research on tropical temperature strains remains limited, resulting in a significant knowledge gap.

The tropical rainforests of Kalimantan Island, noted for their remarkable biodiversity, serve as an underutilised resource for discovering new fungal strains with superior degradative capacities [14][15][16]. The discovery and characterisation of indigenous strains from these areas may yield more effective options for textile wastewater treatment, especially in tropical and subtropical locations. This study analyses Trametes sp. UM 12, obtained from Samarinda Botanical Garden. The research aims encompass assessing its capability for RB5 decolourisation and characterising its enzymatic functions under diverse operational conditions. The results enhance the existing knowledge

on biological treatment methods for textile wastewater and provide insights into the potential of tropical fungal strains for environmental applications.

2. Materials and Methods

2.1. Materials

Reactive Black 5 (RB 5) dye, guaiacol, and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were purchased from Sigma (USA). Peptone and Yeast Extract were purchased from Himedia (India). All other chemicals were provided by Wako (Japan).

2.2. Fungal Culture

The Trametes sp. UM 12 obtained from the Samarinda Botanical Garden was transferred to the chemical technology laboratory of the Engineering Faculty, Mulawarman University. Fungal was grown on petri dishes containing 2% Malt Extract Agar (MEA) supplemented with 50 ppm of chloramphenicol [17]. The petri dishes were incubated for 7 days at room temperature. Phanerochaete crysosporium ATCC 34541, used as a standard reference strain, was a gift from the Biomass Conversion Laboratory of the Research Institute for Sustainable Humanosphere, Kyoto University.

2.3. Laccase Enzyme Assay

Laccase activity was determined spectrophotometrically using crude laccase supernatant. Two mM ABTS in 0.1 M acetate buffer pH 5.0, at A_{420} nm for 1 min. The assay mixture contained 100 µL of supernatant, 400 µL of acetate buffer (pH 5.0), and 100 µLf 2 mM ABTS[18]. One unit (U) of laccase activity was defined as the amount of enzyme required to oxidise 1 µmol of ABTS per minute[18].

2.4. Decolourisation of RB-5

The decolourisation was carried out by adding 50 ppm of RB 5 into the medium containing (g/l): glucose (20), KH_2PO_4 (1), $MgSO_4.7H_2O$ (0.5), $CaCl_2$ (0.01), $FeSO_4.7H_2O$ (0.01), $MnSO_4.4H_2O$ (0.001), $ZnSO_4.7H_2O$ (0.001), $CuSO_4.5H_2O$ (0.002) [19]. The pH was adjusted to 5.0 after sterilisation at 121°C for 15 minutes. Ten mycelial discs (8 mm in diameter) were inoculated into each flask, and the cultures were incubated at 30°C in shaking conditions at 150 rpm for 96 hours. Culture supernatants (1 ml) were collected daily to monitor decolourisation and enzyme activities. All experiments were done in triplicate. The decolourisation of RB 5 dye was monitored spectrophotometrically at 598 nm using a UV-Vis Biobase spectrophotometer. Percentage of decolourisation was calculated by the following Eq. (1) [10][20]:

$$Decolorization\ (\%) = \frac{Initial\ absorbance\ of\ RB5 - Final\ absorbance\ of\ RB5}{Initial\ absorbance\ of\ RB5} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

2.5. Effect of pH and temperature

To determine the effect of pH on dye decolourisation, studies of RB 50 (50 mg L-1) decolourisation were conducted over a pH range of 4 to 7 [1]. The experimental studies were performed as per section 2.4. The cultures were incubated at 30°C in shaking conditions at 150 rpm for 48 hours. To determine the effect of incubation temperature on dye decolourisation, studies of RB 5 decolourisation at a 50 mg L-1 concentration (pH 5) were performed at temperatures ranging from 20 to 40 ◦C 20][21].

2.6. Effect of Agitation

To assess the impact of agitation on RB 50 (50 mg L-1) decolourisation, experiments were conducted. These experiments were incubated under optimal pH and temperature conditions, as determined in a previous study, both in a shaking environment at 150 rpm and in a static (non-agitated) setting for 48 hours.

2.7. Effect of dye concentration

To determine the effect of dye concentration on the decolourisation of RB 5 by *Trametes* sp. UM 12, experiments were conducted using RB 5 concentrations of 100, 250, 500, and 1000 mg/L. The dye was added to the culture medium prepared as described in section 2.4, with the pH adjusted to the optimal pH after sterilisation. Ten mycelial discs (8 mm diameter) of *Trametes* sp. UM 12 were inoculated into each flask containing the respective dye concentrations. Cultures were incubated under optimal conditions of temperature and agitation (as determined previously) for 120 hours. Samples were taken daily to measure decolourisation percentage. All experiments were performed in triplicate. Decolourisation was monitored spectrophotometrically at 598 nm, and the percentage of decolourisation was calculated using Equation (1).

3. Result and Discussion

The decolourisation efficiency of Trametes sp. UM 12 and P. chrysosporium ATCC 34541 against the Reactive Black 5 (RB 5) dye were evaluated over 96 hours of incubation (**Figure 1**). Both species demonstrated decolourisation capability, with the most rapid degradation occurring within the first 48 hours in Trametes sp. UM 12 exhibited higher performance, achieving 48% decolourization at 24 hours, 95% at 48 hours, and 99% by 72 hours. In contrast, P. chrysosporium ATCC 34541 showed moderate decolourisation efficiency, with 40% at 24 hours, 72% at 48 hours, and a maximum of 79% at 96 hours. Trametes sp. UM 12 shows superior decolourisation due to its robust ligninolytic enzyme system, particularly laccase, which effectively cleaves azo bonds in synthetic dyes [22]. Previous studies have reported similar findings, in which the genera Perenniporia, Trametes, and Deconica demonstrated exceptional dye-degrading capabilities of RB 5 due to their enzyme profiles and tolerance to xenobiotic compounds [23]. The moderate performance of P. chrysosporium may be related to its dependence on lignin peroxidase and manganese peroxidase [24], which require specific mediators and optimal conditions [24]. The rapid decolourisation of Trametes sp. UM 12 presents significant advantages for industrial wastewater treatment applications. Fungal bioremediation offers an environmentally friendly and cost-effective alternative with reduced treatment time and operational costs of wastewater treatment [25]. Fungi can address a wide range of pollutants, including heavy metals, persistent organic compounds, dyes, pharmaceuticals, and phenolic pollutants in wastewater, making them highly versatile agents for bioremediation [26]. These findings

demonstrate that Trametes sp. UM 12 is a highly promising candidate for textile wastewater treatment, offering superior bioremediation potential compared to the conventional model organism P. chrysosporium.

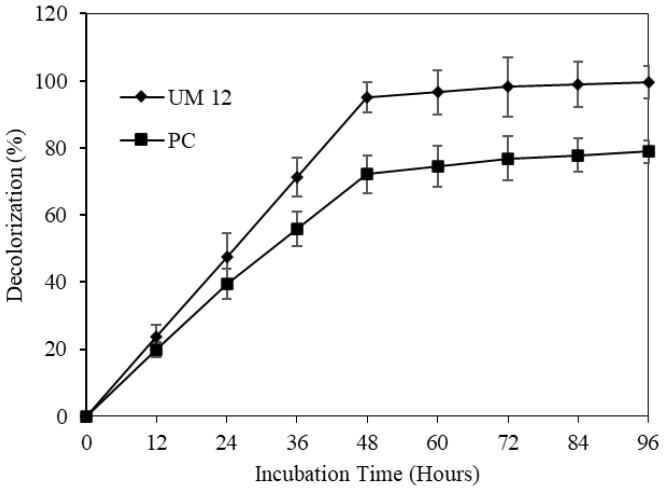

Fig 1. Decolourisation of RB 5 by Trametes sp. (UM 12) and P. chrysosporium ATCC 34541 (PC)

The study investigated how different initial pH levels between 4 and 7 affected Trametes sp. UM 12 decolourisation of RB 5 during 48 hours of incubation (**Figure 2**). The highest results showed that RB 5 decolourisation depended on pH, with pH 5 being optimal for Trametes sp. UM 12 strain removed 99.7% of RB 5 dye from the solution when the pH was set to 5 during 48 hours of incubation. The decolourisation rate at pH 5 reached 99.7% (48 h), resulting in almost complete dye elimination. The decolourisation rate at pH 5 was 6-fold higher than at pH 7 and 3.2-fold higher than at pH 6 over the 48 hours. The decolourisation process showed extreme sensitivity to pH: UM 12 produced 6-fold more decolourisation at pH 5 than at pH 7, and 3.2-fold more at pH 6. The superior performance at pH 5 showed optimal of multiple biochemical parameters essential for laccase function. Fungal laccases, particularly those from Trametes species, possess catalytic copper centres (T1, T2, and T3) whose coordination geometry and redox potential are exquisitely pH-sensitive [27]. At pH 5, the type 1 copper site maintains its optimal Cu^{2+}/Cu^{+} redox potential (0,45-0,71 V) [28][29], facilitating efficient one-electron oxidation of phenolic and non-phenolic substrates through direct or mediator-facilitated mechanisms [30][31]. Furthermore, Trametes and related species grow efficiently in media adjusted to pH values from 4.0 to 7.0 [4][32][33][34]. Studies report that pH 5.5 yields the highest growth rates, with mycelial mass, protein concentration, and laccase activity increasing under these conditions at standard culture temperatures (25–30°C) [33]. Growth and enzyme yields may decline slightly under extreme conditions.

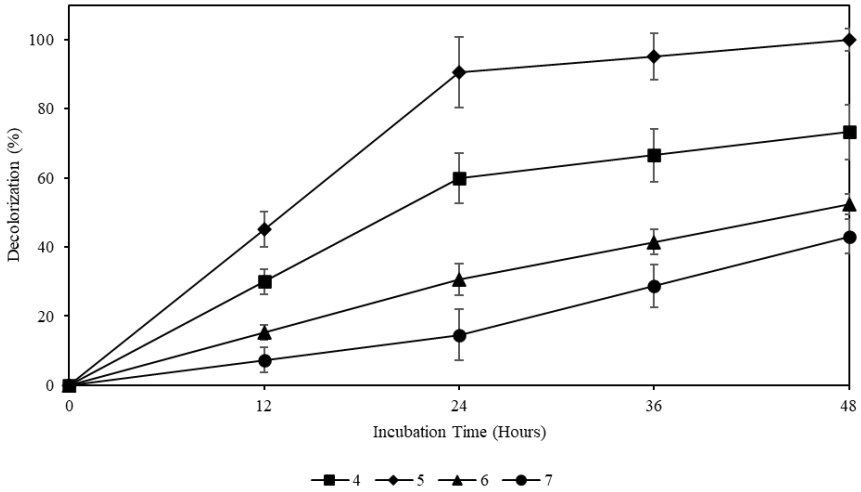

Fig 2. Effect of pH on decolourisation (%) of RB 5 by UM 12

The influence of incubation temperature on RB 5 decolourisation by Trametes sp. UM 12 was evaluated across a temperature range of 20-40°C within 48 hours (**Figure 3**). The results revealed optimal decolourisation performance observed at 30-35°C. Temperature not only affected enzymatic catalytic efficiency but also fundamentally regulated fungal metabolic activity, mycelial growth, and ligninolytic enzyme production, demonstrating the integrated nature of physiological and biochemical parameters in biodegradation processes [32][35][36][37]. Temperature significantly influenced RB 5 decolourisation by Trametes sp. UM 12 over 48 hours. Optimal performance occurred at 30°C and 35°C, achieving 97.1% and 98.4% decolorization, respectively, with peak laccase activity on 12-24 hours. At 20°C, decolourisation only reached 72.4% (48 h), reflecting temperature-limited enzyme production. At 40°C, performance dramatically decreased to 42.1% (48 h). The optimal temperature range of 30-35°C was identified for Trametes sp. UM 12 aligns well with reported optima for related species. Pleurotus exhibits maximum decolourisation of RB 5 at 35-40°C [38], Gloeophyllum at 25°C [4], and Trametes trogii at 30-33°C [39]—the moderate thermotolerance of Trametes sp. UM 12 (optimal at 35°C) categorises it as a typical mesophilic

tropical isolate suitable for ambient-temperature industrial applications but requiring cooling systems if operated in high-temperature industrial settings [40].

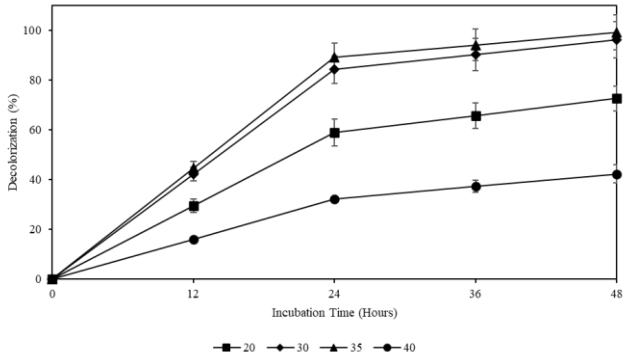

Fig 3. Effect of temperature on decolourisation (%) of RB 5 by UM 12

Agitation in a rotary shaker at 150 rpm significantly enhanced RB5 decolourisation by Trametes sp. UM 12 compared to static conditions over 48 hours (**Figure 4**). Under agitated conditions (150 rpm), the strain achieved 37% (12 h), 52% (24 h), 90% (36 h), and 99,6% (48 h) decolourisation. In contrast, static cultures yielded 27.8% (12 h), 37.4% (24 h), 65.9% (36 h), and 89.2% (48 h). The highest performance under agitation primarily reflects enhanced oxygen mass transfer, which is critical for aerobic fungal metabolism and laccase-catalysed oxidation [34][41]. Additionally, agitation promotes uniform substrate distribution, optimal dispersed mycelial morphology, and maximises enzyme secretion and distribution efficiency 17][42][43].

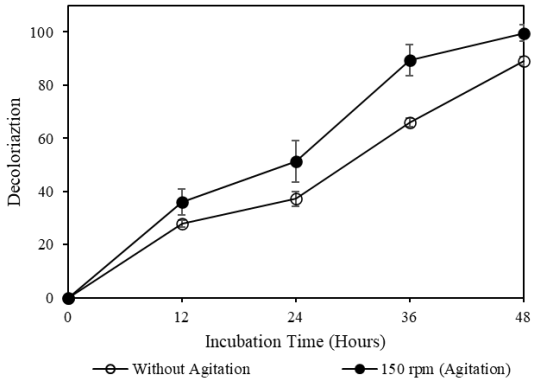

Fig 4. Effect of with and without agitation on decolourisation (%) of RB 5 by UM 12

The initial RB 5 concentration significantly influenced the decolourisation efficiency of Trametes sp. UM 12 over 120 hours (**Figure 5**). At 100 mg/L, optimal performance was achieved with 10.2% (24 h), 38.4% (48 h), 79.8% (72 h), 98.3% (96 h), and 98.7% (120 h) decolourisation, representing near-complete dye removal. At 250 mg/L, efficiency decreased moderately to 7.8% (24 h), 32.7% (48 h), 59.3% (72 h), 84.6% (96 h), and 96.8% (120 h). However, performance declined dramatically at higher concentrations. At 500 mg/L, decolourisation reached only 52.1% (120 h), while at 1000 mg/L, it reached only 31.8% (120 h). The inverse relationship between dye concentration and decolourisation reflects substrate inhibition, enzyme saturation kinetics, and dye toxicity effects on fungal growth [44][45][46].

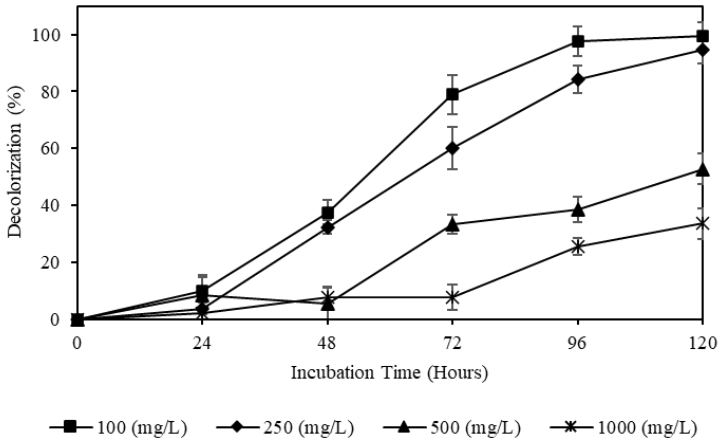

Fig 5. Effect of Dye concentration on decolourization (%) of RB 5 by UM 12

4. Conclusion

The Trametes sp. The UM 12 strain shows exceptional potential for treating textile wastewater containing azo dyes, including Reactive Black 5, as it was isolated from a distinctive tropical habitat. The strain showed superior dye decolourisation performance, completely removing all colour within short incubation periods, exceeding the capabilities of typical fungal strains. The study shows that white-rot fungi, which occur naturally in tropical rainforests, can serve as sustainable wastewater treatment methods in tropical and subtropical regions. The research data enables scientists to create industrial biological treatment systems which use biological methods to extract dye pollutants from industrial waste streams.

Acknowledgement

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